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Tissue Engineering for Bone Regeneration and Osseointegration in the Oral Cavity

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1. Introduction

The alveolar processes of the mandible and maxilla line the alveolus and provide structural support and maintenance for teeth as part of the periodontium, consisting of the periodontal ligament (PDL), cementum, connective tissue, and gingiva. Alveolar bone is especially susceptible to inflammation-induced bone resorption due to high rates of progressive periodontitis—a leading chronic oral inflammatory disease estimated to affect 47.2% of adults in the United States, with a prevalence of 70% for adults aged 65 years and older [1]. Advanced periodontal disease alters alveolar bone morphology and destroys surrounding tooth-supporting tissues, thereby necessitating tooth extraction. Since the existence of alveolar bone is mutually connected to the dentition and other periodontal tissues, the alveolar ridge continues to resorb following tooth removal even if a dental implant is placed into a fresh extraction socket. Physiologically, this is caused by continuous bone remodeling in response to mechanical loading changes that occur with alterations in the applied force and strain distribution to the osseous tissue during mastication, as stipulated by Wolff's Law [2]. Ridge or socket preservation and augmentation using bone grafting materials is a clinically viable approach to maintain any remaining bone following tooth extraction and further condition it in preparation for dental implant placement. Sufficient bone volume, height, and width are necessary to ensure implant stability and osseointegration that can sustain optimal bone-implant contact biomechanical loading. Other dental procedures that

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involve grafting include maxillary sinus floor augmentation, which is employed for patients with bone loss in the posterior maxilla that houses premolar and molar teeth [3]. Bone defects in the oral cavity resulting from trauma, chronic infection, congenital defects, or surgical resection require clinical intervention, most frequently using autologous bone grafting techniques. However, critical limitations of this approach include donor site morbidity and inadequate supply of graft tissue. Tissue engineering approaches using scaffolds alone or in combination with growth factor, cell and/or gene delivery have the potential to address existing challenges in managing bone loss and increase clinical options for controllable regeneration of intraoral osseous tissues.

2. Scaffolds

2.1 Intraoral bone grafts

An autologous bone graft is considered the gold standard due to low risk of immunogenicity or disease transmission that could be associated with an allograft (genetically different donor from the same species) or xenograft (donor from another species). Most importantly, bone transplanted from the patient is native to its host environment and readily associates with the remnant tissue, providing a pre-established population of viable cells and growth factors necessary for osteogenesis. Local sites such as the maxillary tuberosity or mandibular symphysis can be used for harvesting of small autologous grafts [4]. Nevertheless, there are several key reasons for a critical need of alternative grafts capable of substituting the autograft: limited availability of autologous tissue for larger bone defects, donor site morbidity and potential wound-based infections, as well as prolonged operative times [5]. Although lacking in osteogenicity, allografts and xenografts can be prepared to have osteoconductive and osteoinductive properties. Bone allografts are available as fresh/fresh-frozen, freeze-dried, or demineralized and freeze-dried. The mechanical properties of allografts derived from a living donor or cadaveric tissue are changed substantially during extensive tissue processing involving decellularization, sterilization, and preservation for clinical use [6]. Such tissue treatment removes viable cells that are osteogenic and osteoinductive in nature, leaving behind a structurally supportive framework primarily composed of minerals and proteins-termed the extracellular matrix (ECM). The allograft ECM serves as a scaffold for osteoblasts originating from the bone defect into which the graft is placed to facilitate new bone formation. Depending on the method of processing, an allograft can also be osteoinductive if it retains the biological properties necessary to recruit mesenchymal stem cells to the site and stimulate their differentiation into osteoprogenitor cells. One example is demineralized bone matrix (DMB), which has reduced levels of calcium and phosphorus and is primarily type I collagen, but can be considered osteoinductive if it retains factors such as bone morphogenetic proteins (BMPs) and transforming growth factor- β (TGF- β) [7]. As expected, DMB shows an increased rate of resorption relative to a mineralized bone graft during tissue remodeling *in vivo*. In addition, derivation of DBM involves grinding of bone to obtain particulates as opposed to processing the allograft in its native structural form, making it useful for small to moderate defects [8].

Xenografts offer another alternative for bone replacement in dental regeneration, with most products derived from coral, porcine, or bovine sources. A recent study comparing implant

placement into sinus floors augmented with an autologous mandibular bone graft versus a commercially-available bovine xenograft found equivalent implant survival rates over an observational period of 5 years [9]. However, implant survivability depends on many factors, including patient demographics and surgical technique, thereby warranting longer-term evaluations and more comprehensive consideration of factors that may influence the clinical outcome. Extensive meta-analysis of histomorphometric and bone graft healing time results for sinus floor augmentation described in the literature over a period of 16 years concluded that autologous bone grafts result in higher total bone volume levels compared to other bone grafting materials [10]. Another comprehensive systematic review of treatment modalities used to evaluate dental implant survival rates in maxillary sinus grafts employed statistically robust methodology to correct for study effects. It concluded that application of grafting membranes for guided bone regeneration supplementary to a bone graft was more important for implant survival rate over factors such as which bone substitute material was selected for the surgery [11]. These results indicate the difficulty of identifying specific factors that influence final clinical outcomes and underline the fact that there is no unified consensus on whether non-patient derived grafts can perform at the same level as autografts for not only bone regeneration but also implant performance at augmented bone sites. Each case is patient-specific and requires thorough consideration of all contributing factors, including the health of the patient's native bone and its suitability for grafting procedures.

2.2 Natural and synthetic matrices

In addition to standard grafting procedures using bone-derived materials, a number of natural and synthetic materials have become commercially available for use in oral surgery. Scaffolds that are architecturally and/or biologically compatible for bone regeneration are frequently based on one or more of the bone's naturally-occurring proteins or minerals, including organic (predominantly collagen type I) and inorganic (hydroxyapatite, a calcium phosphate mineral) components [12]. Calcium phosphate (CaP) materials are subdivided into ceramics and cements, which vary in their rate of *in vivo* degradation, structure, and mechanical strength. Common synthetic CaP bone substitutes include hydroxyapatite (HA) ceramics, β -tricalcium phosphate (β -TCP) cements, and biphasic calcium phosphates (BCPs) [13, 14]. Fragility and poor fatigue resistance of these ceramics and cements requires their use at non-load bearing bone replacement sites or as coatings on load-bearing metal implants for increased bone-to-implant contact. Coating a dental implant surface (i.e., titanium, stainless steel, or an cobalt-chrome alloy) with CaP-derivatives has been extensively investigated using various surface coating deposition techniques to improve implant stability and rate of osseointegration [15, 16]. In addition to containing minerals native to osseous tissue, these biomaterials retain an interconnected porous architecture--allowing adequate space through increased surface area for bone ingrowth via cell infiltration, blood vessel formation, nutrient/oxygen transport, and waste elimination. Ongoing studies are being performed to determine the optimum porosity for bone ingrowth and corresponding bone substitute resorption rate, since an ideal regenerative scenario would consist of a biomaterial resorption rate timed with new osseous tissue ingrowth [17]. Likewise, there are ongoing investigations to confirm the utility of using CaP bone substitutes for implant coating. For example, recent studies of hydroxyapatite coatings on titanium cups for orthopaedic-based

implants such as femoral stems did not show significant differences between HA-coated and non-coated stems and may not constitute a clinical advantage [18].

Bioactive glass (BG), a silicon oxide with substituted calcium first developed in the 1960s by Professor Larry Hench, is a biocompatible glass-ceramic material approved by the US Food and Drug Administration (FDA) for use as a synthetic intraoral bone graft (termed 45S5 Bioglass®). Upon exposure to aqueous solutions the highly reactive surface converts to a gel layer that mineralizes to form an osteoconductive hydroxy carbonate apatite layer that chemically binds with osseous tissues [19]. Bioglass® also has a reported elastic modulus of 35 GPa that is similar to that of cortical bone ($E_{\text{longitudinal}} = \sim 14\text{--}20$ GPa) [20, 21]. Comparatively, the elastic moduli of HA single crystals and β -TCP are in the range of 54–79 GPa and 120–162 GPa, respectively [22, 23]. This makes BG an attractive option for metal implant coating, reducing the potential for stress shielding and subsequent bone resorption which occurs with decreased bone loading [24]. To date, studies using dental implants coated with BGs have not conclusively shown significant increases in osseointegration relative to other coatings such as HA [25]. However, novel exploratory combinations for dental implant coatings using BG are promising: one example is the incorporation of BGs with HA and the polymer poly(lactide-*co*-glycolide) (PLGA), which indicates rapid bone-like apatite formation *in vitro*, with potential antimicrobial activity on oral bacteria [26].

Polymeric materials that have been commercialized or are currently under investigation for intraoral bone regeneration are either naturally- or synthetically-derived. Natural materials that consist of polymeric networks (i.e., collagen, alginate) have been extensively investigated as composite materials with other bone replacement grafts, including β -TCP and HA [27]. Major advantages of a tissue-sourced polymer such as collagen include its biocompatibility, biodegradability, and ability to readily bind growth factors critical for osteoinduction, including BMPs. Currently, collagen is the most commercially-available natural polymer on the market for use in periodontal bone regeneration as a sponge, membrane, or in particulate form combined with other bone grafts [28]. It is expected that future research will continue to focus on the development of a more diverse array of naturally-derived, fully-resorbable polymeric bone grafts combined with non-immunogenic materials such as alginate and chitosan that can be engineered for growth factor, cell, and/or gene delivery. Such delivery devices may also include synthetically-derived resorbable materials whose physical, mechanical, and degradation properties can be more easily controlled via polymer chemical composition and molecular weight. Historically, oral surgery has utilized non-resorbable synthetic membranes as cell-occlusive barriers for guided bone regeneration, among which the most broadly used material has been expanded polytetrafluoroethylene (ePTFE) [29]. Unnecessary patient discomfort and added cost of a follow-up visit to remove the membrane has increased demand for resorbable alternatives. Examples of widely-investigated resorbable polymers for applications in bone regeneration include poly- α -hydroxy esters such as polyglycolic acid (PGA), polylactic acid (PLA), and PLGA. The ease of processing synthetic materials enables their fabrication into a variety of structurally-diverse forms, including thin films, meshes, fibers, and porous foams. An existing disadvantage of these materials is their bulk erosion *in vivo* due to hydrolysis that can induce foreign body reactions to acidic polymer degradation products, increasing the

potential for fibrous tissue encapsulation during wound healing. Although studies of PLGA utility for guided bone regeneration are still limited, there is evidence that its use as a barrier membrane results in alveolar bone regeneration that is on par with that observed using a collagen-based membrane [30]. Other copolymer combinations that yield useful properties for osseous tissue regeneration include PLGA with poly(ethylene glycol) (PEG), a widely used biocompatible hydrophilic drug delivery carrier. PEG-PLGA thermo-sensitive copolymers can be encapsulated with osteoinductive factors and polymerized into a gel-like structure for delivery into an osseous defect, possibly in combination with other mechanically durable bone grafts [31]. PCL is another biodegradable polymer with significant research indicating its suitability for bone regeneration. An inert material, it shows increased osteoblast adhesion, spreading, and proliferation when coated with CaP or HA [32], and multiple studies have indicated PCL's potential to promote alveolar bone formation in periodontal defects [33, 34]. With greater emphasis placed on materials that can be used for exogenous factor delivery to accelerate and improve existing periodontal tissue defect treatments, polymers such as PCL and PEG which are already FDA-approved as drug delivery devices are expected to be more extensively investigated in pre-clinical models of intraoral bone regeneration in the coming years.

2.3 Engineering scaffolds for intraoral bone regeneration

The development of scaffolds that are optimal for regeneration of osseous tissues requires a design strategy which adheres to established knowledge of the mechanical, chemical, structural, and biological properties of natural bone that make it a functional entity. Therefore, key considerations in such scaffold design include: (1) biocompatibility/non-toxic degradation; (2) bioactivity, enabling cell interaction with material surface; (3) maintenance of a 3-D shape after implantation; (4) adequate porosity and pore diameter/distribution/orientation; (5) mechanical properties similar to tissue targeted for regeneration (i.e., Young's modulus); (6) degradation mechanics (i.e., bulk erosion, surface erosion); (7) degradation rate, ideally matching the rate of tissue regeneration; and (8) osteoconductive/inductive and angiogenic factors to influence infiltrating cell populations and promote blood vessel invasion [35, 36].

While a broad range of instructive carrier materials in various forms have been investigated for bone regeneration, the addition of cells and application of growth factor delivery strategies can significantly influence the regenerative outcome by engineering the environment that closely matches that of the target tissue in its native state (see Figure 1). Current strategies include the use of bone-marrow stromal cells and stem cell varieties including mesenchymal (MSCs), adipose-derived (ADSCs) and induced pluripotent (iPSCs). Analyses of cell-specific markers and transcription factors such as Runx2, alkaline phosphatase (ALP), osteocalcin, osteopontin, and osteonectin allow for the determination of osteogenicity during stem cell differentiation into bone-derived cells [37]. Clinically-applicable cell therapy is focused on the use of patient-derived stem cells that are undifferentiated, given that terminally-differentiated cells are difficult to expand *ex vivo* relative to more highly proliferative stem/progenitor cells. Likewise, the use of stem cells allows for a more completely physiological repair process that involves the differentiation of MSCs or iPSCs not only into bone-derived cells but also cell types involved in

neovascularization, such as endothelial cells. However, despite significant progress and tremendous potential in cell therapy, critical challenges remain in transitioning the use of iPSCs into clinically-applicable approaches. Since the creation of iPSCs involves the reprogramming of somatic cells via transcription factors to produce cells with embryonic stem cell-like properties, there is risk of epigenetic and genetic defect accumulations, immunogenic responses, or tumor formations. While the immunogenic response can be mediated through use of a patient's native somatic cells, overexpression of transcription factors or presence of partially-reprogrammed iPSCs are known to cause teratoma formation, requiring significant efforts to address these safety concerns prior to the consideration of iPSCs for patient-based treatments [38].

With advancements in cell therapy, there has been a simultaneous increase in novel scaffold fabrication techniques that emphasize greater control over surface topography, internal microstructure, and pore interconnectivity. Traditionally, porous scaffolds have been widely explored as bone graft substitutes for cell attachment given the importance of allowing adequate room for tissue ingrowth and vascularization (i.e., pore size of 150-500 μm) [39]. While natural materials retain their bioactivity, synthetic non-immunogenic materials have several advantages for development of clinically-translatable scaffolds, including added flexibility in manufacturing, reproducibility, sterilization, and storage times. Electrospinning and solid freeform fabrication (SFF) are two scaffold fabrication techniques which allow increased control over scaffold morphology: Electrospinning is a polymer-processing technique used for creating polymer fibers on the nano and micron scale to influence cell behavior through structural and physical cues that mimic ECM architecture. In addition to allowing control over a variety of parameters that determine fiber dimension, density, and porosity, electrospinning can readily be used for mass production of fiber-based scaffolds [40]. More recently, 3-D printing technology has been adapted for use in bone tissue engineering via solid freeform fabrication (SFF), a rapid prototyping technique. This process consists of developing a computer-aided design (CAD) file that specifies the exact dimensional features of the desired scaffold which is then transferred to a 3-D printer that reproduces the file to yield a printed version of the design with structural integrity. Selective laser sintering (SLS) is an example of a process that creates objects layer-by-layer using polymeric, ceramic, or metal powders that the machine sinters. During sintering, the powder is heated below the melting point, causing its particle boundaries to fuse together at locations dictated by the CAD-based file. When creating a porous material, the sizes and characteristics of the individual pores within the material are limited by the machine's resolution and ability to support a specific printing material [41]. Studies using SLS for bone regeneration have focused on PCL-based printed scaffolds, showing that these are mechanically-appropriate to support bone tissue formation and can be used as BMP-7 and BMP-2 growth factor carriers following biofunctionalization [42, 43]. This technology is especially applicable for clinically-based studies given that patient-specific anatomical bone defects can be obtained using computed tomography (CT) scans and reproduced to yield a scaffold with appropriate structural dimensions. A recent publication by Park et al proposes a potential future application of image-based PCL SFF-based scaffolds for clinical periodontal regeneration (see Figure 2), indicating that further pre-clinical investigation and

verification of these technologies will bring them closer to translation into clinical practice [44].

While novel methods of scaffold development and cell therapy are being explored for bone regeneration, there is already an established array of clinically-applicable therapeutic factors and delivery strategies. In order to better understand existing clinical applications and the nature of ongoing pre-clinical studies, the remainder of this review focuses on the areas of growth factor delivery for oral bone regeneration and highlights some of the key studies that have moved previously experimental therapies into today's mainstream clinical applications in periodontal regeneration.

3. Growth Factors and Protein Delivery

Growth factor delivery (GFD) is of critical importance in mediating the scaffold environment and subsequent cellular response. Growth factors are soluble polypeptides that bind to cell membrane receptors and influence cellular function, and the inclusion of osteoinductive/conductive factors (BMP, TGF- β , IGF, FGF-2, PDGF) guides the cell differentiation and tissue formation process in bone regenerative therapies. Likewise, vascularization is vital for the sustainability of newly-formed tissue, and necessitates the inclusion of GFs with angiogenic properties (PDGF, VEGF, FGF-2, and TGF- β). Scaffold-based GFD as opposed to bolus injection of the therapeutic factors into the defect site has several advantages, including the potential for improved control over GF release kinetics and localization. A major challenge in GFD involves the need for release profiles that mimic those present during natural tissue repair or morphogenesis. Sequential or simultaneous spatiotemporal delivery of multiple GFs can be achieved using scaffolds based on the therapeutic time window during which GF delivery is optimal for tissue regeneration, and can involve the combination of factors involved in both tissue formation and angiogenesis. For example, sequential delivery of GF combinations consisting of BMP-2 and TGF- β or VEGF and BMP showed increased bone formation when compared to single GFD [45, 46]. GF rate of release is governed by how it is bound to the scaffold, which can involve (1) mixing the GF with scaffold particles, (2) physical encapsulation within the scaffold, (3) chemical immobilization, and (4) affinity-based binding. Current clinical application of GFs for bone regeneration typically involves the use of biomaterial carriers, yet the factor is usually mixed in and physically adsorbed onto the graft particles and lacks more sophisticated modes of delivery that would enable spatiotemporal control over release kinetics or dual GFD. Other modes of delivery currently investigated in preclinical studies are promising: Physical encapsulation of GFs within the scaffold, for example, can be achieved using polymeric microparticles (1-100 μ m) with varying surface areas through which the encapsulated GF diffuses at a rate that is dependent on carrier particle size, with a larger size leading to slower diffusion. GFs can also be conjugated on the scaffold surface via covalent immobilization, while heparin-binding GFs such as BMP-2 can be presented using affinity-based binding by conjugating heparin to biomaterials [47, 48]. These modes of GFD have potential for future applications in clinical studies, allowing for a broad range of release profiles and controlled spatiotemporal presentation of multiple GFs using carriers that serve as temporary support structures for osseous tissue engineering strategies.

Growth factors and proteins act locally on the activity of periodontal cell populations to modulate bone formation and enhance the regenerative response (see Table 1). A diverse range of bone matrix-based proteins have been isolated and are delivered alone or in combination with a synthetically- or naturally-derived bone graft. An overview of some of the factors and proteins that are featured most prominently in oral-based surgery or currently being investigated in preclinical (see Table 2) and clinical studies (see Table 3) is presented here.

3.1 Pre-clinical Studies using Growth Factor and Protein Delivery

3.1.1 Platelet-derived growth factor (PDGF)—PDGF's primary role is the promotion of soft-tissue healing. It was introduced to improve healing of diabetic ulcers, and was later approved for periodontal regeneration [49, 50]. *In vitro* studies show that PDGF stimulates cell populations key for periodontal regeneration, increasing cementoblast DNA synthesis and regulating osteopontin expression [51]. PDGF stimulates PDL cell chemotaxis and mitosis, and has synergistic proliferative effects when combined with allografts [52, 53]. PDL cells may modulate bone formation by increasing osteoblast proliferation and blocking osteoblast differentiation and expression of the mineralized tissue markers osteopontin and osteonectin [54-56]. In canine and primate models, application of PDGF, often combined with insulin-like growth factor (IGF), to periodontal defects resulted in increased bone, cementum, and PDL formation [57-63]. In a canine class III furcation defect model, guided tissue regeneration (GTR) with PDGF-BB and ePTFE membranes stimulated PDL formation in early stages followed by total periodontal regeneration [64]. PDGF/IGF-1 for guided bone regeneration (GBR) at implants placed into extraction sockets showed increased early (3-week) histologic and clinical bone formation [57, 65, 66]. When combined with xenograft, PDGF at immediate implants in canines resulted in enhanced radiographic bone gain when used without a collagen membrane [67]. PDGF also enhanced lateral GBR in dogs when used with a collagen membrane and xenograft or alloplastic graft material. In another canine model testing for vertical ridge augmentation, PDGF in a xenogeneic block graft showed increased histologic bone gain when used without a collagen membrane [68, 69].

3.1.2 Bone morphogenetic proteins—BMPs are members of the transforming growth factor-beta (TGF- β) superfamily and have strong osteoinductive properties, especially BMP-2, -4, -6, -7, and -9 [70, 71]. BMP-2 and BMP-7 stimulate PDL cell differentiation into osteoblasts and increase expression of mineralized tissue markers when combined with PDL cells or osteoblasts *in vitro*, although doses greater than 10 ng/mL may be toxic to cells [72-76]. BMPs have also been shown to downregulate proliferation and mineralization of cementoblasts and gingival fibroblasts. They are used primarily to enhance bone formation for implant site development. When applied to periodontal intrabony defects, BMPs showed enhanced speed and quantity of bone formation but limited cementogenesis, complicated by ankylosis and root resorption [77-83]. Commercially, rhBMP-2 is approved for extraction socket and sinus augmentation. In rat extraction sockets, BMP-2 was shown to increase the speed and quantity of bone formation via its osteoinductive effects [84]. In sinus augmentation models in various species, BMP-2 consistently improved histologic and radiographic bone gain [85-89] and there are indications that it may provide significant

vertical and horizontal ridge augmentation. Delivery of BMP-2 via grafting materials may modulate its effects on bone formation, contour, and quality [90-94]. Possible complications of BMP-2 delivery shown in canine ridge augmentation models include increased incidence of seromas and wound failure [91]. When compared to BMP-2, BMP-4 has been shown to have comparable effects on bone gain and improved bone quality in a rat ridge augmentation model [90]. BMP-7, also known as osteogenic protein-1 (OP-1), has similar applications as BMP-2 [95-101]. BMPs may be suitable for periodontal regeneration as demonstrated in canine class III furcations and its modulatory effects on cementoblast mineralization *in vitro* [95, 102].

3.1.3 Fibroblast growth factor-2—FGF-2 was initially found to stimulate proliferation of bovine fibroblasts. It has effects in soft tissues by inducing proliferation of gingival epithelial cells, gingival and connective tissue fibroblasts, and PDL cells [103-107]. Interestingly, FGF-2 inhibits mineralization and ALP expression by PDL cells but allows them to maintain their differentiation potential and express bone regulatory compound osteopontin [104, 108]. These features, coupled with its strong angiogenic potential, may allow FGF-2 to promote an environment favoring periodontal regeneration [104, 109]. FGF-2 applied topically has been studied in primate and canine class II furcation defect models, where it significantly increased regeneration of PDL, cementum, and bone without adverse effects [106, 110]. GTR with FGF-2 and a collagen membrane resulted in more defect fill in dog class III furcations versus control groups [111].

3.1.4 Growth/differentiation factor-5—Like BMPs, GDF-5 is another member of the TFG- β superfamily and shares a similar structure [112]. It stimulates PDL cell proliferation, early osteoblast differentiation, and extracellular matrix synthesis by both cell types [113, 114]. It has been shown to significantly increase periodontal regeneration in canines and primates with a β -TCP carrier [115, 116]. Various other carriers tested in dog periodontal regeneration showed a primarily positive effect [117, 118]. In implants, GBR with GDF-5/ β -TCP carrier showed increased peri-implant bone, while GDF-5 coated implants had increased stability determined by pull-out test in rabbits [119]. GDF-5 has also been used in mini-pig sinus augmentation, where it enhanced bone formation with a β -TCP carrier [120, 121]. In lateral ridge augmentation with a coated Bio-Oss® block, GDF-5 resulted in increased mineralized tissue formation [122].

3.1.5 Teriparatide—The osteoporosis medication teriparatide consists of parathyroid hormone's first 34 amino acids. *In vitro* and depending on cell state, teriparatide influences PDL cell survival and causes osteoblast-like behavior with increased osteoprotegerin expression [123, 124]. It has been tested in rats with induced osteoporosis, where it caused increased bone mineralization and formation in extraction sockets and prevented periodontal bone loss [125, 126]. In canine GBR, teriparatide improved bone formation around implants [127, 128].

3.2 Clinical Applications of Growth Factor and Protein Delivery

Periodontal regenerative therapies focus on bone regeneration to provide implant site development. Guided bone/tissue regeneration is the most well-documented technique for

targeted bone regeneration and is designed to exclude undesired cells using barrier membranes [129]. Under certain circumstances (i.e., defect shape and size) both procedures have shown high predictability; nonetheless, due to the non-osteogenic characteristics of available biomaterials (xeno-/allo-geneic), some shortcomings have arisen for challenging situations (i.e., vertical bone augmentation). The application of growth factor and protein delivery might overcome these limitations by inducing the proliferation of MSCs to achieve bone formation [130, 131]. Indeed, this approach has shown very promising results from countless pre-clinical studies [132, 133] (see Table 3), which has aroused enthusiasm among clinicians.

Within osseous regeneration, PDGF-BB has been tested for infrabony defects and alveolar bone regeneration. Recently, a multicenter randomized double-masked clinical trial aimed to evaluate the long-term stability of periodontal defects in patients with localized severe periodontitis filled with rhPDGF-BB in a β -TCP scaffold vs. scaffold alone. Results from this study showed that the use of PDGF-BB (0.3 mg/mL) after a 36-month follow-up achieved 87% of bone gain compared to the control group (53.8%). It was also found that, albeit not reaching statistical difference, the steady increase of clinical attachment level and linear bone gain suggest the long-term stability when using this growth factor [134]. These findings were in agreement with results obtained by Jayakumar et al. in a shorter follow-up clinical trial [135]. These demonstrated that after 6 months, a significant increase in bone fill (65.6%) was achieved when using 0.3 mg/mL of PDGF-BB vs. β -TCP alone (47.5%) [50]. Nevins et al. also reported that the use of PDGF-BB in combination with demineralized freeze-dried allogeneic bone graft is capable of attaining complete regeneration of the attachment apparatus for infrabony and Class II furcation defects [136]. Therefore, PDGF-BB seems to promote periodontal regeneration in a safe and effective manner. However, the available data is still limited to draw clear conclusions about its potential. Likewise, PDGF-BB has also been recently used for increasing the predictability of alveolar bone regeneration. Nevins et al. evaluated the effect of PDGF-BB compared with enamel matrix derivative (EMD) and two other grafting materials upon newly formed bone in socket regeneration [137]. Due to the small sample size and weak defect standardization it was not possible to draw a clear line between groups; nevertheless, ridge morphology for implant placement was more convenient for the PDGF-BB group. More recently, the same group [138] studied in a case-series the effect of PDGF-BB in combination with equine/bovine grafting materials on bone regeneration of large extraction site defects. Histologic results revealed new bone formation in association with remaining graft particles and no evidence of inflammatory cell infiltration. Accordingly, horizontal/vertical alveolar bone augmentation might become a more predictable technique with the use of PDGF-BB.

In 2007, rhBMP-2 was approved by FDA as an alternative to autologous bone grafting in alveolar ridge augmentation and sinus elevation procedures [139] due to its osteoinductive potential [140]. Results obtained for sinus augmentation indicate that when compared to autogenous bone, no differences can be observed by means of vertical bone gain and density [141]. Nonetheless, if rhBMP-2 is grafted with autologous bone it seems to increase cell activity, osteoid lines and vascular supply [141, 142]. Contrasting these findings, Kao et al. showed that when rhBMP-2 was blended with bovine-derived xenogeneic graft, less bone formation was found. Authors claimed that this may be due to the enhancement of osteoclast

differentiation by the adjustment of RANKL, a protein involved in bone remodeling and regeneration [143]. Additionally, the safety of the growth factor was demonstrated by lack of an immune response in any of the studies [141-143]. Therefore, agreement was found between human clinical trials and preclinical studies [144]. However, more clinical studies need to be conducted focusing on the effect of the carrier and dose-dependent responses [145]. Other findings indicate that rhBMP-2 preserves alveolar ridge heights while also increasing their horizontal dimensions [146-148]. Fiorellini et al. noted the dose-dependence of the protein, showing that a 1.5 mg/mL dose was more optimal than 0.75 mg/mL. Nevertheless, it is worthy to note that although overall safety of rhBMP-2 was demonstrated, moderate signs of local inflammation were present, which may be a trigger for an impaired healing process.

BMP-7 has been predominantly studied for sinus augmentation. Using a small sample size case study, Corinaldesi et al. compared the use of rhBMP-7 with deproteinized bone (0.5g) vs. deproteinized bone alone (2g). Although there were no observed differences by means of bone gain, newly formed bone was statistically greater for the control group (19.9% vs. 6.6%) 4 months after grafting [149].

Another recently studied growth factor is GDF-5, which has been used for periodontal regeneration, alveolar bone and sinus augmentation procedures. A pilot randomized clinical trial was conducted to study the effect of GDF-5 embedded in β -TCP for infrabony periodontal defects of chronic periodontitis patients. Six months after therapy the clinical attachment gain for the test group was almost double compared to the level gained in the control group (3.4mm vs. 1.7mm), although this was not statistically significant [150]. To date, only one study has appraised the use of rhGDF-5 for sinus augmentation: Koch et al. found that at 4 months GDF-5 behaved similarly to an autologous graft in terms of bone formation (28% vs. 32%) [151]. Notably, larger bone augmentation occurred in the composite group of GDF-5 combined with β -TCP regardless of the time point assessed.

After being evaluated in pre-clinical model and showing its effectiveness in regenerating periodontal defects, FGF-2 has also been tested for human use due to its robust angiogenic and mitogenic potential [110]. Kitamura et al. showed in a Phase 2B multicenter randomized clinical trial that at 36 weeks after periodontal surgical therapy, bone fill was 35% greater when used with 0.3% FGF-2 compared to the carrier alone [152]. An insignificant increase occurred from 36 up to 72 weeks, which may be explained by different patterns of healing (regeneration vs. long-junctional epithelium).

Currently, teriparatide is being investigated for craniofacial regeneration [153]. One clinical trial assessing its effect on periodontal regeneration in moderate to severe chronic periodontitis patients showed that the adjuvant of teriparatide administration combined with vitamin D daily with periodontal surgery had a favorable influence on attachment levels and bone repair. In addition, it is worthy to mention that improvements were correlated with baseline levels of 1.25 dihydroxyvitamin D3 (better results with baseline levels >20ng/dL) [154]. The use of teriparatide might increase the reliability in cases of poor bone density. Kuchler et al. conducted a randomized controlled feasibility study to appraise the effect of 20 μ g of teriparatide daily during 28 days on mandibular dental implants, finding that after a

healing period of 9 weeks, new bone-volume-per-tissue-volume for the teriparatide group was 17.6% and 15.4% for the control group. In addition, it was shown that bone-to-implant contact was shown to be higher in the periosteal and medullary compartment for the test group, but not for the cortical compartment (5% vs. 4.4%, respectively) [155]. Based on these findings, the safety and efficacy of several growth factors have been documented, but more clinical trials are necessary to validate these preliminary studies.

4. Cell and Gene Delivery

To enhance the therapeutic potential of growth factor delivery, gene therapy has emerged as a method of establishing sustained growth factor expression through the transduction of cell populations via viral and non-viral delivery mechanisms that alter gene expression. Currently investigated methods of vector delivery for intraoral bone regeneration and existing pre-clinical studies of their success are addressed below:

4.1 Methods of Gene Delivery

4.1.1 Therapeutic viral gene delivery—Targeted gene delivery can be achieved using viral vectors engineered to infect cells with genetic material that will compensate for defective gene(s) or produce a beneficial protein product, without causing disease. This highly effective cellular uptake and favorable intracellular trafficking abilities make recombinant viral vectors suitable for both *in vivo* and *ex vivo* application. Gene expression following transfection is generally efficacious and long-lasting, ranging from several weeks to months depending on the vector type and target tissue [156]. Furthermore, the genetic material can either be integrated into the host chromosome or transported into the nuclei of infected cells without chromosomal integration.

Recombinant viral vectors include adenovirus, adeno-associated virus (AAV), and retrovirus, among others. Adenoviruses are large, double-stranded DNA viruses that infect non-dividing cells without integrating into the host chromosome, resulting in relatively short-term gene expression. AAVs are ssDNA viruses with a similar biological profile as adenoviruses, but with the primary added benefit of integration competence. Although AAVs have a broad tropism, they require adenovirus co-infection for replication cycle completion. In contrast, lentiviruses – part of the retrovirus family – will infect both dividing and non-dividing cells to provide stable gene expression by integrating into the host genome.

Each of the recombinant viral vectors has its advantages and disadvantages (see Table 4). Risks for potential immunogenicity and insertional mutagenesis continue to raise safety concerns with some recombinant viral vectors, which will be circumvented through an improved understanding of our immune response and the viruses themselves.

4.1.2 Therapeutic non-viral gene delivery—Non-viral methods rely on the combination of nucleic acids with synthetic or natural vectors, as well as physical forces to deliver genetic information to a target cell population [157]. In general, non-viral gene delivery can be utilized to introduce new genetic material or down-regulate the expression of abnormal genes at the mRNA level. Compounds such as cationic lipids and polymers form condensed complexes with negatively charged nucleic acids to protect and facilitate their

cellular uptake and intracellular transport. Additionally, physical forces, including but not limited to electroporation, form transient defects in the plasma and nuclear membranes to further facilitate the transport of genes into the nucleus. Major advantages of non-viral gene delivery include delivery of significantly larger fragments of genetic information and lack of a risk for immunogenicity and infection [158-160]. Further research is required to improve the variability of gene expression and specificity of the cell types being transfected using non-viral methods for gene delivery.

4.2 Safety and Regulatory Considerations of Cell and Gene Therapy

The translation of cell- and gene-based therapies to the clinic is intrinsically linked with reducing or eliminating significant risks associated with the treatment to the patient and improving the clinical outcome. Regulatory policies established by the FDA stipulate that the safety and efficacy of a given treatment must be verified prior to its approval for clinical use. In addition to the safety issues associated with processing cells *ex vivo*, other considerations involve the selected cell source, handling and expansion protocols, including best practices for maintaining cells during transportation, scaffold seeding, and transplantation [161]. While the current safety profile of iPSCs still presents significant barriers to clinical translation, cell-based therapy using MSCs has successfully been applied for treatment of degenerative diseases and investigated as a potential clinically-viable approach for dental-based tissue engineering strategies. For example, human dental pulp-derived mesenchymal stem cells (DPSC) can be processed using current good tissue practices (cGTP) within a 5 day period post-isolation from an extracted tooth, banked via cryopreservation, and recovered prior to treatment [162]. In the presence of a scaffold that serves as a carrier for cell delivery, additional considerations for safe application to the clinic include adherence to good manufacturing practices (cGMP) for scaffold fabrication and sterilization [163]. Additional steps involving scaffold cell-seeding, maintenance, and transfer to the clinic require stringent quality control measures and comprehensive protocols to ensure safety and efficacy. To minimize the associated treatment costs and improve practicality of cell-based therapy in the clinic with or without scaffold carriers, these processes must be streamlined and simplified to avoid unnecessary complications and reduce regulatory burdens.

Clinically-applicable procedures for the regeneration of oral and craniofacial bone using gene therapy are highly dependent on the selection of vectors with appropriate safety profiles. Primary concerns with viral vectors include their potential to illicit an immune response, exhibit insertional mutagenesis, and activate oncogenes, as has been observed with lentiviruses and retroviruses. Safer alternatives are adeno-associated vectors (AAVs) and adenoviruses: AAVs are non-pathogenic, while adenoviruses do not integrate into the cell genome and thereby are not replicated during cell division [164]. While these vectors are currently being tested in FDA-approved human clinical trials for rare disorders, the development of more efficient non-viral gene therapy methods involving DNA, mRNA, or siRNA delivery can be more promising from a regulatory approval perspective for regeneration of common tissue defects [165].

4.3 Pre-clinical Studies for Regeneration of Oral Tissues using Gene Therapy

Adenoviruses have been used experimentally for tissue engineering of tooth-supported bony defects. One example is the use of an adenovirus encoding the PDGF-B gene (Ad-PDGF-B) on a collagen matrix to treat periodontal lesions *in vivo*. Clinical, hematological and blood chemical tests were done without any significant histopathological changes when Ad-PDGF-B was used [166, 167]. Jin et al. demonstrated Ad-BMP7 induced rapid chondrogenesis, osteogenesis and cementogenesis in bridging periodontal alveolar bone defects without significant inflammatory responses [168]. This is an example of the potential that gene therapy-based vectors such as adenoviral BMP have for successfully engineering bone in a preclinical model (see Figure 3) when combined with a transduced cell carrier such as gelatin that provides three-dimensional support for tissue growth during the regenerative process.

AAVs have been used to prevent periodontal disease progression in rats. Cirelli et al., demonstrated the use of pseudotyped adeno-associated virus vector based on serotype 1 (AAV2/1) to deliver TNF receptor-immunoglobulin Fc (TNFR:Fc) to rats subjected to *Porphyromonas gingivalis* (Pg) lipopolysaccharide (LPS)[169]. Animals treated with AAV2/1-TNFR:Fc showed sustained levels of serum TNFR protein and sustained levels of Pg-LPS-mediated bone loss. AAVs have also been successfully used for the treatment of neurological, cardiovascular and autoimmune diseases [170].

Lentiviral transfection was used to investigate follicular dendritic cell secreted protein (FDC-SP) on the inhibition of osteogenic differentiation of human PDL cells. Xiang et al. used FDC-SP transfection on hPDL cell proliferation, osteogenic and fibrogenic phenotypes [171]. Cell proliferation and cell cycle tests indicated that transfection with FDC-SP did not affect hPDL proliferation. Moreover, expression levels of type 1 collagen were upregulated while osteocalcin, osteopontin and bone sialoprotein were downregulated in the transfected cells. Lentiviral vectors are considered excellent genetic vector systems by being both efficient and stable in gene delivery and therapy [172].

With regard to the application of non-viral vectors for growth factor delivery, plasmids have been used successfully to enhance cell survival and engraftment with IGF-1 in smooth muscle cells. Huang et al. used plasmid DNA encoded BMP-4 in critical-sized cranial defects in rats and demonstrated bone regeneration significantly increased both on the edges and center of the defects as compared to the control group of the scaffold only [173].

4.4 Clinical Applications of Cell Delivery

Cell and gene therapy have the potential to greatly improve current methods of bone regeneration through increased bioactivity of scaffolds and localized growth factor delivery. This concept is being comprehensively studied to provide a more predictable armamentarium of available treatment options for local alveolar bone loss. Reviewed here are documented clinical studies using cell-based therapy to the craniofacial complex. Cell-based therapy pre-clinical studies are in the process of being transitioned into clinically-applicable approaches that are safe and effective in regenerating periodontal tissues.

4.4.1 Alveolar bone augmentation/preservation—Existing studies have investigated the safety of cell-based therapy for alveolar bone augmentation: Filho-Cerruti et al. studied the association of platelet-rich plasma (PRP) and mononuclear cells from bone marrow aspirate and bone scaffold for bone augmentation in the maxillae [174]. An overall graft success rate of 94.7% was reported with enough ridge dimension obtained for proper dental implant placement. Documented graft failures were due to infection of the maxillary sinus and lack of integration into the host cortical bone. Bone formation with the presence of osteoblasts scattered throughout the trabeculae were noted histologically, with minimal marginal bone loss noticed during the 4-year study follow-up. Subsequently, Pelegrine et al. evaluated in a case series study the clinical and histomorphometric behavior of upper anterior extraction sockets treated with an autologous bone marrow graft versus no graft material in the control group [175]. Clinical results showed that the use of bone marrow stem cells (BMSCs) minimized alveolar bone loss after tooth extraction compared to control. Nevertheless, 6 months after grafting, similar outcomes were found by means of mineralized bone. More recently, Kaigler et al. conducted a randomized controlled feasibility trial to compare the use of tissue repair cells (BMSCs) with conventional GBR (membrane only with gelatin carrier) for alveolar bone preservation. At the time of implant placement, a second need of grafting was more frequent (6-fold greater implant exposure) for sockets treated with GBR. Additionally, it is also important to note that the regenerated bone in the test group exhibited greater density and higher vascularization with significant acceleration of osteogenesis at 6 weeks.

4.4.2 Sinus augmentation—Vertical bone augmentation in the sinus antrum is oftentimes an imperative to achieve implant stability due to progressive alveolar bone resorption. The use of stem cell therapy as a complement to conventional graft and scaffold materials could improve bone formation and accelerate regeneration. In a histomorphometric study, Gonshor et al. compared bone formation following sinus augmentation procedures using either an allograft cellular bone matrix containing native mesenchymal stem cells or a conventional allograft. Results of the test group revealed a mean of 32.5% and 4.9% for vital bone and remaining graft material, respectively, over a follow up healing period of 3.7 months. Contrastingly, for the control group, only 18.3% of vital bone content was found while an increase of up to 25.8% was noticed of remaining graft [176]. These results were consistent with the results obtained by Rickert et al., who aimed to compare bovine-derived mineral bone seeded with mononuclear stem cells with bovine-derived mineral bone mixed with autogenous bone. Significantly greater bone formation was observed in the test (17.7%) when compared with the control group (12.0%) at 14 weeks [177].

Overall, stem cells seeded in allograft or xenograft scaffold particles are capable of inducing sufficient new bone volume formation to achieve primary implant stability. Within these limitations, the high percentage of vital bone content after a relatively short healing period might encourage clinicians to consider implant therapy at an earlier stage post-grafting [178]. However, the clinical significance in small defects is very finite and therefore, the use of this approach should be further studied in more challenging scenarios with standardized randomized clinical trials.

5. Existing Limitations

Major advances have been made in the reconstruction of intraoral bone defects as a result of improvements in scaffolding matrices and application of bioactive factors that enhance the regenerative response. However, there are existing limitations in the development of optimized scaffolding matrices that meet all the necessary criteria for the regeneration of structurally and physiologically functional osseous tissues. As described in this review, current technologies such as 3-D printing are being adapted for use in the design and development of natural and synthetic matrices that are architecturally similar to bone that allow for controllable features such as chemical composition, porosity, and rate of degradation. Likewise, the selection of materials in scaffold development for intraoral bone regeneration is limited by the attempt to match the properties and rate of regrowth of the developing tissues to the degradation properties of the supporting biomaterial. It remains a challenge to balance the preference for FDA-approved materials that have been more thoroughly investigated for use in humans with materials that may be more mechanically-appropriate for bone regeneration but have been biologically/chemically modified or release degradation products which may hinder the regenerative process *in situ*. This further relates to the established regulatory requirements for translating novel treatments to the clinic, with more complex strategies involving combinations of materials with cell, growth factor, or gene delivery yielding a biologic-device combination product that is more difficult to evaluate as opposed to a stand-alone biologic or device product. While current clinical applications of growth factors in combination with commercialized material carriers are showing significant improvements in clinical treatment, further optimization of delivery systems is necessary to ensure that time-dependent biofactor dose and release kinetics are appropriate for the regenerative repair process. Gene therapy is promising in this area of research and is in need of further evaluation to determine the most appropriate vectors for growth factor gene delivery that is safe and clinically acceptable for further implementation in patient-based studies.

6. Future Directions

There are many exciting opportunities that lie ahead for the reconstruction of craniofacial deficiencies including periodontal, alveolar ridge and large mandibular/maxillary discontinuity defects. The innovations that are ongoing in materials science and in biology have offered many potential avenues in the laboratory and clinic to extend the field of tissue engineering of oral structures such as alveolar bone and soft tissues of the teeth and dental implants. In particular, recent advances in biomaterial design, drug delivery and biologic agents offer less invasive technologies to accelerate and more predictably promote tissue repair and regeneration. The ability of rapid prototyping, three-dimensional printing, electrospinning, and enhanced drug delivery strategies to personalize patient therapies is allowing a more customized approach for oral tissue engineering to benefit both clinicians and patients. Development of scaffolds that act as delivery vehicles for drugs that can be controllably released to counter bone degenerative processes is an example of a future focus for intraoral tissue engineering strategies, as recently evidenced in a study by Ji et al [179] that focused on the incorporation of hydrophilic naringin into electrospun amphiphilic copolymer nanofibers for bone resorption treatment. Further improvements to existing

materials with a history of use in dental and craniofacial applications, such as calcium phosphate-based cements, are a promising venue for addressing their existing limitations through the improvement of mechanical integrity and incorporation of growth factor delivery vehicles. Lee et al [180] present such an approach through the reinforcement of macroporous calcium phosphate cement (CPC) with absorbable fibers and simultaneous delivery of rhBMP2 in the CPC matrix with VEGF or TGF- β 1 in alginate hydrogel microbeads within the matrix. Such combinational approaches to address the need for controlled delivery of growth factors at critical stages of the regenerative process are important in advancing our ability to predictably regrow lost or damaged tissue. Further improvement and streamlining of existing processes and technologies for addressing these tissue engineering strategies will enable their continued entrance into the clinical arena to provide more predictable regenerative medicine therapeutics for enhanced patient care.

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References

1. Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ. Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J Dent Res*. 2012; 91:914–20. [PubMed: 22935673]
2. Hansson S, Halldin A. Alveolar ridge resorption after tooth extraction: A consequence of a fundamental principle of bone physiology. *J Dent Biomech*. 2012; 3:1758736012456543. [PubMed: 22924065]
3. Reynolds MA, Aichelmann-Reidy ME, Branch-Mays GL. Regeneration of periodontal tissue: bone replacement grafts. *Dent Clin North Am*. 2010; 54:55–71. [PubMed: 20103472]
4. Chen FM, Jin Y. Periodontal tissue engineering and regeneration: current approaches and expanding opportunities. *Tissue Eng Part B Rev*. 2010; 16:219–55. [PubMed: 19860551]
5. Silva FM, Cortez AL, Moreira RW, Mazzonetto R. Complications of intraoral donor site for bone grafting prior to implant placement. *Implant Dent*. 2006; 15:420–6. [PubMed: 17172961]
6. Holtzclaw D, Toscano N, Eisenlohr L, Callan D. The safety of bone allografts used in dentistry: a review. *J Am Dent Assoc*. 2008; 139:1192–9. [PubMed: 18762629]
7. Delloye C, Cornu O, Druez V, Barbier O. Bone allografts: What they can offer and what they cannot. *J Bone Joint Surg Br*. 2007; 89:574–9. [PubMed: 17540738]
8. Louis PJ. Bone grafting the mandible. *Oral Maxillofac Surg Clin North Am*. 2011; 23:209–27. v. [PubMed: 21492797]
9. Lutz R, Berger-Fink S, Stockmann P, Neukam FW, Schlegel KA. Sinus floor augmentation with autogenous bone vs. a bovine-derived xenograft – a 5-year retrospective study. *Clinical Oral Implants Research*. 2014:n/a–n/a.
10. Klijn RJ, Meijer GJ, Bronkhorst EM, Jansen JA. A meta-analysis of histomorphometric results and graft healing time of various biomaterials compared to autologous bone used as sinus floor augmentation material in humans. *Tissue Eng Part B Rev*. 2010; 16:493–507. [PubMed: 20334505]
11. Dutenhoefer F, Souren C, Menne D, Emmerich D, Schon R, Sauerbier S. Long-term survival of dental implants placed in the grafted maxillary sinus: systematic review and meta-analysis of treatment modalities. *PLoS One*. 2013; 8:e75357. [PubMed: 24058679]
12. McMahon RE, Wang L, Skoracki R, Mathur AB. Development of nanomaterials for bone repair and regeneration. *J Biomed Mater Res B Appl Biomater*. 2013; 101:387–97. [PubMed: 23281143]
13. Dorozhkin SV. Bioceramics of calcium orthophosphates. *Biomaterials*. 2010; 31:1465–85. [PubMed: 19969343]

14. Vlad MD, Gomez S, Barraco M, Lopez J, Fernandez E. Effect of the calcium to phosphorus ratio on the setting properties of calcium phosphate bone cements. *J Mater Sci Mater Med.* 2012; 23:2081–90. [PubMed: 22639154]
15. Alghamdi HS, Cuijpers VM, Wolke JG, van den Beucken JJ, Jansen JA. Calcium-phosphate-coated oral implants promote osseointegration in osteoporosis. *J Dent Res.* 2013; 92:982–8. [PubMed: 24056224]
16. Lin A, Wang CJ, Kelly J, Gubbi P, Nishimura I. The role of titanium implant surface modification with hydroxyapatite nanoparticles in progressive early bone-implant fixation in vivo. *Int J Oral Maxillofac Implants.* 2009; 24:808–16. [PubMed: 19865620]
17. Bashoor-Zadeh M, Baroud G, Böhner M. Simulation of the in vivo resorption rate of beta-tricalcium phosphate bone graft substitutes implanted in a sheep model. *Biomaterials.* 2011; 32:6362–73. [PubMed: 21658758]
18. Camazzola D, Hammond T, Gandhi R, Davey JR. A Randomized Trial of Hydroxyapatite-Coated Femoral Stems in Total Hip Arthroplasty. *The Journal of Arthroplasty.* 24:33–7. [PubMed: 18534441]
19. Hench LL. The story of Bioglass. *J Mater Sci Mater Med.* 2006; 17:967–78. [PubMed: 17122907]
20. Gerhardt LC, Boccaccini AR. Bioactive Glass and Glass-Ceramic Scaffolds for Bone Tissue Engineering. *Materials.* 2010; 3:3867–910.
21. Novitskaya E, C PY, Hamed E, Jun L, Lubarda V, Jasiuk I, McKittrick J. Recent advances on the measurement and calculation of the elastic moduli of cortical and trabecular bone: a review. *Theoret Appl Mech.* 2011; 38:209–97.
22. Teraoka K, Ito A, Maekawa K, Onuma K, Tateishi T, Tsutsumi S. Mechanical properties of hydroxyapatite and OH-carbonated hydroxyapatite single crystals. *J Dent Res.* 1998; 77:1560–8. [PubMed: 9663442]
23. Viswanath B, Raghavan R, Gurao NP, Ramamurty U, Ravishankar N. Mechanical properties of tricalcium phosphate single crystals grown by molten salt synthesis. *Acta Biomaterialia.* 2008; 4:1448–54. [PubMed: 18448402]
24. Yu L, Li Y, Zhao K, Tang Y, Cheng Z, Chen J, et al. A novel injectable calcium phosphate cement-bioactive glass composite for bone regeneration. *PLoS One.* 2013; 8:e62570. [PubMed: 23638115]
25. van Oirschot BA, Alghamdi HS, Narhi TO, Anil S, Al Farraj Aldosari A, van den Beucken JJ, et al. In vivo evaluation of bioactive glass-based coatings on dental implants in a dog implantation model. *Clin Oral Implants Res.* 2014; 25:21–8. [PubMed: 23078340]
26. Mehdikhani-Nahrkhalaji M, Fathi MH, Mortazavi V, Mousavi SB, Hashemi-Beni B, Razavi SM. Novel nanocomposite coating for dental implant applications in vitro and in vivo evaluation. *J Mater Sci Mater Med.* 2012; 23:485–95. [PubMed: 22127403]
27. Bongio M, van den Beucken JJJP, Leeuwenburgh SCG, Jansen JA. Development of bone substitute materials: from ‘biocompatible’ to ‘instructive’. *Journal of Materials Chemistry.* 2010; 20:8747–59.
28. Ramseier CA, Rasperini G, Batia S, Giannobile WV. Advanced reconstructive technologies for periodontal tissue repair. *Periodontol 2000.* 2012; 59:185–202. [PubMed: 22507066]
29. Bashutski JD, Wang HL. Periodontal and endodontic regeneration. *J Endod.* 2009; 35:321–8. [PubMed: 19249588]
30. Hua N, Ti VL, Xu Y. Biodegradable Effect of PLGA Membrane in Alveolar Bone Regeneration on Beagle Dog. *Cell Biochem Biophys.* 2014
31. Dreifke MB, Ebraheim NA, Jayasuriya AC. Investigation of potential injectable polymeric biomaterials for bone regeneration. *J Biomed Mater Res A.* 2013; 101:2436–47. [PubMed: 23401336]
32. Li X, Xie J, Yuan X, Xia Y. Coating electrospun poly(epsilon-caprolactone) fibers with gelatin and calcium phosphate and their use as biomimetic scaffolds for bone tissue engineering. *Langmuir.* 2008; 24:14145–50. [PubMed: 19053657]
33. Lee CH, Hajibandeh J, Suzuki T, Fan A, Shang P, Mao JJ. Three-dimensional printed multiphase scaffolds for regeneration of periodontium complex. *Tissue Eng Part A.* 2014; 20:1342–51. [PubMed: 24295512]

34. Costa PF, Vaquette C, Zhang Q, Reis RL, Ivanovski S, Hutmacher DW. Advanced tissue engineering scaffold design for regeneration of the complex hierarchical periodontal structure. *J Clin Periodontol*. 2014; 41:283–94. [PubMed: 24304192]
35. Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: recent advances and challenges. *Crit Rev Biomed Eng*. 2012; 40:363–408. [PubMed: 23339648]
36. Hollister SJ. Porous scaffold design for tissue engineering. *Nat Mater*. 2005; 4:518–24. [PubMed: 16003400]
37. Fisher S, Franz-Odenaal T. Evolution of the bone gene regulatory network. *Curr Opin Genet Dev*. 2012; 22:390–7. [PubMed: 22663778]
38. Hong SG, Winkler T, Wu C, Guo V, Pittaluga S, Nicolae A, et al. Path to the clinic: Assessment of iPSC-based cell therapies in vivo in a non-human primate model. *Cell reports*. 2014; 7:1298–309. [PubMed: 24835994]
39. Scaglione S, Giannoni P, Bianchini P, Sandri M, Marotta R, Firpo G, et al. Order versus Disorder: in vivo bone formation within osteoconductive scaffolds. *Sci Rep*. 2012; 2
40. Ho ST, Hutmacher DW, Ekaputra AK, Hitendra D, Hui JH. The evaluation of a biphasic osteochondral implant coupled with an electrospun membrane in a large animal model. *Tissue Eng Part A*. 2010; 16:1123–41. [PubMed: 19863255]
41. Mazzoli A. Selective laser sintering in biomedical engineering. *Med Biol Eng Comput*. 2013; 51:245–56. [PubMed: 23250790]
42. Xia Y, Zhou P, Cheng X, Xie Y, Liang C, Li C, et al. Selective laser sintering fabrication of nano-hydroxyapatite/poly-epsilon-caprolactone scaffolds for bone tissue engineering applications. *Int J Nanomedicine*. 2013; 8:4197–213. [PubMed: 24204147]
43. Williams JM, Adewunmi A, Schek RM, Flanagan CL, Krebsbach PH, Feinberg SE, et al. Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering. *Biomaterials*. 2005; 26:4817–27. [PubMed: 15763261]
44. Park CH, Rios HF, Taut AD, Padial-Molina M, Flanagan CL, Pilipchuk SP, et al. Image-based, fiber guiding scaffolds: a platform for regenerating tissue interfaces. *Tissue Eng Part C Methods*. 2014; 20:533–42. [PubMed: 24188695]
45. Cui Q, Dighe AS, Irvine JN Jr. Combined angiogenic and osteogenic factor delivery for bone regenerative engineering. *Curr Pharm Des*. 2013; 19:3374–83. [PubMed: 23432677]
46. Bai Y, Yin G, Huang Z, Liao X, Chen X, Yao Y, et al. Localized delivery of growth factors for angiogenesis and bone formation in tissue engineering. *Int Immunopharmacol*. 2013; 16:214–23. [PubMed: 23587487]
47. Romagnoli C, D'Asta F, Brandi ML. Drug delivery using composite scaffolds in the context of bone tissue engineering. *Clin Cases Miner Bone Metab*. 2013; 10:155–61. [PubMed: 24554923]
48. Lee SH, Shin H. Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering. *Adv Drug Deliv Rev*. 2007; 59:339–59. [PubMed: 17499384]
49. Wieman TJ, Smiell JM, Su Y. Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. A phase III randomized placebo-controlled double-blind study. *Diabetes Care*. 1998; 21:822–7. [PubMed: 9589248]
50. Nevins M, Giannobile WV, McGuire MK, Kao RT, Mellonig JT, Hinrichs JE, et al. Platelet-derived growth factor stimulates bone fill and rate of attachment level gain: results of a large multicenter randomized controlled trial. *J Periodontol*. 2005; 76:2205–15. [PubMed: 16332231]
51. Saygin NE, Tokiyasu Y, Giannobile WV, Somerman MJ. Growth factors regulate expression of mineral associated genes in cementoblasts. *J Periodontol*. 2000; 71:1591–600. [PubMed: 11063392]
52. Bartold PM, Raben A. Growth factor modulation of fibroblasts in simulated wound healing. *J Periodontal Res*. 1996; 31:205–16. [PubMed: 8926571]
53. Sant'Ana AC, Marques MM, Barroso TE, Passanezi E, de Rezende ML. Effects of TGF-beta1, PDGF-BB, and IGF-1 on the rate of proliferation and adhesion of a periodontal ligament cell lineage in vitro. *J Periodontol*. 2007; 78:2007–17. [PubMed: 18062122]

54. Franchimont N, Durant D, Rydzziel S, Canalis E. Platelet-derived growth factor induces interleukin-6 transcription in osteoblasts through the activator protein-1 complex and activating transcription factor-2. *J Biol Chem.* 1999; 274:6783–9. [PubMed: 10037779]
55. Strayhorn CL, Garrett JS, Dunn RL, Benedict JJ, Somerman MJ. Growth factors regulate expression of osteoblast-associated genes. *J Periodontol.* 1999; 70:1345–54. [PubMed: 10588498]
56. Yu X, Hsieh SC, Bao W, Graves DT. Temporal expression of PDGF receptors and PDGF regulatory effects on osteoblastic cells in mineralizing cultures. *Am J Physiol.* 1997; 272:C1709–16. [PubMed: 9176163]
57. Lynch SE, Buser D, Hernandez RA, Weber HP, Stich H, Fox CH, et al. Effects of the platelet-derived growth factor/insulin-like growth factor-I combination on bone regeneration around titanium dental implants. Results of a pilot study in beagle dogs. *J Periodontol.* 1991; 62:710–6. [PubMed: 1753324]
58. Lynch SE, Williams RC, Polson AM, Howell TH, Reddy MS, Zappa UE, et al. A combination of platelet-derived and insulin-like growth factors enhances periodontal regeneration. *J Clin Periodontol.* 1989; 16:545–8. [PubMed: 2778088]
59. Park JB, Matsuura M, Han KY, Norderyd O, Lin WL, Genco RJ, et al. Periodontal regeneration in class III furcation defects of beagle dogs using guided tissue regenerative therapy with platelet-derived growth factor. *J Periodontol.* 1995; 66:462–77. [PubMed: 7562336]
60. Sigurdsson TJ, Nygaard L, Tatakis DN, Fu E, Turek TJ, Jin L, et al. Periodontal repair in dogs: evaluation of rhBMP-2 carriers. *Int J Periodontics Restorative Dent.* 1996; 16:524–37. [PubMed: 9242091]
61. Giannobile WV, Hernandez RA, Finkelman RD, Ryan S, Kiritsy CP, D'Andrea M, et al. Comparative effects of platelet-derived growth factor-BB and insulin-like growth factor-I, individually and in combination, on periodontal regeneration in *Macaca fascicularis*. *J Periodontal Res.* 1996; 31:301–12. [PubMed: 8858534]
62. Rutherford RB, Niekrash CE, Kennedy JE, Charette MF. Platelet-derived and insulin-like growth factors stimulate regeneration of periodontal attachment in monkeys. *J Periodontal Res.* 1992; 27:285–90. [PubMed: 1640350]
63. Sigurdsson TJ, Tatakis DN, Lee MB, Wikesjo UM. Periodontal regenerative potential of space-providing expanded polytetrafluoroethylene membranes and recombinant human bone morphogenetic proteins. *J Periodontol.* 1995; 66:511–21. [PubMed: 7562341]
64. Cho MI, Lin WL, Genco RJ. Platelet-derived growth factor-modulated guided tissue regenerative therapy. *J Periodontol.* 1995; 66:522–30. [PubMed: 7562342]
65. Becker W, Lynch SE, Lekholm U, Becker BE, Caffesse R, Donath K, et al. A comparison of ePTFE membranes alone or in combination with platelet-derived growth factors and insulinlike growth factor-I or demineralized freeze-dried bone in promoting bone formation around immediate extraction socket implants. *J Periodontol.* 1992; 63:929–40. [PubMed: 1453308]
66. Stefani CM, Machado MA, Sallum EA, Sallum AW, Toledo S, Nociti H Jr. Platelet-derived growth factor/insulin-like growth factor-I combination and bone regeneration around implants placed into extraction sockets: a histometric study in dogs. *Implant Dent.* 2000; 9:126–31. [PubMed: 11314699]
67. Al-Hazmi BA, Al-Hamdan KS, Al-Rasheed A, Babay N, Wang HL, Al-Hezaimi K. Efficacy of using PDGF and xenograft with or without collagen membrane for bone regeneration around immediate implants with induced dehiscence-type defects: a microcomputed tomographic study in dogs. *J Periodontol.* 2013; 84:371–8. [PubMed: 22524331]
68. Schwarz F, Ferrari D, Podolsky L, Mihatovic I, Becker J. Initial pattern of angiogenesis and bone formation following lateral ridge augmentation using rhPDGF and guided bone regeneration: an immunohistochemical study in dogs. *Clin Oral Implants Res.* 2010; 21:90–9. [PubMed: 20070751]
69. Simion M, Rocchietta I, Kim D, Nevins M, Fiorellini J. Vertical ridge augmentation by means of deproteinized bovine bone block and recombinant human platelet-derived growth factor-BB: a histologic study in a dog model. *Int J Periodontics Restorative Dent.* 2006; 26:415–23. [PubMed: 17073351]
70. Urist MR. Bone: formation by autoinduction. *Science.* 1965; 150:893–9. [PubMed: 5319761]

71. Cheng H, Jiang W, Phillips FM, Haydon RC, Peng Y, Zhou L, et al. Osteogenic activity of the fourteen types of human bone morphogenetic proteins (BMPs). *J Bone Joint Surg Am.* 2003; 85-A:1544–52. [PubMed: 12925636]
72. Kobayashi M, Takiguchi T, Suzuki R, Yamaguchi A, Deguchi K, Shionome M, et al. Recombinant human bone morphogenetic protein-2 stimulates osteoblastic differentiation in cells isolated from human periodontal ligament. *J Dent Res.* 1999; 78:1624–33. [PubMed: 10520967]
73. Marcopoulou CE, Vavouraki HN, Dereka XE, Vrotsos IA. Proliferative effect of growth factors TGF-beta1, PDGF-BB and rhBMP-2 on human gingival fibroblasts and periodontal ligament cells. *J Int Acad Periodontol.* 2003; 5:63–70. [PubMed: 12887144]
74. Muthukuru M. Bone morphogenic protein-2 induces apoptosis and cytotoxicity in periodontal ligament cells. *J Periodontol.* 2013; 84:829–38. [PubMed: 22839695]
75. Cheifetz S, Li IW, McCulloch CA, Sampath K, Sodek J. Influence of osteogenic protein-1 (OP-1;BMP-7) and transforming growth factor-beta 1 on bone formation in vitro. *Connect Tissue Res.* 1996; 35:71–8. [PubMed: 9084644]
76. Sampath TK, Maliakal JC, Hauschka PV, Jones WK, Sasak H, Tucker RF, et al. Recombinant human osteogenic protein-1 (hOP-1) induces new bone formation in vivo with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation in vitro. *J Biol Chem.* 1992; 267:20352–62. [PubMed: 1328198]
77. Blumenthal NM, Koh-Kunst G, Alves ME, Miranda D, Sorensen RG, Wozney JM, et al. Effect of surgical implantation of recombinant human bone morphogenetic protein-2 in a bioabsorbable collagen sponge or calcium phosphate putty carrier in intrabony periodontal defects in the baboon. *J Periodontol.* 2002; 73:1494–506. [PubMed: 12546100]
78. Choi SH, Kim CK, Cho KS, Huh JS, Sorensen RG, Wozney JM, et al. Effect of recombinant human bone morphogenetic protein-2/absorbable collagen sponge (rhBMP-2/ACS) on healing in 3-wall intrabony defects in dogs. *J Periodontol.* 2002; 73:63–72. [PubMed: 11852905]
79. Saito A, Saito E, Handa R, Honma Y, Kawanami M. Influence of residual bone on recombinant human bone morphogenetic protein-2-induced periodontal regeneration in experimental periodontitis in dogs. *J Periodontol.* 2009; 80:961–8. [PubMed: 19485827]
80. Selvig KA, Sorensen RG, Wozney JM, Wikesjo UM. Bone repair following recombinant human bone morphogenetic protein-2 stimulated periodontal regeneration. *J Periodontol.* 2002; 73:1020–9. [PubMed: 12296587]
81. Barboza EP, Caula AL, Caula Fde O, de Souza RO, Geolas Neto L, Sorensen RG, et al. Effect of recombinant human bone morphogenetic protein-2 in an absorbable collagen sponge with space-providing biomaterials on the augmentation of chronic alveolar ridge defects. *J Periodontol.* 2004; 75:702–8. [PubMed: 15212353]
82. Wikesjo UM, Guglielmoni P, Promsudthi A, Cho KS, Trombelli L, Selvig KA, et al. Periodontal repair in dogs: effect of rhBMP-2 concentration on regeneration of alveolar bone and periodontal attachment. *J Clin Periodontol.* 1999; 26:392–400. [PubMed: 10382580]
83. Wikesjo UM, Lim WH, Thomson RC, Cook AD, Wozney JM, Hardwick WR. Periodontal repair in dogs: evaluation of a bioabsorbable space-providing macroporous membrane with recombinant human bone morphogenetic protein-2. *J Periodontol.* 2003; 74:635–47. [PubMed: 12816296]
84. Matin K, Nakamura H, Irie K, Ozawa H, Ejiri S. Impact of recombinant human bone morphogenetic protein-2 on residual ridge resorption after tooth extraction: an experimental study in the rat. *Int J Oral Maxillofac Implants.* 2001; 16:400–11. [PubMed: 11432660]
85. Cha JK, Lee JS, Kim MS, Choi SH, Cho KS, Jung UW. Sinus augmentation using BMP-2 in a bovine hydroxyapatite/collagen carrier in dogs. *J Clin Periodontol.* 2014; 41:86–93. [PubMed: 24117528]
86. Choi Y, Lee JS, Kim YJ, Kim MS, Choi SH, Cho KS, et al. Recombinant human bone morphogenetic protein-2 stimulates the osteogenic potential of the Schneiderian membrane: a histometric analysis in rabbits. *Tissue Eng Part A.* 2013; 19:1994–2004. [PubMed: 23544996]
87. Hanisch O, Tatakis DN, Rohrer MD, Wohrle PS, Wozney JM, Wikesjo UM. Bone formation and osseointegration stimulated by rhBMP-2 following subantral augmentation procedures in nonhuman primates. *Int J Oral Maxillofac Implants.* 1997; 12:785–92. [PubMed: 9425759]

88. Liu Y, Enggist L, Kuffer AF, Buser D, Hunziker EB. The influence of BMP-2 and its mode of delivery on the osteoconductivity of implant surfaces during the early phase of osseointegration. *Biomaterials*. 2007; 28:2677–86. [PubMed: 17321590]
89. Nevins M, Kirker-Head C, Nevins M, Wozney JA, Palmer R, Graham D. Bone formation in the goat maxillary sinus induced by absorbable collagen sponge implants impregnated with recombinant human bone morphogenetic protein-2. *Int J Periodontics Restorative Dent*. 1996; 16:8–19. [PubMed: 8631615]
90. Arosarena O, Collins W. Comparison of BMP-2 and -4 for rat mandibular bone regeneration at various doses. *Orthod Craniofac Res*. 2005; 8:267–76. [PubMed: 16238607]
91. Jovanovic SA, Hunt DR, Bernard GW, Spiekermann H, Wozney JM, Wikesjo UM. Bone reconstruction following implantation of rhBMP-2 and guided bone regeneration in canine alveolar ridge defects. *Clin Oral Implants Res*. 2007; 18:224–30. [PubMed: 17348887]
92. Kawakatsu N, Oda S, Kinoshita A, Kikuchi S, Tsuchioka H, Akizuki T, et al. Effect of rhBMP-2 with PLGA/gelatin sponge type (PGS) carrier on alveolar ridge augmentation in dogs. *J Oral Rehabil*. 2008; 35:647–55. [PubMed: 18482353]
93. Linde A, Hedner E. Recombinant bone morphogenetic protein-2 enhances bone healing, guided by osteopromotive e-PTFE membranes: an experimental study in rats. *Calcif Tissue Int*. 1995; 56:549–53. [PubMed: 7648485]
94. Shimazu C, Hara T, Kinuta Y, Moriya K, Maruo Y, Hanada S, et al. Enhanced vertical alveolar bone augmentation by recombinant human bone morphogenetic protein-2 with a carrier in rats. *J Oral Rehabil*. 2006; 33:609–18. [PubMed: 16856959]
95. Giannobile WV, Ryan S, Shih MS, Su DL, Kaplan PL, Chan TC. Recombinant human osteogenic protein-1 (OP-1) stimulates periodontal wound healing in class III furcation defects. *J Periodontol*. 1998; 69:129–37. [PubMed: 9526911]
96. Leknes KN, Yang J, Qahash M, Polimeni G, Susin C, Wikesjo UM. Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-2: radiographic observations. *Clin Oral Implants Res*. 2008; 19:1027–33. [PubMed: 18828819]
97. Liu Y, Han JX, Xiao SQ, Wang SL, Wang M. Study of recombinant human osteogenic protein-1 expressed in prokaryocyte on the repair of extracted socket in rabbits. *J Biomed Mater Res A*. 2006; 77:324–30. [PubMed: 16404713]
98. Margolin MD, Cogan AG, Taylor M, Buck D, McAllister TN, Toth C, et al. Maxillary sinus augmentation in the non-human primate: a comparative radiographic and histologic study between recombinant human osteogenic protein-1 and natural bone mineral. *J Periodontol*. 1998; 69:911–9. [PubMed: 9736374]
99. Ripamonti U, Heliotis M, Rueger DC, Sampath TK. Induction of cementogenesis by recombinant human osteogenic protein-1 (hop-1/bmp-7) in the baboon (*Papio ursinus*). *Arch Oral Biol*. 1996; 41:121–26. [PubMed: 8833601]
100. Terheyden H, Jepsen S, Moller B, Rueger D. Sinus floor augmentation with simultaneous implant insertion using recombinant human osteogenic protein-1. *Laryngorhinootologie*. 2001; 80:47–51. [PubMed: 11272248]
101. Terheyden H, Jepsen S, Moller B, Tucker MM, Rueger DC. Sinus floor augmentation with simultaneous placement of dental implants using a combination of deproteinized bone xenografts and recombinant human osteogenic protein-1. A histometric study in miniature pigs. *Clin Oral Implants Res*. 1999; 10:510–21. [PubMed: 10740460]
102. Hakki SS, Foster BL, Nagatomo KJ, Bozkurt SB, Hakki EE, Somerman MJ, et al. Bone morphogenetic protein-7 enhances cementoblast function in vitro. *J Periodontol*. 2010; 81:1663–74. [PubMed: 20681807]
103. Dereka XE, Markopoulou CE, Mamalis A, Pepelassi E, Vrotsos IA. Time- and dose-dependent mitogenic effect of basic fibroblast growth factor combined with different bone graft materials: an in vitro study. *Clin Oral Implants Res*. 2006; 17:554–9. [PubMed: 16958696]
104. Hidaka T, Nagasawa T, Shirai K, Kado T, Furuichi Y. FGF-2 induces proliferation of human periodontal ligament cells and maintains differentiation potentials of STRO-1(+)/CD146(+) periodontal ligament cells. *Arch Oral Biol*. 2012; 57:830–40. [PubMed: 22244620]

105. Hughes-Fulford M, Li CF. The role of FGF-2 and BMP-2 in regulation of gene induction, cell proliferation and mineralization. *J Orthop Surg Res.* 2011; 6:8. [PubMed: 21306641]
106. Murakami S, Takayama S, Ikezawa K, Shimabukuro Y, Kitamura M, Nozaki T, et al. Regeneration of periodontal tissues by basic fibroblast growth factor. *J Periodontal Res.* 1999; 34:425–30. [PubMed: 10685372]
107. Takayama S, Yoshida J, Hirano H, Okada H, Murakami S. Effects of basic fibroblast growth factor on human gingival epithelial cells. *J Periodontol.* 2002; 73:1467–73. [PubMed: 12546097]
108. Terashima Y, Shimabukuro Y, Terashima H, Ozasa M, Terakura M, Ikezawa K, et al. Fibroblast growth factor-2 regulates expression of osteopontin in periodontal ligament cells. *J Cell Physiol.* 2008; 216:640–50. [PubMed: 18412142]
109. Murakami S. Periodontal tissue regeneration by signaling molecule(s): what role does basic fibroblast growth factor (FGF-2) have in periodontal therapy? *Periodontol 2000.* 2011; 56:188–208. [PubMed: 21501244]
110. Murakami S, Takayama S, Kitamura M, Shimabukuro Y, Yanagi K, Ikezawa K, et al. Recombinant human basic fibroblast growth factor (bFGF) stimulates periodontal regeneration in class II furcation defects created in beagle dogs. *J Periodontal Res.* 2003; 38:97–103. [PubMed: 12558943]
111. Rossa C Jr, Marcantonio E Jr, Cirelli JA, Marcantonio RA, Spolidorio LC, Fogo JC. Regeneration of Class III furcation defects with basic fibroblast growth factor (b-FGF) associated with GTR. A descriptive and histometric study in dogs. *J Periodontol.* 2000; 71:775–84. [PubMed: 10872959]
112. Lee J, Wikesjo UM. Growth/differentiation factor-5: pre-clinical and clinical evaluations of periodontal regeneration and alveolar augmentation - review. *J Clin Periodontol.* 2014
113. Nakamura T, Yamamoto M, Tamura M, Izumi Y. Effects of growth/differentiation factor-5 on human periodontal ligament cells. *J Periodontal Res.* 2003; 38:597–605. [PubMed: 14632923]
114. Koch FP, Weinbach C, Hustert E, Al-Nawas B, Wagner W. GDF-5 and BMP-2 regulate bone cell differentiation by gene expression of *MSX1*, *MSX2*, *Dlx5*, and *Runx2* and influence *OCN* gene expression in vitro. *Int J Periodontics Restorative Dent.* 2012; 32:285–93. [PubMed: 22408773]
115. Emerton KB, Drapeau SJ, Prasad H, Rohrer M, Roffe P, Hopper K, et al. Regeneration of periodontal tissues in non-human primates with rhGDF-5 and beta-tricalcium phosphate. *J Dent Res.* 2011; 90:1416–21. [PubMed: 21940517]
116. Lee JS, Wikesjo UM, Jung UW, Choi SH, Pippig S, Siedler M, et al. Periodontal wound healing/regeneration following implantation of recombinant human growth/differentiation factor-5 in a beta-tricalcium phosphate carrier into one-wall intrabony defects in dogs. *J Clin Periodontol.* 2010; 37:382–9. [PubMed: 20447262]
117. Kim TG, Wikesjo UM, Cho KS, Chai JK, Pippig SD, Siedler M, et al. Periodontal wound healing/regeneration following implantation of recombinant human growth/differentiation factor-5 (rhGDF-5) in an absorbable collagen sponge carrier into one-wall intrabony defects in dogs: a dose-range study. *J Clin Periodontol.* 2009; 36:589–97. [PubMed: 19538332]
118. Kim YT, Wikesjo UM, Jung UW, Lee JS, Kim TG, Kim CK. Comparison between a beta-tricalcium phosphate and an absorbable collagen sponge carrier technology for rhGDF-5-stimulated periodontal wound healing/regeneration. *J Periodontol.* 2013; 84:812–20. [PubMed: 22897651]
119. Simank HG, Stuber M, Frahm R, Helbig L, van Lenthe H, Muller R. The influence of surface coatings of dicalcium phosphate (DCPD) and growth and differentiation factor-5 (GDF-5) on the stability of titanium implants in vivo. *Biomaterials.* 2006; 27:3988–94. [PubMed: 16595152]
120. Gruber RM, Ludwig A, Merten HA, Achilles M, Poehling S, Schliephake H. Sinus floor augmentation with recombinant human growth and differentiation factor-5 (rhGDF-5): a histological and histomorphometric study in the Goettingen miniature pig. *Clin Oral Implants Res.* 2008; 19:522–9. [PubMed: 18371105]
121. Gruber RM, Ludwig A, Merten HA, Pippig S, Kramer FJ, Schliephake H. Sinus floor augmentation with recombinant human growth and differentiation factor-5 (rhGDF-5): a pilot study in the Goettingen miniature pig comparing autogenous bone and rhGDF-5. *Clin Oral Implants Res.* 2009; 20:175–82. [PubMed: 19077151]

122. Schwarz F, Rothamel D, Herten M, Ferrari D, Sager M, Becker J. Lateral ridge augmentation using particulated or block bone substitutes biocoated with rhGDF-5 and rhBMP-2: an immunohistochemical study in dogs. *Clin Oral Implants Res.* 2008; 19:642–52. [PubMed: 18492078]
123. Kraus D, Jager A, Abuduwali N, Deschner J, Lossdorfer S. Intermittent PTH(1-34) signals through protein kinase A to regulate osteoprotegerin production in human periodontal ligament cells in vitro. *Clin Oral Investig.* 2012; 16:611–8.
124. Lossdorfer S, Gotz W, Jager A. Parathyroid hormone modifies human periodontal ligament cell proliferation and survival in vitro. *J Periodontal Res.* 2006; 41:519–26. [PubMed: 17076776]
125. Kawane T, Takahashi S, Saitoh H, Okamoto H, Kubodera N, Horiuchi N. Anabolic effects of recombinant human parathyroid hormone (1 - 84) and synthetic human parathyroid hormone (1 - 34) on the mandibles of osteopenic ovariectomized rats with maxillary molar extraction. *Horm Metab Res.* 2002; 34:293–302. [PubMed: 12173069]
126. Marques MR, da Silva MA, Manzi FR, Cesar-Neto JB, Nociti FH Jr, Barros SP. Effect of intermittent PTH administration in the periodontitis-associated bone loss in ovariectomized rats. *Arch Oral Biol.* 2005; 50:421–9. [PubMed: 15748695]
127. Jung RE, Cochran DL, Domken O, Seibl R, Jones AA, Buser D, et al. The effect of matrix bound parathyroid hormone on bone regeneration. *Clin Oral Implants Res.* 2007; 18:319–25. [PubMed: 17386063]
128. Valderrama P, Jung RE, Thoma DS, Jones AA, Cochran DL. Evaluation of parathyroid hormone bound to a synthetic matrix for guided bone regeneration around dental implants: a histomorphometric study in dogs. *J Periodontol.* 2010; 81:737–47. [PubMed: 20429653]
129. Hammerle CH, Jung RE. Bone augmentation by means of barrier membranes. *Periodontol* 2000. 2003; 33:36–53. [PubMed: 12950840]
130. Jung RE, Glauser R, Scharer P, Hammerle CH, Sailer HF, Weber FE. Effect of rhBMP-2 on guided bone regeneration in humans. *Clin Oral Implants Res.* 2003; 14:556–68. [PubMed: 12969359]
131. Urist MR, Maeda H, Shamie AN, Teplica D. Endogenous bone morphogenetic protein expression in transplants of urinary bladder. *Plast Reconstr Surg.* 1998; 101:408–15. discussion 16-7. [PubMed: 9462774]
132. Sawada Y, Hokugo A, Nishiura A, Hokugo R, Matsumoto N, Morita S, et al. A trial of alveolar cleft bone regeneration by controlled release of bone morphogenetic protein: an experimental study in rabbits. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2009; 108:812–20. [PubMed: 19782619]
133. Wikesjo UM, Qahash M, Thomson RC, Cook AD, Rohrer MD, Wozney JM, et al. rhBMP-2 significantly enhances guided bone regeneration. *Clin Oral Implants Res.* 2004; 15:194–204. [PubMed: 15008931]
134. Nevins M, Kao RT, McGuire MK, McClain PK, Hinrichs JE, McAllister BS, et al. Platelet-derived growth factor promotes periodontal regeneration in localized osseous defects: 36-month extension results from a randomized, controlled, double-masked clinical trial. *J Periodontol.* 2013; 84:456–64. [PubMed: 22612364]
135. Jayakumar A, Rajababu P, Rohini S, Butchibabu K, Naveen A, Reddy PK, et al. Multi-centre, randomized clinical trial on the efficacy and safety of recombinant human platelet-derived growth factor with beta-tricalcium phosphate in human intra-osseous periodontal defects. *J Clin Periodontol.* 2011; 38:163–72. [PubMed: 21133980]
136. Camelo M, Nevins ML, Schenk RK, Lynch SE, Nevins M. Periodontal regeneration in human Class II furcations using purified recombinant human platelet-derived growth factor-BB (rhPDGF-BB) with bone allograft. *Int J Periodontics Restorative Dent.* 2003; 23:213–25. [PubMed: 12854772]
137. Nevins ML, Camelo M, Schupbach P, Nevins M, Kim SW, Kim DM. Human buccal plate extraction socket regeneration with recombinant human platelet-derived growth factor BB or enamel matrix derivative. *Int J Periodontics Restorative Dent.* 2011; 31:481–92. [PubMed: 21845243]

138. Nevins ML, Reynolds MA, Camelo M, Schupbach P, Kim DM, Nevins M. Recombinant human platelet-derived growth factor BB for reconstruction of human large extraction site defects. *Int J Periodontics Restorative Dent*. 2014; 34:157–63. [PubMed: 24600651]
139. McKay WF, Peckham SM, Badura JM. A comprehensive clinical review of recombinant human bone morphogenetic protein-2 (INFUSE Bone Graft). *Int Orthop*. 2007; 31:729–34. [PubMed: 17639384]
140. Urist MR. Bone: formation by autoinduction. 1965. *Clin Orthop Relat Res*. 2002;4–10. [PubMed: 11937861]
141. Boyne PJ, Marx RE, Nevins M, Triplett G, Lazaro E, Lilly LC, et al. A feasibility study evaluating rhBMP-2/absorbable collagen sponge for maxillary sinus floor augmentation. *Int J Periodontics Restorative Dent*. 1997; 17:11–25. [PubMed: 10332250]
142. Triplett RG, Nevins M, Marx RE, Spagnoli DB, Oates TW, Moy PK, et al. Pivotal, randomized, parallel evaluation of recombinant human bone morphogenetic protein-2/absorbable collagen sponge and autogenous bone graft for maxillary sinus floor augmentation. *J Oral Maxillofac Surg*. 2009; 67:1947–60. [PubMed: 19686934]
143. Kao DW, Kubota A, Nevins M, Fiorellini JP. The negative effect of combining rhBMP-2 and Bio-Oss on bone formation for maxillary sinus augmentation. *Int J Periodontics Restorative Dent*. 2012; 32:61–7. [PubMed: 22254226]
144. Torrecillas-Martinez L, Monje A, Picos MA, Ortega-Oller I, Suarez F, Galindo-Moreno P, et al. Effect of rhBMP-2 upon maxillary sinus augmentation: a comprehensive review. *Implant Dent*. 2013; 22:232–7. [PubMed: 23648577]
145. de Freitas RM, Spin-Neto R, Junior EM, Pereira LA, Wikesjo UM, Susin C. Alveolar Ridge and Maxillary Sinus Augmentation Using rhBMP-2: A Systematic Review. *Clin Implant Dent Relat Res*. 2013
146. Howell TH, Fiorellini J, Jones A, Alder M, Nummikoski P, Lazaro M, et al. A feasibility study evaluating rhBMP-2/absorbable collagen sponge device for local alveolar ridge preservation or augmentation. *Int J Periodontics Restorative Dent*. 1997; 17:124–39. [PubMed: 9497707]
147. Cochran DL, Jones AA, Lilly LC, Fiorellini JP, Howell H. Evaluation of recombinant human bone morphogenetic protein-2 in oral applications including the use of endosseous implants: 3-year results of a pilot study in humans. *J Periodontol*. 2000; 71:1241–57. [PubMed: 10972640]
148. Fiorellini JP, Buser D, Riley E, Howell TH. Effect on bone healing of bone morphogenetic protein placed in combination with endosseous implants: a pilot study in beagle dogs. *Int J Periodontics Restorative Dent*. 2001; 21:41–7. [PubMed: 11829034]
149. Corinaldesi G, Piersanti L, Piattelli A, Iezzi G, Pieri F, Marchetti C. Augmentation of the floor of the maxillary sinus with recombinant human bone morphogenetic protein-7: a pilot radiological and histological study in humans. *Br J Oral Maxillofac Surg*. 2013; 51:247–52. [PubMed: 22766268]
150. Windisch P, Stavropoulos A, Molnar B, Szendroi-Kiss D, Szilagyi E, Rosta P, et al. A phase IIa randomized controlled pilot study evaluating the safety and clinical outcomes following the use of rhGDF-5/beta-TCP in regenerative periodontal therapy. *Clin Oral Investig*. 2012; 16:1181–9.
151. Koch FP, Becker J, Terheyden H, Capsius B, Wagner W. A prospective, randomized pilot study on the safety and efficacy of recombinant human growth and differentiation factor-5 coated onto beta-tricalcium phosphate for sinus lift augmentation. *Clin Oral Implants Res*. 2010; 21:1301–8. [PubMed: 20546250]
152. Kitamura M, Akamatsu M, Machigashira M, Hara Y, Sakagami R, Hirofuji T, et al. FGF-2 stimulates periodontal regeneration: results of a multi-center randomized clinical trial. *J Dent Res*. 2011; 90:35–40. [PubMed: 21059869]
153. Chan HL, McCauley LK. Parathyroid hormone applications in the craniofacial skeleton. *J Dent Res*. 2013; 92:18–25. [PubMed: 23071071]
154. Bashutski JD, Eber RM, Kinney JS, Benavides E, Maitra S, Braun TM, et al. Teriparatide and osseous regeneration in the oral cavity. *N Engl J Med*. 2010; 363:2396–405. [PubMed: 20950166]
155. Kuchler U, Luvizuto ER, Tangl S, Watzek G, Gruber R. Short-term teriparatide delivery and osseointegration: a clinical feasibility study. *J Dent Res*. 2011; 90:1001–6. [PubMed: 21555773]

156. Walther W, Stein U. Viral vectors for gene transfer: a review of their use in the treatment of human diseases. *Drugs*. 2000; 60:249–71. [PubMed: 10983732]
157. Al-Dosari MS, Gao X. Nonviral gene delivery: principle, limitations, and recent progress. *AAPS J*. 2009; 11:671–81. [PubMed: 19834816]
158. Nelson CE, Gupta MK, Adolph EJ, Guelcher SA, Duvall CL. siRNA Delivery from an Injectable Scaffold for Wound Therapy. *Adv Wound Care (New Rochelle)*. 2013; 2:93–9. [PubMed: 24527332]
159. Rios HF, Lin Z, Oh B, Park CH, Giannobile WV. Cell- and gene-based therapeutic strategies for periodontal regenerative medicine. *J Periodontol*. 2011; 82:1223–37. [PubMed: 21284553]
160. Wegman F, Oner FC, Dhert WJ, Alblas J. Non-viral gene therapy for bone tissue engineering. *Biotechnol Genet Eng Rev*. 2013; 29:206–20. [PubMed: 24568281]
161. Jung Y, Bauer G, Nolte JA. Concise Review: Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells: Progress Toward Safe Clinical Products. *Stem cells (Dayton, Ohio)*. 2012; 30:42–7.
162. Perry BC, Zhou D, Wu X, Yang FC, Byers MA, Chu TMG, et al. Collection, Cryopreservation, and Characterization of Human Dental Pulp-Derived Mesenchymal Stem Cells for Banking and Clinical Use. *Tissue Engineering Part C, Methods*. 2008; 14:149–56. [PubMed: 18489245]
163. Hollister SJ, Murphy WL. Scaffold Translation: Barriers Between Concept and Clinic. *Tissue Engineering Part B, Reviews*. 2011; 17:459–74. [PubMed: 21902613]
164. Bleich NK, Kallai I, Lieberman JR, Schwarz EM, Pelled G, Gazit D. Gene therapy approaches to regenerating bone. *Advanced drug delivery reviews*. 2012; 64:1320–30. [PubMed: 22429662]
165. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. *Nat Rev Genet*. 2014; 15:541–55. [PubMed: 25022906]
166. Chang PC, Cirelli JA, Jin Q, Seol YJ, Sugai JV, D'Silva NJ, et al. Adenovirus encoding human platelet-derived growth factor-B delivered to alveolar bone defects exhibits safety and biodistribution profiles favorable for clinical use. *Hum Gene Ther*. 2009; 20:486–96. [PubMed: 19199824]
167. Jin Q, Anusaksathien O, Webb SA, Printz MA, Giannobile WV. Engineering of tooth-supporting structures by delivery of PDGF gene therapy vectors. *Mol Ther*. 2004; 9:519–26. [PubMed: 15093182]
168. Jin QM, Anusaksathien O, Webb SA, Rutherford RB, Giannobile WV. Gene therapy of bone morphogenetic protein for periodontal tissue engineering. *J Periodontol*. 2003; 74:202–13. [PubMed: 12666709]
169. Cirelli JA, Park CH, MacKool K, Taba M Jr, Lustig KH, Burstein H, et al. AAV2/1-TNFR:Fc gene delivery prevents periodontal disease progression. *Gene Ther*. 2009; 16:426–36. [PubMed: 19078994]
170. Warrington KH Jr, Herzog RW. Treatment of human disease by adeno-associated viral gene transfer. *Hum Genet*. 2006; 119:571–603. [PubMed: 16612615]
171. Xiang L, Ma L, He Y, Wei N, Gong P. Transfection with follicular dendritic cell secreted protein to affect phenotype expression of human periodontal ligament cells. *J Cell Biochem*. 2014; 115:940–8. [PubMed: 24357406]
172. Logan AC, Lutzko C, Kohn DB. Advances in lentiviral vector design for gene-modification of hematopoietic stem cells. *Curr Opin Biotechnol*. 2002; 13:429–36. [PubMed: 12459333]
173. Huang YC, Simmons C, Kaigler D, Rice KG, Mooney DJ. Bone regeneration in a rat cranial defect with delivery of PEI-condensed plasmid DNA encoding for bone morphogenetic protein-4 (BMP-4). *Gene Ther*. 2005; 12:418–26. [PubMed: 15647766]
174. Filho Cerruti H, Kerkis I, Kerkis A, Tatsui NH, da Costa Neves A, Bueno DF, et al. Allogeneous bone grafts improved by bone marrow stem cells and platelet growth factors: clinical case reports. *Artif Organs*. 2007; 31:268–73. [PubMed: 17437494]
175. Pelegrine AA, da Costa CE, Correa ME, Marques JF Jr. Clinical and histomorphometric evaluation of extraction sockets treated with an autologous bone marrow graft. *Clin Oral Implants Res*. 2010; 21:535–42. [PubMed: 20337664]

176. Gonshor A, McAllister BS, Wallace SS, Prasad H. Histologic and histomorphometric evaluation of an allograft stem cell-based matrix sinus augmentation procedure. *Int J Oral Maxillofac Implants*. 2011; 26:123–31. [PubMed: 21365047]
177. Rickert D, Sauerbier S, Nagursky H, Menne D, Vissink A, Raghoobar GM. Maxillary sinus floor elevation with bovine bone mineral combined with either autogenous bone or autogenous stem cells: a prospective randomized clinical trial. *Clin Oral Implants Res*. 2011; 22:251–8. [PubMed: 20831758]
178. Schmelzeisen R, Gutwald R, Oshima T, Nagursky H, Vogeler M, Sauerbier S. Making bone II: maxillary sinus augmentation with mononuclear cells--case report with a new clinical method. *Br J Oral Maxillofac Surg*. 2011; 49:480–2. [PubMed: 20678831]
179. Ji Y, Wang L, Watts DC, Qiu H, You T, Deng F, et al. Controlled-release naringin nanoscaffold for osteoporotic bone healing. *Dent Mater*. 2014; 30:1263–73. [PubMed: 25238705]
180. Lee K, Weir MD, Lippens E, Mehta M, Wang P, Duda GN, et al. Bone regeneration via novel macroporous CPC scaffolds in critical-sized cranial defects in rats. *Dent Mater*. 2014; 30:e199–207. [PubMed: 24768062]
181. Bsoul S, Terezhalmay G, Abboud H, Woodruff K, Abboud SL. PDGF BB and bFGF stimulate DNA synthesis and upregulate CSF-1 and MCP-1 gene expression in dental follicle cells. *Arch Oral Biol*. 2003; 48:459–65. [PubMed: 12749918]
182. Mumford JH, Carnes DL, Cochran DL, Oates TW. The effects of platelet-derived growth factor-BB on periodontal cells in an in vitro wound model. *J Periodontol*. 2001; 72:331–40. [PubMed: 11327060]
183. Zhao M, Berry JE, Somerman MJ. Bone morphogenetic protein-2 inhibits differentiation and mineralization of cementoblasts in vitro. *J Dent Res*. 2003; 82:23–7. [PubMed: 12508040]
184. Kemoun P, Laurencin-Dalicieux S, Rue J, Farges JC, Gennero I, Conte-Auriol F, et al. Human dental follicle cells acquire cementoblast features under stimulation by BMP-2/-7 and enamel matrix derivatives (EMD) in vitro. *Cell Tissue Res*. 2007; 329:283–94. [PubMed: 17443352]
185. Zhao M, Xiao G, Berry JE, Franceschi RT, Reddi A, Somerman MJ. Bone morphogenetic protein 2 induces dental follicle cells to differentiate toward a cementoblast/osteoblast phenotype. *J Bone Miner Res*. 2002; 17:1441–51. [PubMed: 12162498]
186. Murphy MG, Mailhot J, Borke J, Wataha J, Sharawy M, Smith A. The effects of rhBMP-2 on human osteosarcoma cells and human gingival fibroblasts in vitro. *J Oral Implantol*. 2001; 27:16–24. [PubMed: 11326537]
187. Laflamme C, Curt S, Rouabhia M. Epidermal growth factor and bone morphogenetic proteins upregulate osteoblast proliferation and osteoblastic markers and inhibit bone nodule formation. *Arch Oral Biol*. 2010; 55:689–701. [PubMed: 20627196]
188. Lallier TE, Spencer A. Use of microarrays to find novel regulators of periodontal ligament fibroblast differentiation. *Cell Tissue Res*. 2007; 327:93–109. [PubMed: 17024420]
189. Hakki SS, Nohutcu RM, Hakki EE, Berry JE, Akkaya MS, Somerman MJ. Dexamethasone and basic-fibroblast growth factor regulate markers of mineralization in cementoblasts in vitro. *J Periodontol*. 2005; 76:1550–8. [PubMed: 16171446]
190. Fujisawa K, Miyamoto Y, Nagayama M. Basic fibroblast growth factor and epidermal growth factor reverse impaired ulcer healing of the rabbit oral mucosa. *J Oral Pathol Med*. 2003; 32:358–66. [PubMed: 12787043]
191. Reyes-Botella C, Vallecillo-Capilla MF, Ruiz C. Effect of different growth factors on human cultured osteoblast-like cells. *Cell Physiol Biochem*. 2002; 12:353–8. [PubMed: 12438771]
192. Yoshimoto T, Yamamoto M, Kadomatsu H, Sakoda K, Yonamine Y, Izumi Y. Recombinant human growth/differentiation factor-5 (rhGDF-5) induced bone formation in murine calvariae. *J Periodontol Res*. 2006; 41:140–7. [PubMed: 16499717]
193. Sumita Y, Honda MJ, Ueda M, Asahina I, Kagami H. Differential effects of growth differentiation factor-5 on porcine dental papilla- and follicle-derived cells. *Growth Factors*. 2010; 28:56–65. [PubMed: 19909214]
194. Takahashi D, Odajima T, Morita M, Kawanami M, Kato H. Formation and resolution of ankylosis under application of recombinant human bone morphogenetic protein-2 (rhBMP-2) to class III furcation defects in cats. *J Periodontol Res*. 2005; 40:299–305. [PubMed: 15966907]

195. Sigurdsson TJ, Fu E, Tatakis DN, Rohrer MD, Wikesjo UM. Bone morphogenetic protein-2 for peri-implant bone regeneration and osseointegration. *Clin Oral Implants Res.* 1997; 8:367–74. [PubMed: 9612141]
196. Miranda DA, Blumenthal NM, Sorensen RG, Wozney JM, Wikesjo UM. Evaluation of recombinant human bone morphogenetic protein-2 on the repair of alveolar ridge defects in baboons. *J Periodontol.* 2005; 76:210–20. [PubMed: 15974844]
197. Terheyden H, Jepsen S, Vogeler S, Tucker M, Rueger DC. Recombinant human osteogenic protein 1 in the rat mandibular augmentation model: differences in morphology of the newly formed bone are dependent on the type of carrier. *Mund Kiefer Gesichtschir.* 1997; 1:272–5. [PubMed: 9384794]
198. McAllister BS, Margolin MD, Cogan AG, Taylor M, Wollins J. Residual lateral wall defects following sinus grafting with recombinant human osteogenic protein-1 or Bio-Oss in the chimpanzee. *Int J Periodontics Restorative Dent.* 1998; 18:227–39. [PubMed: 9728105]
199. Weng D, Poehling S, Pippig S, Bell M, Richter EJ, Zuh O, et al. The effects of recombinant human growth/differentiation factor-5 (rhGDF-5) on bone regeneration around titanium dental implants in barrier membrane-protected defects: a pilot study in the mandible of beagle dogs. *Int J Oral Maxillofac Implants.* 2009; 24:31–7. [PubMed: 19344022]
200. Leknes KN, Yang J, Qahash M, Polimeni G, Susin C, Wikesjo UM. Alveolar ridge augmentation using implants coated with recombinant human growth/differentiation factor -5 (rhGDF-5). Radiographic observations. *Clin Oral Implants Res.* 2012
201. Kwon DH, Bennett W, Herberg S, Bastone P, Pippig S, Rodriguez NA, et al. Evaluation of an injectable rhGDF-5/PLGA construct for minimally invasive periodontal regenerative procedures: a histological study in the dog. *J Clin Periodontol.* 2010; 37:390–7. [PubMed: 20447263]
202. Fischer J, Kolk A, Wolfart S, Pautke C, Warnke PH, Plank C, et al. Future of local bone regeneration - Protein versus gene therapy. *J Craniomaxillofac Surg.* 2011; 39:54–64. [PubMed: 20434921]
203. Gower RM, Shea LD. Biomaterial Scaffolds for Controlled, Localized Gene Delivery of Regenerative Factors. *Adv Wound Care (New Rochelle).* 2013; 2:100–6. [PubMed: 24527333]
204. Liu TB, Fedak PW, Weisel RD, Yasuda T, Kiani G, Mickle DA, et al. Enhanced IGF-1 expression improves smooth muscle cell engraftment after cell transplantation. *Am J Physiol Heart Circ Physiol.* 2004; 287:H2840–9. [PubMed: 15331370]
205. Cam C, Segura T. Matrix-based gene delivery for tissue repair. *Curr Opin Biotechnol.* 2013; 24:855–63. [PubMed: 23680305]
206. Iwanaga K, Tominaga K, Yamamoto K, Habu M, Maeda H, Akifusa S, et al. Local delivery system of cytotoxic agents to tumors by focused sonoporation. *Cancer Gene Ther.* 2007; 14:354–63. [PubMed: 17273182]
207. Kotopoulos S, Dimceviski G, Gilja OH, Hoem D, Postema M. Treatment of human pancreatic cancer using combined ultrasound, microbubbles, and gemcitabine: a clinical case study. *Med Phys.* 2013; 40:072902. [PubMed: 23822453]

Highlights

- Bone regeneration using scaffolds with cell/growth factor delivery is reviewed.
- Pre-clinical/clinical results using bone regenerative therapies are discussed.
- Novel methods of scaffold fabrication and growth factor delivery highlighted.
- Intraoral bone regeneration requires strategic scaffold design parameters.

Figure 1.

A combination of various approaches in scaffold material selection, cell therapy, and growth factor delivery is required to achieve optimal tissue regeneration that mimics the mechanical, chemical, structural, and biological properties of natural bone that make it a physiologically functional entity. Abbreviations: [*Scaffolds*] Polyglycolic acid (PGA), polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(ethylene glycol) (PEG), hydroxyapatite (HA), β -tricalcium phosphate (β -TCP); [*Cell Therapy*] Osterix (Osx), alkaline phosphatase (ALP), osteoprotegerin (OPG), bone sialoprotein (BSP); [*Growth Factors*] bone morphogenetic protein (BMP), transforming growth factor-beta (TGF- β), insulin-like growth factor (IGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF).

Figure 2.

Solid free-form fabrication (SFF), or rapid prototyping, is emerging as a clinically-viable approach for tissue engineering of anatomically precise scaffolds for periodontal, including intraoral bone, regeneration. This method is based on obtaining a CT scan of the patient-specific defect, generating a CAD-based file of a scaffold with an appropriate fit to the defect, and 3-D printing the final version to obtain a polymer-based scaffold with structural integrity that can be used as a cell therapy and growth factor delivery platform to enhance the regenerative process. Reproduced with permission from Park CH et al [43].

Figure 3.

Successful regeneration of alveolar bone and surrounding periodontal tissues using gene therapy vectors such as adenoviral BMP-7 (Ad-BMP-7) has been achieved in animal models. Bone regeneration and bridging was observed with the use of ex vivo BMP-7 gene transfer using a gelatin-based cell carrier in a rat wound model consisting of a large mandibular alveolar bone defect. Comparatively, transduction of syngeneic dermal fibroblasts using green fluorescent protein (Ad-GFP) or noggin (Ad-noggin) did not result in ectopic bone formation (*left panel*). Mature cartilage and newly-formed bone was observed at Day 21 using Ad-BMP-7 gene transfer (*right panel*). Reproduced with permission from Jin Q-M et al [168].

Table 1Effects of growth factors on periodontal cells *in vitro*.

Growth factor	Cell type	Effect	Studies
PDGF	Cementoblasts	<ul style="list-style-type: none"> Increased DNA synthesis and osteopontin mRNA expression 	[50]
	Dental follicle cells	<ul style="list-style-type: none"> Stimulated DNA synthesis and expression of CSF-1 and MCP-1 	[173]
	Gingival fibroblasts	<ul style="list-style-type: none"> Increased mitosis No increase in proliferation or chemotaxis 	[72, 174]
	Osteoblasts	<ul style="list-style-type: none"> Increased proliferation and interleukin-6 transcription Inhibited differentiation Blocked osteopontin, osteonectin 	[53-55]
	PDL cells	<ul style="list-style-type: none"> Stimulated proliferation (with or without allograft) Induced matrix synthesis and increased cell migration and mitosis 	[51, 52, 64, 72]
BMP2	Cementoblasts	<ul style="list-style-type: none"> Inhibited differentiation and mineralization 	[175]
	Dental follicle cells	<ul style="list-style-type: none"> Stimulated osteoblast/cementoblast differentiation Increased mineralization and ALP 	[176, 177]
	Gingival fibroblasts	<ul style="list-style-type: none"> Decreased mitosis At high doses, inhibited mineralization and OCN 	[72, 178]
	Osteoblasts	<ul style="list-style-type: none"> Increased proliferation, mineralization, and expression of ALP and OCN 	[179]
	PDL cells	<ul style="list-style-type: none"> Increased mineralization and expression of mineralization markers At doses > 10ng/mL, induced apoptosis/cytotoxicity Stimulated osteoblast differentiation Decreased mitosis 	[71-73]
BMP-7	Cementoblasts	<ul style="list-style-type: none"> Increased mineralization and mineralized tissue markers 	[101]
	Dental follicle cells	<ul style="list-style-type: none"> Increased mineralization and ALP expression 	[176]

Growth factor	Cell type	Effect	Studies
FGF-2	Osteoblasts	<ul style="list-style-type: none"> Increased proliferation, mineralization, and expression of ALP and OCN 	[74, 75, 179]
	PDL cells	<ul style="list-style-type: none"> Reduced proliferation Induced ALP 	[180]
	Cementoblasts	<ul style="list-style-type: none"> Stimulated DNA synthesis Decreased mineralization and expression of OCN Modulated expression of OPN 	[181]
	Dental follicle cells	<ul style="list-style-type: none"> Stimulated DNA synthesis and expression of CSF-1 and MCP-1 	[173]
	Gingival epithelial cells	<ul style="list-style-type: none"> Increased proliferation 	[106]
	Gingival fibroblasts	<ul style="list-style-type: none"> Increased proliferation 	[182]
	Osteoblasts	<ul style="list-style-type: none"> Promoted differentiation Induced proliferation Decreased mineralization gene expression 	[104, 183]
	PDL cells	<ul style="list-style-type: none"> Increased proliferation (alone and combined with DFDBA or FDBA), migration, and extracellular matrix production Maintained differentiation potential Stimulated OPN Inhibited ALP expression, mineralization, and OCN 	[102, 103]
	CT fibroblasts	<ul style="list-style-type: none"> Increased proliferation 	[184]
	Dental follicle cells	<ul style="list-style-type: none"> Reduced ALP activity 	[185]
GDF-5	Osteoblasts	<ul style="list-style-type: none"> Increased early differentiation and matrix production Modulated proliferation 	[113, 184]
	PDL cells	<ul style="list-style-type: none"> Increased proliferation and matrix synthesis Decreased ALP activity 	[112]
	PDL cells	<ul style="list-style-type: none"> Modified proliferation & survival and expression of mineralized markers (dependent on maturation state) 	[122, 123]
	PDL cells		
	PDL cells		
Teriparatide	PDL cells	<ul style="list-style-type: none"> Modified proliferation & survival and expression of mineralized markers (dependent on maturation state) 	[122, 123]

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ALP: alkaline phosphatase, BMP: bone morphogenetic protein, CSF-1: colony stimulating factor-1, FGF-2: fibroblast growth factor-2, GDF-5: growth/differentiation factor-5, MCP-1: macrophage chemotactic protein-1, OCN: osteocalcin, OPN: osteopontin, PDGF: platelet-derived growth factor, PDL: periodontal ligament

Table 2
Preclinical animal models of growth factor delivery for periodontal and implant applications.

Growth factor	Model	Animal	Results	Studies
PDGF-BB	Furcation defect	Canine	<ul style="list-style-type: none"> Stimulated PDL formation (early stage) Promoted periodontal regeneration (late stage) 	[63]
	GBR at implants	Canine	<ul style="list-style-type: none"> With IGF-1, significantly increased histologic bone-implant contact and peri-implant bone fill With IGF-1, increased early (3 week) bone formation at immediate implants With ePTFE membrane and IGF-1, increased bone gain and histologic parameters versus membrane alone or membrane + DFDBA 	[56, 64, 65]
	Periodontal defect	Canine	<ul style="list-style-type: none"> After flap surgery, increased new bone, cementum and PDL With IGF-1, promotes periodontal regeneration 	[57-59, 62]
		Non-human primate	<ul style="list-style-type: none"> Significantly increased new attachment 	[60-62, 76]
	Ridge augmentation	Canine	<ul style="list-style-type: none"> With block graft, increased histologic bone formation With bone mineral and collagen membrane, supports lateral bone formation With biphasic calcium phosphate and collagen membrane, supports GBR With xenograft scaffold, promoted bone regeneration similar in quality to native bone With xenograft, improved radiographic results when used without collagen membrane 	[67, 68] [66]
rhBMP-2	Extraction socket	Rat	<ul style="list-style-type: none"> Increased speed and quantity of bone formation Induced proliferation and differentiation of mesenchymal cells 	[83]
	Furcation defect	Feline	<ul style="list-style-type: none"> Early ankylosis may resolve with polymer carrier 	[186]
	GBR at implants	Canine	<ul style="list-style-type: none"> Resorbable and ePTFE membranes delay early (1 month) bone formation but may result in increased or similar 3-month bone formation vs. no membrane Increased bone augmentation for implants placed in extraction sockets and for implant-site circumferential and fenestration defects BMP2-coated implants provide increased bone formation, histologic bone apposition, and osseointegration Improved late (3-month) bone formation for ACS versus PLGA carrier 	[82, 95, 132, 147, 187]

Growth factor	Model	Animal	Results	Studies
	Periodontal defect	Non-human primate	<ul style="list-style-type: none"> Increased bone and cementum regeneration 	[76]
		Canine	<ul style="list-style-type: none"> Increased quantity and speed of bone formation Bone quantity formed correlated with residual bone height Limited cementum regeneration Induced ankylosis and root resorption No benefit for calcium phosphate cement carrier 	[77-79, 81, 82]
		Rat	<ul style="list-style-type: none"> Significant horizontal and vertical bone augmentation ePTFE membranes improve bone contour Increased bone formation with ACS+ bone graft material Potential carriers: absorbable collagen sponge, hyaluronic acid polymer, collagen-calcium hydroxyapatite-TCP complex, PLGA/gelatin sponge 	[89, 91-93]
		Canine	<ul style="list-style-type: none"> Increased histologic and radiographic bone formation, +/- bone graft Increased incidence of seromas and wound failure Possible decreased bone quality when combined with bone graft With xenograft block, supported bone formation Late-stage implant stability comparable to native bone 	[80, 90, 91, 121]
		Non-human primate	<ul style="list-style-type: none"> Increased ridge width and bone quality in TCP/HA/ACS and CaP cement carriers 	[188]
	Sinus augmentation	Canine	<ul style="list-style-type: none"> Enhanced histologic bone formation 	[84]
		Rabbit	<ul style="list-style-type: none"> Increased bone volume for collagenated BCP/BCP carriers 	[85]
		Goat	<ul style="list-style-type: none"> Increased radiographic bone formation 	[88]
		Non-human primate	<ul style="list-style-type: none"> Increased vertical bone gain 	[86]
		Mini-pig	<ul style="list-style-type: none"> BMP-2 coatings did not improve peri-implant bone gain 	[87]
BMP-4	Ridge augmentation	Rat	<ul style="list-style-type: none"> Improved bone quality and comparable quantity versus BMP-2 	[89]
BMP-7	Furcation defect	Canine	<ul style="list-style-type: none"> Significantly increased histologic bone, cementum, and new attachment in class III furcations 	[94]

Growth factor	Model	Animal	Results	Studies
	GBR at implants	Canine	<ul style="list-style-type: none"> • Implant-coating applications 	[95]
		Non-human primate	<ul style="list-style-type: none"> • Stimulated cementum formation 	[98]
	Ridge augmentation	Rat	<ul style="list-style-type: none"> • Increased bone formation in xenograft block versus control 	[189]
	Sinus augmentation	Non-human primate	<ul style="list-style-type: none"> • Comparable radiographic and histologic bone formation and residual lateral wall defect reduction versus bone graft 	[97, 190]
		Mini-pig	<ul style="list-style-type: none"> • With xenograft, increases speed and quality of osseointegration at simultaneous implants 	[99, 100]
	Socket augmentation	Rabbit	<ul style="list-style-type: none"> • Histologic increased speed of healing by 4-6 weeks • Significantly increased ALP activity and calcium 	[96]
	Furcation defect	Canine	<ul style="list-style-type: none"> • Significantly increased regeneration of cementum, PDL, and bone vs. controls 	[109, 110]
	GBR at implants	Canine	<ul style="list-style-type: none"> • Improves peri-implant GBR (β-TCP) • GDF-5 coating may increase bone formation (dose-dependent) 	[191, 192]
	Implant coating	Rabbit	<ul style="list-style-type: none"> • Improved implant stability as determined by pull-out test 	[118]
	Periodontal defect	Canine	<ul style="list-style-type: none"> • Significantly increased perio. regeneration in PLGA (dose-dependent), β-TCP, ACS carriers, with bone formation for β-TCP stable up to 24 wks • Beta-TCP/PLGA carrier may cause ankylosis 	[115-117, 193]
		Non-human primate	<ul style="list-style-type: none"> • Supported periodontal regeneration with β-TCP carrier 	[114]
	Ridge augmentation	Canine	<ul style="list-style-type: none"> • With xenograft block, supported bone regeneration 	[121]
	Sinus augmentation	Mini-pigs	<ul style="list-style-type: none"> • Enhanced bone formation with β-TCP 	[119, 120]
Teriparatide	Extraction socket	Osteopenic rats	<ul style="list-style-type: none"> • Increased bone mineral density and anabolic effects 	[124]
	GBR at implants	Canine	<ul style="list-style-type: none"> • Significantly improved bone formation 	[126, 127]
	Periodontal defect	Ovariectomized rats	<ul style="list-style-type: none"> • Preventative effects on periodontal bone loss 	[125]

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ACS: absorbable collagen sponge, ALP: alkaline phosphatase, BCP: biphasic calcium phosphate, BMP: bone morphogenetic protein, bTCP: beta tricalcium phosphate, ePTFE: expanded polytetrafluoroethylene, FGF: fibroblast growth factor, GBR: guided bone regeneration, GDF: growth/differentiation factor, HA: hydroxyapatite, IGF: insulin-like growth factor, PDGF: platelet-derived growth factor, PDL: periodontal ligament, PLA: polylactic acid, PGA: polyglycolic acid

Table 3

Clinical application of growth factor and protein delivery in periodontics, osseointegration, and pre-prosthetic surgical procedures.

Growth factor/protein	Indication	Evidence level	Efficacy and safety	References
PDGF-BB	Periodontal defects	★★★	<ul style="list-style-type: none"> • Gain in PD, CAL and BOP • Increasing of radiographic bone level 	[49, 134-137]
	Furcation involvements	★	<ul style="list-style-type: none"> • Gain in PD, CAL and BOP • Increasing of radiographic bone level 	
	Alveolar bone augmentation/preservation	★	<ul style="list-style-type: none"> • Accelerate the healing process 	
BMP-2	Alveolar bone augmentation/preservation	★★	<ul style="list-style-type: none"> • Accelerate the healing process • Histological findings high proportion of newly formed bone 	[140-143, 145-148]
	Sinus augmentation	★★	<ul style="list-style-type: none"> • In combination with allogeneic/autologous bone graft: histologic findings similar to autologous bone and adequate vertical bone gain (BMP-2) • In combination with bovine-derived xenogeneic graft: histologic findings show less newly-bone formation compared to xenograft alone but adequate vertical bone gain (BMP-2, BMP-7) 	
GDF-5	Periodontal defect	★	<ul style="list-style-type: none"> • Gain in CAL 	[149, 150]
	Sinus augmentation	★	<ul style="list-style-type: none"> • Histologic results similar to autologous bone • Adequate vertical bone gain 	
FGF-2	Periodontal defects	★	<ul style="list-style-type: none"> • Early radiographic bone fill 	[151]
Teriparatide	Peri-implant bone	★	<ul style="list-style-type: none"> • Gain in PD and CAL • Increasing radiographic bone level 	[153, 154]
			<ul style="list-style-type: none"> • Greater bone-to-implant contact in the periosteal and medullary compartment • Minimal effect on cortical compartment 	

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Growth factor/protein	Indication	Evidence level	Efficacy and safety	References
			• Slight higher bone-volume-per-tissue-volume	

Table 4

Viral and non-viral vectors utilized in tissue engineering [159].

Vector	Type	Advantages	Disadvantages	Pre-Clinical Studies
Adenovirus	viral	High transduction rate; transfection of wide range of cell types	High immunogenic potential; transient gene expression [159, 194, 195]	Chang et al., 2009 [160]; Jin et al., 2003 [162]
Lentivirus	viral	Non-immunogenic; sustained gene expression	Risk for insertional mutagenesis; transduction limited to dividing cells [159, 194, 195]	Xiang et al., 2014; [165], Logan et al., 2002 [166]
Adeno-associated virus	viral	Little/no immunogenicity; transduction in both dividing and non-dividing cells; sustained gene expression	Accommodates only very small size transgenes; production of high titers is difficult [159, 194, 195]	Cirelli et al., 2009 [163]; Warrington et al., 2006 [164]
Plasmid	non-viral	Non-immunogenic; transfection of wide range of cell types; localized gene expression	Very low transfection efficiency; transient gene expression [159, 195]	Liu et al., 2004 [196]; Huang et al., 2005 [167]
Nucleic acid/polymer complexes	non-viral	Targeted down regulation of gene expression	Low transfection efficiency; very transient effects on gene expression; potential for immunogenicity [157, 197]	Iwanaga et al., 2007 [198]; Kotopoulos et al., 2013 [199]