Tissue engineering in mandibular reconstruction: osteogenesis-inducing scaffolds

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Abstract

Currently, the gold standard for aesthetic and functional reconstruction of critical mandibular defects is an autologous fibular flap; however, this carries risk of donor site morbidity, and is not a promising option in patients with depleted donor sites due to previous surgeries. Tissue engineering presents a potential solution in the design of a biomimetic scaffold that must be osteoconductive, osteoinductive, and support osseointegration. These osteogenesis-inducing scaffolds are most successful when they mimic and interact with the surrounding native macro- and micro-environment of the mandible. This is accomplished via the regeneration triad: (1) a biomimetic, bioactive osteointegrative scaffold, most likely a resorbable composite of collagen or a synthetic polymer with collagen-like properties combined with beta-tri calcium phosphate that is 3D printed according to defect morphology; (2) growth factor, most frequently bone morphogenic protein 2 (BMP-2); and (3) stem cells, most commonly bone marrow mesenchymal stem cells. Novel techniques for scaffold modification include the use of nano-hydroxyapatite, or combining a vector with a biomaterial to create a gene activated matrix that produces proteins of interest (typically BMP-2) to support osteogenesis. Here, we review the current literature in tissue engineering in order to discuss the success of varying use and combinations of scaffolding materials (i.e., ceramics, biological polymers, and synthetic polymers) with stem cells and growth factors, and will examine their success in vitro and in vivo to induce and guide osteogenesis in mandibular defects.

Keywords: Osteogenic scaffolds, mandibular reconstruction, tissue engineering, regeneration triad, bone morphogenic protein, bone marrow mesenchymal stem cells, beta-tri calcium phosphate, gene activated matrix

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INTRODUCTION

A mandibular defect is the loss of a lower jaw bone segment that produces a gap within the bone of 2 cm or more, resulting in a continuity or non-continuity mandibular defect. These defects primarily arise from tumor resection, infection, physical trauma, and osteomyelitis. Such a critical defect will not heal on its own or regenerate more than 10% of the lost bone within the lifetime of the patient. Not only is mandibular bone important for craniofacial aesthetics, but also for the support of muscles of mastication, facial expression and speech. Therefore, the choice of scaffold to repair the defect must allow for sufficient muscle attachment to restore oral and maxillofacial function, which has been shown to have significant impact on the patient's quality of life. Thus, to achieve successful reconstruction, care must be taken to restore both aesthetics and functional capacity.

The autologous fibular free flap is currently the workhorse for mandibular defect repair which, along with other autologous free vascularized tissue transfer, is considered the “gold standard” for mandibular reconstruction because of their osteogenic, osteoinductive, and osteoconductive properties, in combination with the avoidance of an immune reaction. These grafts also contain live stem or osteoprogenitor cells that themselves migrate, proliferate, and potentiate bone healing. The major concern with using a fibular free flap is donor site morbidity, which has been reported to occur in 31.2% of patients. These complications include wound-healing disturbance, paresthesias, cold intolerance, motor weakness of the lower leg muscles, pain, edema, poor aesthetics, and gait disturbance, and has been reported to lead to long term morbidities in 17% of patients, and severe disability in 4% of patients. To circumvent this problem, cadaver grafts may be an attractive option, however, osteoclastic resorption, risk of disease transmission (viral) and immune reaction make this a less than ideal alternative. Additionally, synthetic grafts designed from metals or polymers are not bioactive and do not bond to bone or support bone cell function, and can also induce the formation of fibrous tissue at the interface between the implant and bone, which can interfere with bone healing and cause bone resorption, fracture, and eventual failure of the implant.

If advancement is to be made beyond these methods in an effort to prevent such suffering to the patient, the following factors seem to be important in the design of a biotechnology capable of adequately closing a critical osseous defect: (1) a scaffold to allow bone growth on its surface (osteogenesis); (2) growth factors that induce osteogenesis (osteoinduction); (3) cells that will support osteogenesis; and (4) vascular supply and integration for the delivery of oxygen and nutrients to developing and native tissue. Of these, vascularization has been a limiting factor for the use of scaffolds in mandibular repair, since both in vitro and in vivo construct implantation lack pre-existing vasculature. Because of these multifactorial considerations, tissue engineering might provide the solution to this problem.

The critical focus of first-generation biomaterial design was passive biocompatibility; it was not until second-generation biomaterials that biointeractivity for the stimulation of active tissue regeneration emerged. Third-generation biomaterials are bioresponsive, e.g., they can activate genes to influence all aspects of proliferation and differentiation of cells. This assembly of scaffold material, scaffold structure (i.e., pore size), cells and growth factors reveals the multidisciplinary nature of tissue engineering, which is the intersection of material science, mechanical engineering, clinical medicine, and genetics. In mandibular reconstruction, the primary goals of tissue engineering include reducing donor site morbidity, operative time, and operative complexity. If non-vascularized flaps can be used (i.e., patients who have not been and are not planned to undergo radiation), favorable results have been reported with the adjunct use of tissue engineering for mandibular reconstruction. Furthermore, modern regenerative medicine builds on tissue engineering designs to direct the surrounding native cellular environment toward a healing process, thereby making use of foreign biological material to recreate cells and rebuild tissues.
In order to accomplish this, an effective bone scaffold must satisfy the following requirements: osteoconductivity, osteoinductivity and osseointegration\(^{[27]}\). Osteoinductivity is the ability of a material to recruit multipotent cells and encourage their differentiation into an osteoblastic lineage\(^{[28]}\). This is typically accomplished adding both growth factors and stem cells, such that growth factors signal to surrounding mesenchymal stem cells to differentiate into chondroblasts and osteoblasts to form new bone\(^{[29,30]}\). In the context of mandibular reconstruction, stem cells have potential to regenerate oral and dental tissues, such as bone, dentin, cementum, periodontal ligaments, mucosa, and salivary glands\(^{[22]}\). Mesenchymal stem cells are the most common source of osteoprogenitor cell used, and may be derived from bone marrow, adipose tissue, and dental and periodontal tissue, and their differentiation is guided by growth factors [such as bone morphogenic protein (BMP)]. Such involvement and interaction between growth factors are essential to the process of native bone healing, including vascular endothelial growth factor (VEGF), fibroblastic growth factors, insulin-like growth factors, platelet-derived growth factor, and BMP, to name a few\(^{[31]}\). During osteogenesis, an osteoconductive material will allow the growth of bone not only on the scaffold surface, but also into pores and channels, such that both cortical and cancellous bone are formed around and within the framework\(^{[32]}\). Such materials may also be designed to be resorbed in order to encourage growth of native bone. Osseointegration is the degree to which the native bone and the implant favorably interact, and such incorporation of a graft is influenced by many factors, such as the type of bone scaffold used and the site of implantation\(^{[33]}\). Thus, the general principle underlying third generation biomaterials is the regeneration triad: (1) an extracellular matrix (ECM) scaffold, which can be made of varying material to create a porous 3D structure that may be seeded with; (2) growth factors; and (3) stem cells\(^{[34,35]}\). Ideally, scaffolds should be designed to provide regenerative signals to surrounding cells, while simultaneously improving cell adhesion, proliferation, and differentiation\(^{[36]}\), and mechanical rigidity or flexibility\(^{[37]}\).

Thus, there is extensive flexibility in assembling a scaffold. The choice of scaffold material itself can be varied, and sometimes may be used successfully on its own or in combination with other materials. Furthermore, modification of the scaffold material by coating its surface with nanoparticles, an ECM molecule (such as collagen), or a growth factor (such as BMP-2) has been shown to improve tissue properties\(^{[38]}\). In this review, we will explore the success of varying combinations of the above scaffolding materials, and will examine their success in vivo and in vitro in inducing and guiding osteogenesis in mandibular defects.

**SCAFFOLD MATERIALS AND STRUCTURE**

Beyond the biocompatibility of a scaffold, as has been argued by Chocholata et al.\(^{[21]}\), the most important aspect of scaffold design is its three dimensional structure, namely the degree of pore interconnectivity and pore size, both of which effect the degree of cell attachment and three dimensional regeneration of tissue, as well as cell growth, proliferation, and differentiation, diffusion of waste and the degradation products of scaffolds. The goal of these materials is to initiate or enhance bone formation - if pore size is too small, it can hinder cell migration, and if too large will result in suboptimal binding of cells to the scaffold\(^{[18,39]}\). For maximal osteoconductivity, the ideal pore size as described by Ghayor and Weber\(^{[40]}\) based on in vivo data is 0.7-1.2 mm, and the size of connections between pores should be between 0.5-1.2 mm; sizes larger than this are detrimental to osteoconductivity. During osteointegration, these porous spaces are initially populated by capillaries, perivascular tissues, and osteoprogenitor cells, followed by incorporation of the porous structure within the newly formed bone\(^{[41]}\). Additionally, the scaffold must be designed to degrade at an appropriate rate so that there is enough time for bone regeneration\(^{[42]}\). This is especially relevant in pediatric patients, where the future growth of the mandible must be considered. In this case, fixation of the mandible using titanium locking reconstruction plates does not allow for mandibular growth over time, and might result in facial asymmetry and problems with occlusion as the patient grows\(^{[24]}\). Resorbable plates have been developed in order to address this, but their drawbacks include postoperative plate...
fracture and the development of delayed foreign-body reactions, and this potential harm to the patient’s well-being might discourage their use; consequently, the focus on “resorbable” material has consequently shifted to “bioabsorbable” scaffolding, which combines biodegradation with osteoconduction\(^{[43,44]}\). Lastly, the mechanical properties of the material must sufficiently mimic the native tissue at the implantation site in order to support functionality\(^{[45]}\). These factors will vary with scaffolding material, and will be described below.

A key requirement of effective tissue engineering is constructing a cellular environment that mimics critical aspects of the \textit{in vivo} setting through proper control of the materials and mechanical setting as well as the chemical environment. The macroscopic structure of bone consists of a cortical outer layer encasing porous trabecular bone\(^{[29]}\). However, it is the nanoscopic structure of bone that yields its mechanical, biological and chemical properties, and this heterogenous structure is importantly irregular and anisotropic\(^{[46,47]}\). The ECM of bone is comprised of 60% mineral [hydroxyapatite (HA)] and 30% organic matrix\(^{[48]}\). The organic components give bone tissue its flexibility, and mainly consist of collagen (type I collagen, type III and type IV collagen), and together with fibrin and over 200 types of noncollagenous matrix proteins (glycoproteins, proteoglycans, sialoproteins, \textit{etc}.), collagen forms the native scaffold for mineral deposition\(^{[25,48]}\). These HA\(_{3}\)(PO\(_4\))\(_2\)\((OH)\) nanocrystals, inlayed between individual collagen fibers, give bone its mechanical strength and rigidity\(^{[49]}\). Due to this structure, bone tissue can be treated as a ceramic-organic bio-nanocomposite complex\(^{[48]}\).

In an effort to design biomimetic material, natural (some authors also called these biological) scaffolds use existing ECM materials, and may be protein-based (e.g., collagen, fibrin) and polysaccharide-based (e.g., chitosan, alginate, glycosaminoglycans, hyaluronic acid)\(^{[50-52]}\). Such material also contains cross-linking agents (e.g., glutaraldehyde, water-soluble carbodiimide), which can be adjusted to modify degradation rates\(^{[37]}\). One method to achieve both porosity and biocompatibility is to mimic the collagen network of the ECM of bone using nanofibrous scaffolds\(^{[53]}\). This can be constructed using electrospun (PLLA) scaffolds, which when coated with HA has been shown to induce calcium deposition and mineralization and the formation of higher order bone structures such as trabeculi and bone marrow, when combined with stem cells\(^{[54]}\). It has also been shown that electrospun PLLA can be combined with a porous collagen membrane to guide bone regeneration\(^{[55]}\).

Single material scaffolds have shown promise in reconstructing mandibular defects. These materials include: biological polymers (collagen, chitosan), ceramics [beta-tri calcium phosphate (\(\beta\)-TCP), calcium HA, biphosphate calcium phosphate (BCP)], and synthetic polymers [polycaprolactone (PCL), PLA, PGA, PLGA]\(^{[56]}\). The advantages to ceramics are that they are osteoconductive and biocompatible. Herford \textit{et al}.
\(^{[57]}\) generated a ceramic compression resistant osteoconductive matrix that was 15% HA and 85% \(\beta\)-TCP that showed a significantly higher bone density and space maintenance than BMP2 combined with resorbable collagen sponge. However, one of the main concerns in the application of HA bone grafts is poor resorption, and several studies have reported fibrous encapsulation around HA ceramic particles inside alveolar bone\(^{[58-60]}\). In a 12 mm full thickness mandibular defect in a rabbit model using \(\beta\)-TCP ceramic, Lopez \textit{et al}.
\(^{[61]}\) found that new bone accounted for half of the defect site repair at 8 weeks post-scaffold implantation, although no stem cell seeding or BMP signaling was used to direct osteoblast differentiation, instead using the properties of the biomaterial itself to direct endogenous healing mechanisms. Such calcium phosphate ceramics (\(\beta\)-TCP and BCP) are promising because of their biocompatibility and drug delivery potential, and they have been shown to be osteoconductive with sufficient mechanical strength, and they can be reliably used in 3D printing methodology\(^{[62,63]}\). However, calcium phosphate is insufficiently osteoinductive and requires supplementation with growth factors to induce new bone formation\(^{[64]}\). These scaffolds do have lower mechanical strength compared to allografts because they are designed to be degradable such that it can be replaced by new bone; however, the extent of new bone formation, lack of
host-host bridging, and engraftment is similar. In preclinical animal studies, autogenous bone precursor cells seeded onto calcium phosphate ceramic scaffolds, pyrolyzed bovine bone, or calcium carbonate has been comparable to autograft bone in mandibular reconstruction in terms of biomechanical testing, bone bridging, and bone ingrowth.

The second major category is the synthetic polymer (PCL, PLLA, PLA-PEG, PGA, PLGA, PLGA-PEG, etc.). This material is promising because it allows 3D printing of complex structures that are biodegradable, bioactive, and undergo controlled degradation. However, PCL is not ideal for mandible tissue engineering due to inferior mechanical properties such as a low compressive strength.

The third category of material is the natural polymer (collagen, chitosan, silk fibroin, alginate, gelatin, etc.). Although biocompatibility with natural scaffolds is obviously excellent, there remain issues with potential immunogenicity in some cases. Because they do not induce antigen-antibody reactions, decellularized tissue matrices obtained from processing discarded donor tissue is an attractive solution. When bone matrix is demineralized via removal of HA, the remaining bony matrix is comprised mainly of collagen - this biocompatible, bioactive biomaterial has the ability to induce bone morphogenesis via BMP signaling, particularly in stem cells, and can be used as a film, gel, or sponge. Although they have similar osteoinductive and osteoconductive properties as autologous grafts, they lack the corresponding osteogenic properties. Additional major downsides are sourcing, processing, immunogenicity, and disease transmission, as well as lack of mechanical strength to withstand the forces exerted by the muscles of mastication.

In order to address this, Kakabadze et al. reports development of a novel biologically active bone graft using decellularized cancellous bovine femur seeded with human bone marrow mesenchymal stem cells (BMSCs) and growth factors, which was applied clinically to repair a large mandibular defect following primary tumor resection that successfully repaired the defect and showed maintained mandibular bone volume at 5 months post-op. Importantly, like the use of autologous bone, this graft construction requires use of a barrier membrane to prevent fibrous tissue invasion, and decellularized human amnion/chorion membrane was chosen by the authors due to its osseointegrative properties.

However, the shortcomings of using a single material in scaffold construction include: poor strength for biologically-derived materials, brittleness for inorganic materials, and poor cell compatibility and insufficient mechanical strength for synthetic polymers. Because of this, combining two or more materials to create a composite scaffold has shown improvement in material properties and biocompatibility. Most often, the polymer of choice is type I collagen, which is most often coated on scaffolds made from PCL, HA, and TCP in order to aim to mimic the structure of native bone. Additionally, biomimetic Mg-MgHA/collagen-based scaffolds have been shown to greatly improve osteoblast differentiation. When choosing between ceramics to add compressive strength, it should be noted that compared to β-TCP, HA has low absorption kinetics in vivo (1%-2% per year at 5 years postimplantation). An HA-collagen or β-TCP-collagen scaffold can be 3D printed, and the combination of biocompatibility, compressive strength, and resorption rate in vivo and in vitro allows for bone replacement over time, and the degradation rate of the material can be altered by increasing the macroscopic surface area by decreasing the strut diameter or altering micro/nano porosity.

The scaffold surface may also be modified by the addition of nanoparticles. Most commonly, nano-HA is combined with PCL and chitosan scaffolding. Nano-HA is of interest because it has been shown to increase the mechanical properties and improve the protein adsorption capacity of the polymer, while also acting as a substrate for cell attachment and migration during bone regeneration. Polyamide66 is a synthetic polymer chosen by Cai et al. to combine with HA due to its biocompatibility, high tensile...
strength, and its similarity to collagen in chemical structure and functional groups\textsuperscript{[74]}. When combined with BMSCs in a mandibular defect, this scaffold showed greater biocompatibility and osteoconductivity with the surrounding host bone compared with commercial porous polyethylene (MEDPOR) constructs seeded with BMSCs\textsuperscript{[77]}. One of the fundamental hurdles of bone-tissue engineering is vascularization of tissue. Zhu \textit{et al.}\textsuperscript{[79]} fabricated pre-vascularized tissues using a method derived from rapid 3D printing, termed microscale continuous optical bioprinting, in which two types of biocompatible and photopolymerizable hydrogels - glycidal methacrylate-HAp and gelatin methacrylate scaffolds - were pre-designed with vascular channels into which endothelial cells and mesenchymal cells were printed, which resulted in the spontaneous formation of a functional endothelial network both \textit{in vitro} and \textit{in vivo}.

Graphene and its derivatives, such as graphene oxide and reduced graphene oxide, is also a promising scaffold material because it is not only biocompatible, but also has been shown to regulate cell behavior, help in differentiation, and improve adhesion, growth and proliferation of cells\textsuperscript{[21]}. Graphene is built by layering SP2 bonded carbon atoms with atomic graphite in a honeycomb lattice structure\textsuperscript{[80]}. When combined with natural and synthetic biomaterials, graphene has been shown to increase osteogenic potential and mechanical strength of the scaffold\textsuperscript{[80,81]}. However, graphene has been shown to be toxic at higher concentrations and is not reliably biodegradable, warranting further investigation before clinical trials\textsuperscript{[80,81]}.

**STEM CELLS AND GROWTH FACTORS**

Most tissue engineering utilizes living cells, and supplying enough cells is obviously a critically important issue. Cells are typically derived from: (1) donor tissue, which is often in very limited supply; (2) stem or progenitor cells. Stem cells possess two major properties that make them attractive for deriving large cell quantities: (1) their high proliferative capacity; (2) their multipotency, or ability to differentiate into cells of multiple lineages\textsuperscript{[17]}. Bone marrow stoma contains progenitor cells with osteogenic potential, which are referred to as bone marrow stromal cells, or BMSCs\textsuperscript{[82]}. BMSCs are a major seed cell source for bone tissue engineering due to their well-known capability of self-renewal (which is an outcome of asymmetric division), and differentiation into the osteoblastic lineage \textit{in vitro} and \textit{in vivo}\textsuperscript{[83-85]}. Scaffolding has been shown to be capable to support ectopic bone formation when seeded with BMSCs in a mouse model, and the repair of large segmental defects\textsuperscript{[86,87]}. Moreover, many previous studies have succeeded in repairing bone defects by using BMSCs in animal models as well as in humans\textsuperscript{[88]}.

The procedure to extract autologous BMSCs is painful and associated with potential complications, so effort has been made to explore the use of adipose derived stem cells (ADSCs). Although ADSCs have a higher cell yield, the literature suggests they possess an inferior osteogenic capacity compared to BMSCs, so they are not as desirable in mandibular reconstruction\textsuperscript{[89]}. Dental pulp stem cells are also of interest due to their ease of access, low donor site morbidity, and ability to differentiate into fibroblasts, nerve cells, endothelial cells, and odontoblasts in order to facilitate creation of new connective tissue\textsuperscript{[89]}. Raspini \textit{et al.}\textsuperscript{[90]} showed that dental pulp stem cells combined with bioactive glass scaffold that was treated with osteogenic medium \textit{in vitro} showed good biocompatibility and osteogenic induction, making it a promising combination for hard tissue regeneration in the cranio-maxillofacial skeleton. However, the comparative efficacy of these cells between laboratory study and patient intervention remains to be seen\textsuperscript{[91]}.

When bone is transplanted, it is degraded and replaced through a process termed “creeping substitution”, and this degradation process releases calcium phosphates and osteoinductive proteins that amplify bone regeneration\textsuperscript{[41]}. BMPs are a member of the transforming growth factor-beta (TGF-β) superfamily that
induces the formation of bone and cartilage. In order to mimic this endogenous microenvironment, BMPs are often combined with MSCs in order to amplify their bone-forming potential. This use of MSCs with BMPs to repair mandibular bony defects has shown its effectiveness in animal models. Jiang et al. showed that transfection of BMSCs with hBMP-4 enhances their inherent osteogenic capacity in mandibular defect repair. Zhou et al. showed rhBMP-2 combined with prefabricated tissue engineered vascularized bone flaps produced in vivo induced successful reconstruction of the mandibular defect. Chen et al. found that loading a demineralized bone matrix with a formulated collagen-targeting BMP-2 induced better bone formation compared to rhBMP-2, and the authors note remarkable osteoinductive properties with homogenous bone formation. Additionally, BMPs may be combined with non-vascularized bone grafts, such as cadaveric fibula or other non-vascularized bone grafts, to stimulate osteogenesis. Such a design has shown capability to reconstruct mandibular defects up to 12 cm. It should be noted that BMP is contraindicated in cancer, because it is thought to stimulate cancer growth.

The importance of scaffold selection when using BMP-2 and BMP-7 has been well documented. The material must allow sustained diffusion of BMPs throughout the environment and provide matrix for in-growth of osteoprogenitor cells and blood vessels, and the properties of scaffolds constructed with BMP and ceramics, synthetic polymers, or biological polymers differ. Currently, collagen is the gold standard delivery system for BMPs. Composite scaffolds are also promising for BMP use, such as PLA/PEG/HAP which is osteoconductive, or a PLGA-collagen hybrid, which has osteoinductive activity and long stimulation effect. In terms of novel carriers, nanoparticles and microparticles are becoming increasingly popular due to localized and sustained delivery of BMPs, which can be designed with natural polymers, synthetic polymers, or ceramics. Quinlan et al. loaded alginate and PLGA MPs with rhBMP-2 in order to incorporate the polymer into porous HAp-collagen scaffold for bone regeneration, which showed new bone formation in a rat model in vivo. Dual-interacting polymeric nanoparticles were prepared by Seo et al. to form nanocomplexes with BMP-2, which resulted in sustained BMP-2 release and significant bone generation.

BMPs combined with biomaterial appears equivalent to autogenous osteogenic tissue. In humans, native human BMPs, xenogeneic BMPs, rhBMP-2, or rhBMP-7 were reported to yield complete mandibular bony defect bridging without simultaneous use of autogenous osteogenic issue in 29 out of 34 patients. It has long been thought that bone growth cytokines could be reliably used in lieu of traditional bone grafting. While tissue-engineered autogenous osteogenic tissues without application of osteoinductive BMPs has been reported to restore mandibular continuity (n = 16 patients), osteoinductive rhBMP-2 loaded onto various scaffolding materials without concomitant transplantation of autogenous osteogenic tissue has also been shown to restore mandibular continuity.

Other growth factors that have been explored for promoting osteogenesis include recombinant human platelet-derived growth factor, TGF-β, fibroblast growth factor, recombinant human growth/differentiation factor-5, VEGF, and insulin-like growth factor. However, BMPs remain the most frequently used compared to other growth factors. Beside their ability to induce osteogenic differentiation in stem cells, BMPs can accelerate the healing process. However, it should be noted that in a calvaria defect model, BMP-2 and VEGF had similar bone healing capacities, with FGF-2 displaying a significantly higher bone regeneration capacity; however, the healing rate was lower than with BMP-2 and VEGF. BMP-2 and VEGF also showed increased angiogenic response upon healing. It should also be noted that undesirable clinical outcomes with BMPs have been shown, namely extreme bone proliferation (albeit in a calvarial model), ectopic bone formation, radiculitis, and potential stimulation of neoplasms. Because of this, investigation into β-TCP ceramic scaffold coated with an adenosine A2 receptor indirect agonist augmented bone growth as effectively as rhBMP-2 in a 3 mm defect. Adenosine A2A receptor signaling appears to be important for osteoclast differentiation both in vitro and in vivo, and has been shown to promote bone regeneration.
GENE THERAPY

Gene therapy makes use of native nuclear machinery in order to synthesize a protein of interest via the process of transduction, in which a viral vector is typically used \[111\]. In this way, growth factor can be produced in the region of the defect, and has been reported to support mineralized tissue formation \[112\]. Therefore, expression in the host cell lasts longer (weeks to years) compared to pharmaceutical compounds or recombinant protein, which ranges from several hours to days. This allows continuous production of biologically active molecules, thereby mimicking the endogenous physiological healing response in the microenvironment of the defect \[113,114\]. Viral vectors remain preferred to non-viral vectors because they have been rendered replication-incompetent, and non-viral vectors have insufficient transfection efficiencies \[115,116\].

In order to induce de novo bone formation in the maxillofacial region *in vivo*, the genes of interest range from soluble growth factors (PDGF, FGFs), morphogens (BMPs), angiogenetic factors (VEGF), intracellular regulators (LIM mineralization protein-1), transcription factors (Runx2) associated with bone and cartilage-related gene expression \[117,118\]. Due to their ability to initiate and sustain the entirety of the bone formation process, BMPs are the preferred candidates for local gene therapy for bone regeneration \[119\].

Although gene therapy can be administered via systemic or local injection, gene therapy may be delivered with a biomaterial. This combination of a vector and biomaterial is referred to as a gene activated matrix that acts as a scaffold for delivery of the vector to the area of interest \[120\]. This method may be especially attractive in the repair of mandibular defects, in which cells may be removed from the donor site, be genetically modified and implanted onto the scaffold of choice, and re-implanted into the defect \[121\]. Interestingly, BMSCs have been successfully transfected by various vector systems in order to improve their proliferation and differentiation capacities \[117\]. A meta-analysis by Fliefel *et al.* \[115\] which considered majority animal-model studies found evidence that gene therapy improves bone formation in maxillofacial defects. These results have not yet been confirmed in human subjects; thus, it remains an exciting approach to mandibular defect repair that warrants future research and randomized clinical trials \[115\].

CONCLUSION

Tissue engineering for mandibular reconstruction is most successful when it can mimic and interact with the surrounding native macro- and micro-environment in order to induce and support osteogenesis. Based on the current literature, an optimal mandibular scaffold is comprised of three elements: (1) a biomimetic, bioactive osteointegrative scaffold, most likely a resorbable composite of collagen or a synthetic polymer with collagen-like properties with β-TCP that is 3D printed according to defect morphology; (2) growth factor, most frequently BMP; and (3) stem cells, most commonly BMSCs. Overall, the use of a tissue engineered scaffold may prevent common complications of mandibular defect repair with fibular free flap, such as donor site morbidity, and may provide an approach for patients with depleted donor sites due to previous surgeries.

DECLARATIONS

Authors’ contributions

Made substantial contributions to conception and design, analysis, interpretation, and preparation of the review and manuscript: Nelms L.

Assisted with manuscript preparation, as well as provided administrative, technical, and material support: Palmer WJ.

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