

Matrices and scaffolds for drug delivery in dental, oral and craniofacial tissue engineering[☆]

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Abstract

Current treatments for diseases and trauma of dental, oral and craniofacial (DOC) structures rely on durable materials such as amalgam and synthetic materials, or autologous tissue grafts. A paradigm shift has taken place to utilize tissue engineering and drug delivery approaches towards the regeneration of these structures. Several prototypes of DOC structures have been regenerated such as temporomandibular joint (TMJ) condyle, cranial sutures, tooth structures and periodontium components. However, many challenges remain when taking in consideration the high demand for esthetics of DOC structures, the complex environment and yet minimal scar formation in the oral cavity, and the need for accommodating multiple tissue phenotypes. This review highlights recent advances in the regeneration of DOC structures, including the tooth, periodontium, TMJ, cranial sutures and implant dentistry, with specific emphasis on controlled release of signaling cues for stem cells, biomaterial matrices and scaffolds, and integrated tissue engineering approaches.

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1. Introduction

The complexity of dental, oral and craniofacial (DOC) structures presents special challenges for the regeneration of cartilage, bone, muscle, tendons, cranial sutures, temporomandibular joints (TMJ), salivary glands, periodontium and teeth (Fig. 1). A wide range of drug delivery matrices and scaffolds has been applied to accommodate the needs of regenerating DOC tissues. Cells that are responsible for tissue regeneration may be either delivered via biomaterial carriers or recruited *in vivo* by signaling molecules. Drug delivery by controlled release provides several critical advantages because bioactive factors are transported to the desirable milieu in biocompatible carriers, released in a controlled manner and may locally regulate multiple processes of cell chemotaxis, attachment, proliferation, differentiation, and morphogenesis. In the absence of drug delivery and controlled release, bioactive factors may undergo rapid diffusion and denature shortly after *in vivo* delivery, and often fail to induce the intended effects on target cells and tissues. Although control-released drugs or bioactive factors may also diffuse and denature, the continuous dosing provides sustained effects where subsequent doses act on cells that have already been sensitized by previous growth factor exposure.

The auspice of regenerative medicine has rested on the delivery of stem cells towards the healing of diseased, traumatized or ageing tissues and organs. At this time, we believe that drug delivery is a highly effective adjunctive approach for cell based therapies. There is also the potential that controlled delivery of bioactive factors may circumvent the needs for cell delivery in certain defects, and may induce the homing of stem cells, progenitor cells and/or tissue-forming cells in traumatized or diseased tissues. The advantage of cell homing over *ex vivo* cell manipulation and delivery is the minimized laboratory and graft preparation as well as a commercially attractive potential for “off-the-shelf” products.

The nature of DOC structures determines that the regeneration process involves complex and composite tissues [1,2]. For example, the regeneration of the arthritic temporomandibular joint (TMJ) condyle involves at least two tissue phenotypes: cartilage and bone. Biological repair of dental caries frequently involves both enamel and dentin. When carious lesions infect the tooth pulp, healing involves the repair and regeneration of

multiple cell types, such as neurons, odontoblasts, vascular cells and fibroblasts. Periodontal disease involves the periodontal ligament, cementum and alveolar bone. Salivary gland consists of ductal and acini cells interfacing with connective tissue cells in a vascularized environment. Accordingly, DOC regeneration frequently requires the manipulation of multiple tissue phenotypes [1,2]. Some of the features of DOC structures with regard to tissue engineering and tissue regeneration are as follows:

- High demand for esthetics
- Many tissues are highly vascularized
- Often infectious environment of the oral cavity
- Minimal scar formation in the oral cavity
- Many DOC tissues are potential sources for stem cells
- Multiple tissue phenotypes integrated or nearby
- Demand for composite tissue engineering
- Need to accommodate multiple tissue functions
- Most tissues are superficial or readily exposed
- Practiced by multiple medical and dental specialties

A number of tissue engineering projects geared towards DOC structures may serve as prototypes for tissue engineered structures elsewhere in the body. For example, the temporomandibular joint (TMJ) represents a prototype for the regeneration of other synovial joints, given its relatively smaller size in comparison with the hip or knee joints [2–4]. Success in regenerating a functional TMJ may be then scaled up to larger joints of the appendicular skeletal system. Similarly, many of the current tissue engineering techniques that target other regeneration systems may be applicable to DOC regeneration (Fig. 1). However, the scale or the depth of previous and existing work in the areas of dental tissue engineering is far inferior to what is needed for the regeneration of these structures. This review addresses the structural and functional requirements for DOC regeneration with special emphasis on matrices, scaffolding, drug delivery and biomaterial approaches.

2. Teeth and periodontium

Common conditions requiring dental treatment include caries, pulp and odontogenic infections, periodontal disease, tooth impactions, and malocclusion. Current clinical therapies

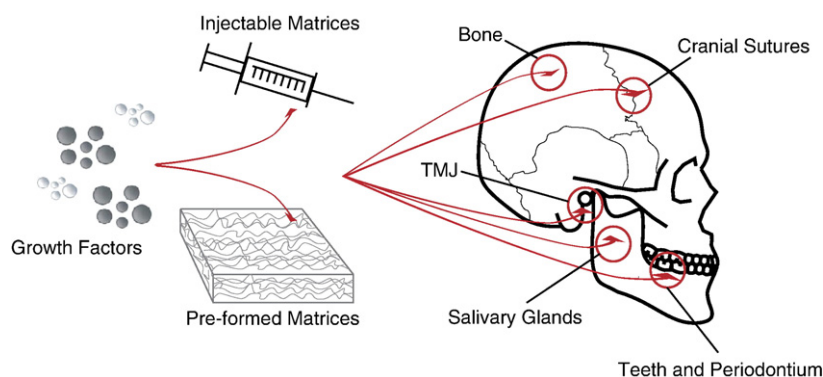


Fig. 1. Drug delivery in dental, oral and craniofacial tissue engineering. Growth factors and other bioactive molecules may be delivered via *in situ* forming injectable matrices or pre-shaped implants for the regeneration of teeth, periodontium, cranial sutures, salivary glands, temporomandibular joints, and calvarial bone.

for dental diseases are metallic or synthetic materials. Many of the dental diseases are unexplored fields in terms of biologically derived regeneration. For example, tooth decay, the second most prevalent disease in the United States, affects over 90% of the population. Tooth decay is initiated by acidogenic bacterial infection in plaque and can range from superficial enamel defects to widespread carious destruction. Partial or complete loss of tooth structure, may require restorations, tooth extraction, surgical titanium implants or tooth replacement using denture-based prostheses. Dental material in use broadly includes metal alloys, cements, acid-based polymer products, and synthetic materials such as composites resins. Also in use are known carcinogens such as mercury in amalgam and formocresol used in pulp therapy. Most restorative dental materials have a limited lifespan and may need to be repaired or replaced periodically. Tissue engineering may provide a viable alternative for the repair of tooth structures including the enamel, dentin, cementum and the tooth pulp. Fortunately, several groups have begun to explore approaches toward the ultimate regeneration of tooth as an organ [5–9]. Although the regeneration of an entire tooth as an organ is an admirable goal, it is also a long-term goal with numerous hurdles, not the least of which is that the eruption of native teeth is poorly understood. Even when a tooth is engineered in the alveolar bone, the eruption mechanism is likely a road block towards regenerating a functional and occluding tooth. On the other hand, if a tooth is regenerated *ex vivo* and ready for implantation, reconnection of apical vessels and nerves would be a critical challenge. Although it is expected that a great deal of biology is learned towards whole tooth engineering, it is predictable that the regeneration of separate tooth structures would be more fruitful at this time.

The anatomy of DOC tissues demonstrates distinct yet integrated structures. Externally, enamel coats the tooth, providing a compact and hard surface. During tooth development, enamel matrix is produced by the ameloblasts that derive from neural crest cells that migrate to form the oral ectoderm. Following enamel deposition, ameloblasts cease to exist in post-natal tissues. This is in contrast to the existence of adult or somatic stem cells in post-natal mammals such as mesenchymal stem cells [4,10–12]. To date, there is little experimental evidence that ameloblasts can be isolated from one of the adult stem cell lineages. On the engineering side, enamel is a generously designed structure and is the hardest mineralized tissue in the body. The elastic modulus of enamel is approximately 12.2×10^6 psi with some spatial variability between apical and lateral aspects [13]. Without surprise, strong glass-, ceramic- or metal-based substitutes have been used as enamel replacements such as amalgam and composites.

A second dental cell type is the odontoblast that is responsible for the formation of dentin that underlies the enamel and is the bulk of the tooth. Odontoblasts derive from neural crest derived mesenchymal cells. Similar to the enamel, the mechanical properties of dentin are strenuous and require adequate substitutes and fixation [14]. In the context of tissue engineering, scaffolds and matrices for enamel and dentin must withstand the minimal forces necessary during mastication and

occlusion shortly after reconstruction. Cementum coats the dentin in the root portion of the tooth and is formed by cementoblasts that also derive from neural crest derived mesenchymal cells. Cementum is considered a part of the periodontium and attaches to the alveolar bone via collagen fibers. Cementum is typically thinner, softer and more permeable than enamel, leading to greater susceptibility to abrasion upon root exposure to the oral environment. In contrast to the overlaying hard tissues of dentin, enamel and cementum, the dental pulp is soft tissue that resides in the central core of the tooth. The pulp, also partially originating from neural crest derived mesenchymal cells, has a rich vascular and nerve supply.

Erupted teeth are supported in bony sockets of the alveolar bone via fibers of the periodontal ligament (PDL). The PDL, similar in function to ligaments and tendons in the musculo-skeletal system such as ACL, transmits mechanical forces and acts as a cushion during mastication, although the PDL has different structural attributes. Each of the hard tissues of the tooth including the enamel, dentin and cementum is formed by apparently a homogeneous cell type. For example, the dentin is formed apparently by odontoblasts alone. In contrast, the PDL and the dental pulp each is formed by a number of diverse cell populations. The PDL is formed by the interactions of cementoblasts, fibroblasts, osteoblasts in addition to blood vessel borne cells, whereas the dental pulp is formed by odontoblasts, fibroblasts, vascular and lymphatic system cells, as well as neurons. Accordingly, the selection of matrices and scaffolds in dental tissue regeneration must reflect these specific conditions and cell lineages, in addition to functional and mechanical requirements.

The multifaceted structure of teeth requires multidirectional engineering perspectives which likely include composite materials that satisfy the numerous requirements for functionality. These perspectives must then converge to the formation of regenerative tissues suitable for rapid established functionality post-intervention. Accordingly, previous efforts in DOC tissue engineering have encompassed a wide range of biomaterials, including biopolymers, ceramics, and metals. Additionally, the stimulatory, scaffolding, or structural functions of biomaterials can be improved by the synchronous activity of bioactive molecules. Drug delivery technology has been described extensively in tissue engineering procedures and is directly applicable in dental regeneration. Controlled delivery of growth factors have been demonstrated to promote alveolar bone regeneration, periodontal ligament formation, as well as pulp cell activation as described in the following paragraphs.

2.1. Molecular fingerprints during tooth development and regeneration

Similarly to hair and glands, teeth develop from ectoderm in vertebrate embryos with critical roles played by interactions between the ectoderm and the underlying mesenchyme [15]. Each step during tooth morphogenesis, ectoderm thickening, followed by the bud, cap, bell and late bell stages, hosts sequential and reciprocal molecular events that regulate the

initiation, morphogenesis, differentiation and mineralization, as well as root formation and eruption of the tooth. Most of the signaling molecules regulating ectoderm and mesenchyme interactions during tooth development belong to the transforming growth factor- β (TGF- β), fibroblast growth factor (FGF), Hedgehog, and Wnt families [15].

The sequential morphogenesis steps are characterized by the formation of signaling centers in the epithelium. Initially, the dental placodes are found during budding, and are characterized by the expression of activin, FGF and BMP-4 (bone morphogenetic protein-4). Placode development is further regulated by Wnts and ectodysplasin. Budding begins along with condensation of mesenchymal stem cells. At this point, the transcription factors Runx2 and Fgf3 regulate epithelial morphogenesis during the bud to cap transition. BMP-4 is also involved in enamel knot formation at the tip of the bud, by inducing expression of p21, resulting in knot cells leaving the cell cycle. The enamel knot expresses many other signaling molecules such as Shh, Bmp-2, Bmp-4, and Bmp-7, Fgf-3, Fgf-4, Fgf-9 and Fgf-20, and Wnt-3, Wnt 10a, and Wnt-10b [15]. SHH signaling is also crucial for the development of epithelial cervical loops flanking the enamel knots. Patterning of the tooth crown is regulated by the secondary enamel knots, which conserve the molecular expression signature and determine the sites for epithelial sheet folds and cusp development. The exact recapitulation of the molecular fingerprints during tooth formation may not be necessary in the process of engineering tooth structures. It is prudent however, to consider the spatial and temporal activity of cytokines and morphogens in order to establish optimal signaling cascades using drug delivery technologies. The delivery of commonly used growth factors such as FGF, BMP and TGF- β are discussed below in the context of regeneration of the dental pulp, as well as dentin and periodontal structures. A summary of drug delivery approaches for DOC regeneration is highlighted in Table 1.

2.2. Dental repair by delivery of matrices and growth factors

The dental pulp is a well vascularized and innervated connective tissue that shows some potential for healing in response to various stimuli and injury [16]. Typically, following pulpal exposure, pulp capping is a common procedure that induces dentine bridge formation and has proven to be an efficient model for repair and wound healing [17–20]. In order to maintain tooth structural integrity after trauma or corrective procedures, calcium hydroxide is sometimes used as a direct capping agent for the pulp [21]. Calcium hydroxide may contribute to reactionary dentin formation as a cavity liner as well as contributing to repair of pulp exposures [22]. However, endodontic treatment post-calcium hydroxide capping may become difficult when formation of necrotic and acute or chronic inflammatory zones are observed [23–26]. The high alkaline pH that calcium hydroxide promotes at the surface of the exposed pulp causes a controlled burn and subsequent scar, below which reparative cells are recruited in the central part of the pulp [21]. Given the replication potential of such reparative cells, it is unlikely that these cells are mature odontoblasts, since

Table 1

Summary of delivery systems for growth factors involved in dentin, pulp, periodontium, cranial sutures and temporomandibular joint regeneration

Tissue type	Growth factor	Delivery vehicle	References
Dentin and pulp	TGF- β 1,2 and 3	Agarose beads	[57]
	BMP-7	Agarose beads	[58]
	BMP-2/ BMP-4	Collagen sponge	[36]
	TGF- β 1	Alginate gels	[60]
Periodontium	FGF-2	Gelatinous carrier	[105]
	FGF-2	Sandwich collagen Membrane	[180]
	IGF-1	Dextran-co-gelatin hydrogel microspheres	[108]
	BMP-12	Collagen sponge	[111]
	BMP-2	Slow dissolving collagen membranes	[116,117]
Cranial sutures	PDGF-BB	β TCP	[53]
	TGF- β 3	Collagen gel	[181]
	TGF- β 3	PLGA microspheres	[182]
Temporomandibular joint	BMP-2	Collagen carrier	[135]
	BMP-2	PLA/PGA copolymer-gelatin sponge complex	[143]
	TGF- β 3	Thermo-reversible hydrogel/hyaluronic acid	[146]
	TGF- β 1	Oligo(poly(ethylene glycol) fumarate) (OPF)	[148]

these typically are terminally differentiated. Consequently, there is the possibility for the existence of “dental pulp stem cells”, which have high proliferation and differentiation potential. These cells aggregate and proliferate adjacent to the wounded area and initiate repair forming osteodentin [21]. Further characterization of pulp stem cells indicate some similarities to bone marrow derived progenitor cells, such as extended self-renewal capabilities as well as multiple lineage differentiation [16,27–30]. Previous studies have demonstrated the differentiation potential of pulp derived cells into many lineage specific cells including dentin and cementum producing cells such as odontoblasts and cementoblasts [31–35]. TGF β s such as BMPs induce differentiation of pulp cells into odontoblasts [36–38]. Human dental pulp cells (hDPCs) may be cultured in various surfaces in order to regulate their differentiation. For instance, hDPCs cultured on dentin surfaces assume odontoblast-like morphologies with cytoplasmic processes that extend the dentinal tubule [33]. The high expandability potential of DPCs was demonstrated in comparable levels to immortal cell lines (NIH 3T3). Additionally, DPCs may be differentiated into mineral nodule forming cells with exposure to dexamethasone and 1,25-dihydroxyvitamin D3.

Alternatively, hyaluronic acid (HA) has been proposed as a substitute to calcium hydroxide, based on previous data demonstrating the stimulatory effects of high molecular weight HA on early healing of rat long bones in addition to circumventing the necrotic and inflammatory shortcomings of calcium hydroxide [39]. Results indicate that capping pulp exposures of rat molars with HA promotes the formation of reparative dentine that extended throughout the pulp chamber

after 30–60 days, following differentiation of fibroblastic and odontoblast-like cells and globular calcified nodules formation at 1 week as well as the formation of a layer of reparative dentine by odontoblast-like cells over the dentine walls at 2 weeks [39]. Such findings indicating alternatives for pulp capping may open possibilities for regenerative drug delivery. Hyaluronic acid has been previously tested as a delivery scaffold for BMP-2 [40]. The release profile of BMP-2 from hyaluronic acid scaffolds shows gradual sustained release up to 28 days (~32% of total encapsulated BMP-2) when compared to fast release collagen gels (100% after 14 days). BMP-2 was released in bioactive form from hyaluronic acid as shown by alkaline phosphatase (ALP) activity induction of a pluripotent cell line indicating osteogenic differentiation. Hyaluronic acid is a versatile biocompatible and bioresorbable material, which is enzymatically degraded. Multiple growth factors may also be loaded concurrently in HA scaffolds such as BMP-2, IGF-1 (insulin-like growth factor-1), and TGF- β 2 [41]. Ten days post-subcutaneous implantation of HA in rats, the newly formed tissue was primarily cartilage in BMP-2/IGF-1 loaded groups and mostly bone in the BMP-2/TGF- β 2 groups.

Several studies implicate platelet-derived growth factor (PDGF) during the development of the ectomesenchyme and the differentiation of dental pulp cells [42–46]. Furthermore, PDGF is involved during growth of the pulpal mesenchyme in developing mouse molars. Given its important roles during tooth development and repair, PDGF delivery in dental systems has been explored. Matrices used in PDGF delivery for the regeneration of several other organ systems have been described including self-assembled nanofibers [47], chitosan-poly(L-lactide) (PLLA) composite matrices and chitosan coated on PLLA matrices [48], porous chondroitin-4-sulfate (CS)-chitosan sponge [49], ethylene vinyl acetate copolymer (EVAc) coated stainless-steel Kirschner wire (K-wire) [50], as well as PLGA microspheres [51].

In the context of DOC tissue engineering, the three PDGF dimers, PDGF AA, AB, and BB were cultured with dental pulp cells from rat incisors resulting in differential regulation. All three dimers suppressed alkaline phosphatase (ALP) activity, osteocalcin expression, calcium content, as well as the formation of dentin-like nodules. However, PDGF AB and BB stimulated expression of dentin sialoprotein (DSP) despite the lack of dentin-like nodules, whereas PDGF AA inhibited DSP. These diverse effects must be considered during odontoblast differentiation from pulp cells. Continuous exposure of genetically delivered PDGF-A has inhibitory effects on cementogenesis of immortalized cementoblasts implanted subcutaneously in severe combined immunodeficient (SCID) mice via upregulation of osteopontin and enhancement of multinucleated giant cells (MNGCs) [52]. In contrast, delivery of an antagonist to PDGF signaling inhibited mineralization of tissue-engineered cementum possibly due to the downregulation of bone sialoprotein and osteocalcin as well as stimulation of MNGCs. These results indicate that although PDGF-A delays mineral formation by cementoblasts, it may still be critically required for mineral neogenesis. Thus, controlled delivery for specific PDGF dimers will likely advance our knowledge of the temporal requirements for each molecule and

lead to the optimization of their pattern of expression in engineered tissues. Accordingly, PDGF-BB has been delivered to patients with severe periodontal osseous defects during reconstructive procedures using β -tricalcium phosphate (β TCP) constructs. Administration of recombinant human PDGF-BB demonstrated pronounced increased levels of vascular endothelial growth factor (VEGF) as well as bone turnover markers in the wound fluid at 3 weeks [53]. Although diffusive delivery of soaked PDGF in β TCP resulted in promising regenerative responses, more complex delivery systems are likely necessary for more rapid, improved and less inflammatory repair. Ceramic based materials such as tricalcium phosphate (TCP) are useful for bone tissue engineering, including alveolar bone, due to their structural similarity to the inorganic portion of bone. TCP is protein free and does not induce immunologic activity. In conjunction to 3-D printing technology, customized TCP constructs may be fabricated to have the pre-designed shape of osteogenic defects, and degrade by the action of osteoclasts or via chemical dissolution by interstitial fluids, thus desirable during natural remodeling of bone [54].

2.3. *Advanced delivery systems — Multiple factor and dose dependence*

The need for complex drug delivery systems has been previously addressed; however still remains in its infancy. For instance, the complex delivery of PDGF and VEGF has been implicated in the formation of angiogenic networks for vascular tissue engineering [55]. Drug delivery studies typically rely on the delivery of single factors, which may not suffice signaling requirements, given that the development of tissues and organs is typically driven by the action of multiple growth factors [55]. Here, two molecules involved during angiogenesis, VEGF and PDGF, were delivered with distinct release kinetics from composite biomaterials. VEGF is an early required molecule during endothelial cell differentiation, whereas PDGF influence is expected later during maturation of blood vessels, by recruiting mural cells including smooth muscle cells. Consequently, their distinct temporal dependence during angiogenesis call for complex drug delivery systems with distinct release profiles from a single composite engineered construct. In order to address this need, VEGF was simply mixed with particulate PLG for rapid release, and PDGF-BB was encapsulated in PLG microspheres for prolonged sustained release. The differential release profile of multiple bioactive molecules is an important concept in dental drug delivery, not only in angiogenesis but also for the orchestrated morphogenesis of DOC structures.

Applications for multiple signaling are illustrated by the differentiation of dental papillae cells using combinations of TGF β 1 and BMP-2 molecules [56]. TGF β 1, BMP-2 and the ethylenediaminetetraacetic acid (EDTA)-soluble fraction of dentin proteins from rabbit incisor teeth stimulated odontoblast differentiation of day-17 first lower mouse molar dental papillae cells. Multiple TGF- β isoforms have also been delivered concurrently using agarose beads as delivery vehicles. Growth factor loaded beads were placed over odontoblast cultures from

rat incisor teeth and stimulated predentine secretion [57,58]. Expression of the TGF β isoforms TGF β 1, TGF β 2 and TGF β 3, result in the sequestration of odontoblasts in the dentin matrix. Although TGF β 2 showed minimal effects, mitogenic effects on the cells of the subodontoblast layer were observed in proximity to the sites of TGF β 3 application. Additionally, tertiary dentine formation typically follows administration of BMP-7 when applied to dentine slices from monkey teeth [58].

In addition to complex delivery systems, growth factor release must account for the probable dose dependence of various cell types. For instance, 2 μ g of BMP-2 delivered in collagen sponges to amputated pulp in dogs show mineralized osteodentine-like tissue containing embedded osteodentocytes 70 days after implantation with only some unmineralized fibrous tissue and pulp-like loose connective tissue [36]. However, when lower doses (660 or 220 ng) of BMP-2 are applied, only unmineralized fibrous and pulp tissues were observed. Furthermore, little pulp tissue proliferation was observed with the administration of TGF- β 1, suggesting a possible inhibitory effect in pulp regeneration. Nevertheless, it is imperative to also note that when TGF- β 1 is implanted short term at mechanically exposed pulps of dog molar and canine teeth, differentiation of odontoblast-like cells and stimulation of primary odontoblasts is observed [59].

Given the apparent role of TGF- β 1 in pulp cell regulation, alginate gels have been used as delivery matrices in the dentin-pulp complex [60]. Alginates provide extracellular matrices with hydration properties that facilitate cellular wound healing activities. Alginate gels were fabricated using calcium chloride TGF- β 1 solutions. TGF- β 1 loaded gels were cultured on the pulpal cut surface of bisected human tooth slices showing two different forms of repair responses, reactionary dentinogenesis and reparative dentinogenesis. The former was represented by upregulation of matrix secretion by preexisting odontoblasts, and the latter induced de novo dentinogenesis along the cut pulpal surface. Moreover, a delivered TGF- β 1 dose response was observed revealing the intermediate dose of 50 ng as most efficient, yielding the largest predentin width. Similarly to other growth factors, TGF- β 1 has been delivered using many biocompatible materials, including chitosan/collagen scaffolds [61], chitosan microspheres [62], poly(D,L-lactic-co-glycolic acid) (PLGA) microspheres [63], tri-co-polymer scaffold with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) [64], injectable oligo(poly(ethylene glycol) fumarate) (OPF) and gelatin microparticles [65].

2.4. Cell–scaffold interactions

The choice of scaffolding material plays pivotal role in dental structure morphogenesis. For instance, dental pulp cells grown in collagenous, ceramic or metal (titanium) matrices, may develop differential expression of dental matrix markers such as dentin sialophosphoprotein (DSPP) [31]. These cells initially express STRO-1, a typical marker for multipotential cells such as bone marrow derived mesenchymal stem cells. Although many similarities were observed between the biomaterials used, after implantation, limited mineralization

was found only in the ceramic scaffold. It is postulated that in contrast with other studies, the open porosity of the ceramic HA/TCP sintered construct could have induced a proliferative response resulting in a DSPP positive yet connective tissue-like structure.

Other extracellular matrix (ECM) components such as fibronectin have been shown to mediate the binding of signaling molecules and regulate cell attachment, which aid in regulating the interactions between cells and ECM resulting in the reorganization of preodontoblasts cytoskeleton during pulpal wound healing [66,67]. Additionally, collagens also regulate preodontoblast rearrangement and aid in the attachment of new odontoblasts to pulp tissue [68]. Bone sialoprotein when used for the restoration of pulp defects appears to stimulate the differentiation of cells which secrete a homogeneous dentin-like deposit and organized extracellular matrix more efficiently than other capping materials such as calcium hydroxide, inducing the development of thick reparative dentinal tissue in the pulp [69]. Thus, the choice of drug delivery scaffolds and matrices must take into account the individual contribution of the biomaterials themselves during the regeneration process.

Recently, synthetic materials have been fabricated into nanometer scale structures in attempts to simulate the matrix environment in which seeded cells can be accommodated to proliferate and differentiate towards desired lineages [70–73]. Nanofibers formed by electrospinning have been shown to mimic the ECM environment to various degrees when cultured with several cell types [74–78]. Poly (D,L-lactide-co-glycolide) (PLGA) has been approved for several biomedical applications in humans and is widely used as scaffold materials in tissue engineering [79–81]. The porosity and tensile properties of electrospun PLGA nanofibers have been characterized [82]. Osteoblast adhesion has been shown to occur when seeded in PLGA nanofibers [83]. Poly(ϵ -caprolactone) (PLC) nanofibers accommodated the differentiation of hMSCs into adipogenic, osteogenic and chondrogenic cells [84]. We have recently demonstrated that human mesenchymal stem cells can proliferate and maintain differentiation characteristics towards osteoblasts and chondrocytes when seeded in PLGA nanofibers fabricated by electrospinning (Fig. 2C). PLGA of 85:15 ratio was electrospun into non-woven fibers with an average diameter of 760 ± 210 nm. The average Young's modulus of electrospun PLGA nanofibers was 42 ± 26 kPa, per nanoindentation with atomic force microscopy. Human MSCs (hMSCs) were seeded at a density of 2×10^6 cells/mL in PLGA nanofiber sheets. After 2 week culture on PLGA nanofiber scaffold, hMSCs remained as precursors upon immunoblotting with hKL12 antibody. The overwhelming majority of hMSCs was viable and proliferating in PLGA nanofiber scaffolds up to the tested 14 days, as assayed by live/dead tests, DNA content and BrdU. In a separate experiment, hMSCs seeded in PLGA nanofiber scaffolds were differentiated into chondrogenic (hMSC-Ch) and osteogenic (hMSC-Ob) cells. SEM taken up to 7 days after cell seeding revealed that hMSCs, hMSC-Ob and hMSC-Ch apparently attached to PLGA nanofibers. Histological assays revealed that hMSCs continuously differentiated into chondrogenic cells and osteogenic cells after 2 week incubation

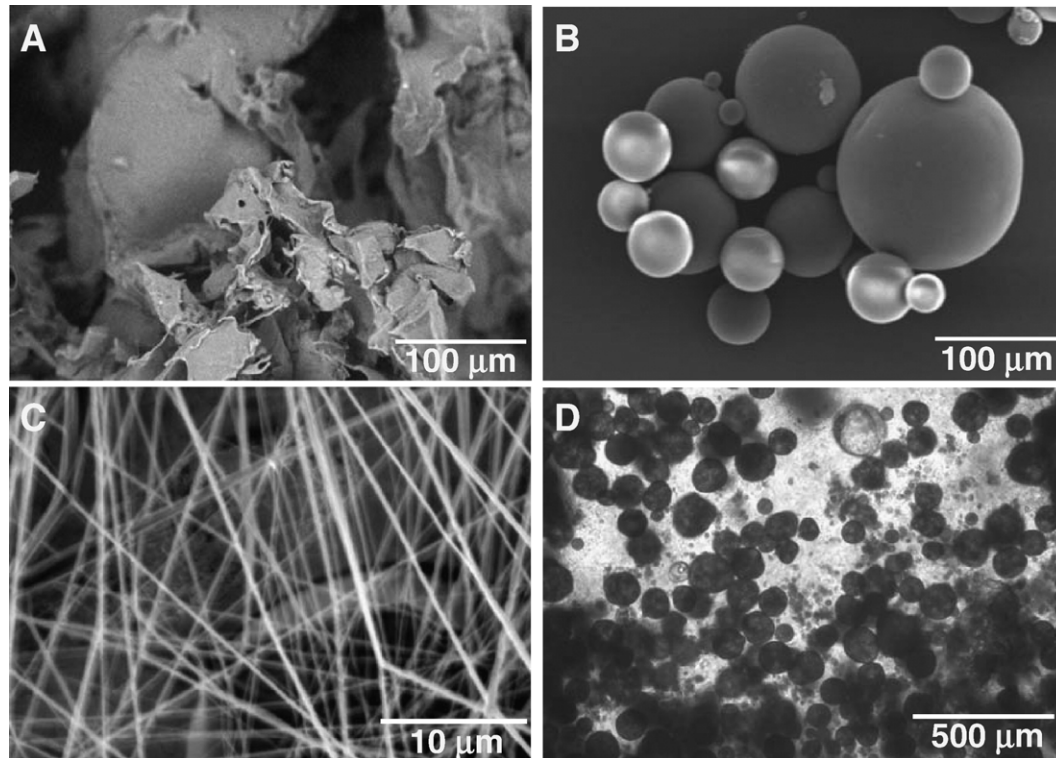


Fig. 2. Various forms of poly-lactic-co-glycolic acid (PLGA) based matrices and scaffolds for dental, oral and craniofacial tissue engineering. A. Porous PLGA sponge fabricated using salt-leaching techniques. B. PLGA microspheres encapsulating growth factors showing smooth spherical surface and wide range of diameters. C. PLGA nanofibers fabricated using electrospinning techniques. D. PLGA microspheres in chitosan-based gels for advanced controlled delivery and cell interaction. A,B,C: scanning electron microscopy (SEM). D: phase contrast image.

on PLGA nanofibers. Taken together, these data represent an original investigation of continuous differentiation of hMSCs into chondrogenic and osteogenic cells in PLGA nanofiber scaffolds.

2.5. Periodontal regeneration

The periodontium consists of cementum, periodontal ligament (PDL), and alveolar bone and serves to transmit mechanical forces and as supporting structure and anchorage for the teeth into the mandible and maxilla. Periodontal disease is characterized by inflammation of periodontal tissues, eventually leading to degeneration of the periodontium [85]. If periodontal disease is left untreated, tooth loss can occur [86]. Periodontal tissue engineering should address the restoration of the PDL along with its orientation and fibrous infiltration into exposed tooth roots and surrounding bone, as well as regeneration of cementum by cementoblasts adjacent to the root, and restoration of the alveolar bone [87]. Previous matrices utilized in regeneration of periodontal structures include bone autografts, allografts, cell occlusive barrier membranes, bioglass, beta-tricalcium phosphate (β TCP), polytetrafluorethylene, poly lactic-co-glycolic acid (PLGA), collagen membranes, and enamel matrix derivatives [88–96]. Undoubtedly, the incorporation of bioactive molecules in successful matrix materials may enhance their varied regenerative capacities. Thus, the effects of many growth factors on periodontal tissue cells must be

evaluated for their implication in periodontal tissue engineering using drug delivery technologies.

When choosing a scaffold matrix material for periodontal regeneration and drug delivery, careful consideration of the natural ECM in the periodontal connective tissues may elucidate some candidates. Collagens, proteoglycans, as well as non-collagenous glycoproteins are typically found in the periodontium, and some have been reviewed in the perspective of dental regeneration. For instance, powerful morphogens such as BMP-2 bind to heparin, heparin sulfate, types I and IV collagens, type II procollagen, fibrillins, proteoglycans, and noggin, chordin, and DAN decreasing the rate of proteolysis [97,98]. In the context of mineralized tissue regeneration such as alveolar bone and cementum, hydroxyapatite is a natural contender for a delivery vehicle due to its affinity for proteins, and in fact, when compared to collagens, TCP, glass beads, polymethylmethacrylate, and a bone cement, hydroxyapatite was the most suitable for bone induction along with insoluble collagens.

The initiation of adequate periodontal regeneration may be assisted by an array of growth factors such as platelet-derived growth factor (PDGF), transforming growth factor- β s (TGF β s) and insulin-like growth factor-1 (IGF-1) [86,99,100]. PDGF receptors α and β are expressed in regenerating periodontal soft and hard tissues [101]. PDGF may be used as a mitogen for fibroblasts and progenitors during periodontal regeneration and has been shown to promote matrix synthesis and cell attachment

to tooth dentinal surfaces [86,102–104]. Subsequently, fibroblast growth factor-2 (FGF2) and TGF β contribute to the further proliferation of local cells. Previous reports on the effects of basic fibroblast growth factor on human gingival epithelial cells as well as during wound healing and periodontal regeneration of alveolar bone defects, promoted supplementary work experiments for FGF-2 delivery in periodontal repair. FGF-2 delivered topically with gelatinous carriers in furcation defects in dogs induced increased new bone formation rate, new trabecular bone formation rate, as well as new cementum formation rate [105–107]. Additionally, IGF-1 has been previously demonstrated to improve periodontal regeneration using controlled delivery approaches. IGF-1 was encapsulated in dextran-co-gelatin hydrogel microspheres by polyionic complexation and delivered to Class III furcation defects in molars of dogs for controlled release. Adequate width of regenerative PDL, regular Sharpey's fibers, and alveolar bone reconstruction was observed after 4–8 weeks [108].

During alveolar bone regeneration, bone morphogenetic proteins (BMPs) are expressed and may be responsible for the osteogenic differentiation of migrated progenitors [109]. BMPs are typically effective osteogenic molecules that may induce ectopic bone formation [110]. Previous studies have demonstrated that BMP-12 loaded in collagen sponges improved periodontal ligament regeneration [111]. Although BMP-2 was more efficient for bone regeneration, application of BMP-12 showed functionally oriented PDL bridging new bone and cementum. Additionally BMP-7, has been shown to induce bone regeneration adjacent to endosseous dental implants, as well as maxillary sinus floor augmentation [112–114]. Interestingly, co-application of BMP-2 and BMP-7 for the regeneration of furcation defects in the mandibular molars of baboons, does not enhance alveolar regeneration when compared to the morphogens administered alone [115]. BMP-2 alone showed greater mineral bone and osteoid formation and remodeling, whereas administration of BMP-7 alone yielded significant cementogenesis. Delivery of BMP-2 from slow dissolving collagen membranes demonstrate improved cementogenesis over faster collagen gel delivery [116,117]. The slowed diffusion rate of the morphogen may play a role in localizing and maximizing the effects of released BMP-2, resulting in more adequate cementum formation.

3. Cranial sutures

Cranial sutures are the soft tissue interface between mineralized calvarial bones and are the immobile joints of the skull. The suture proper and sutural margins consist of multiple cell lineages, which may include fibroblast-like cells and matrices sandwiched between osteoblast-lined bone formation fronts [118,119]. During skull development, calvarial growth occurs at the cranial sutures, however they are kept “open” and unossified for continuous growth. At skeletal maturity, these joints fuse and osteogenic growth ceases. The intracranial layer that overlies the brain, the dura mater, serves as the main regulator of cranial suture function [120,121]. Cranial sutures express very intricate patterns of molecules in a highly regulated

temporospatial manner. During suture patency, cranial sutures express high levels of TGF- β 3, especially along the edges of the bone fronts [122]. As the skull reaches maturity, TGF- β 3 expression is decreased, and TGF- β 2 becomes highly expressed along the osteogenic fronts. When this signaling balance becomes chronologically erroneous, premature osseous fusion may occur, causing a condition called craniosynostosis. One in every ~2500 human newborns suffers from craniosynostosis. Affected children may develop visible craniofacial disfigurements, increased intracranial pressure and severe neurological disorders such as mental retardation, blindness and seizure. Our understanding of the normal molecular events during cranial morphogenesis however, may allow the regulation of sutural function by controlled delivery of TGF- β s. As described for various molecules involved in dental regeneration, controlled release of cytokines involved in craniofacial development include lipid nanoparticles, chitosan or gelatin-based particles, collagen, ceramics, and porous glass [123]. Given the long-term need for cranial suture regulation throughout the development of the skull in early childhood, poly-lactic-co-glycolic acid (PLGA) microspheres offer great advantage over rapid release systems, due to their potential for prolonged release as well as injectable dimensions.

Synthetic polyesters such as PLGA and polycaprolactone (PCL) have been extensively used in several tissue engineering models including craniofacial repair [124–127]. PLGA is a prime candidate for use in regenerative medicine and dentistry due to its biocompatibility, controlled structural and mechanical properties, tailored degradation rates, and the potential as growth factor delivery vehicles. Poly-lactic acid (PLA), poly-glycolic (PGA), and their copolymers (PLGA), are principal tools for scaffold biomaterial fabrication and drug delivery. PLGA may be fabricated in various forms, including sheets, blocks, microspheres, and nanofibers with designed architecture and degradation rates (Fig. 2). PLGA is degraded by hydrolysis and its byproducts are safely eliminated in the body by the Krebs's cycle, causing minimal inflammatory responses. PLGA is also combined with other polymers to form composite materials of optimized properties, such as polyethylene glycol (PEG) — PLGA composites. Composite polymers may improve mechanical strength, further control degradation rates, and stimulate cell growth. Many growth factors and hormones such as BMPs, TGF β s, FGFs, and insulin have been encapsulated in PLGA microspheres for controlled delivery [123]. The work of the authors have involved the microencapsulation and sustained release of TGF- β 3 from PLGA microspheres, as well as microspheres embedded in thermosensitive hydrogels (Fig. 2B,D). Sustained release of TGF- β 3 was observed up to 36 days, at which point approximately 35% of encapsulated growth factor had been released in a controlled fashion [123]. The fabricated controlled delivery system for TGF- β 3 was implemented in a tissue engineered cranial suture rodent model, similar to the corrective surgical procedure in human craniosynostosis patients. Here, the osteogenically fused posterior interfrontal suture of rats was surgically removed to release any bony contact between the frontal bones creating a gap of empirical size between them, simulating the gaps created

in craniostyostosis procedures. However, in the tissue engineering rat model, instead of leaving an empty gap as in present conventional approaches, an implant designed for preventing re-ossification was locally delivered. The implant consisted of autologous bone marrow derived stem cells and TGF- β 3 encapsulated in PLGA microspheres in a pre-sized collagen sponge carrier (Fig. 3A). Results indicate a decrease in bone deposition and the formation of a bone–fibrous tissue–bone interface analogous to normal cranial sutures after implantation of TGF- β 3 loaded constructs (Fig. 3C). Placebo treated cranial defects resulted in progressive bone healing after 4 weeks (Fig. 3B). Although this approach may need further investigations to achieve more controlled regulation of new tissue formation, it is the first attempt to develop technologies for a tissue-engineered implant in craniostyostosis treatments. The continuous release of TGF- β 3 may replicate the correct signaling pathways that occur during normal cranial morpho-

genesis offsetting the molecular mishaps of craniostyostosis (e.g. untimely downregulation of TGF- β 3 and upregulation of TGF- β 2). These findings demonstrate a regenerated cranial suture from autologous stem cells, and replacement of a synostosed cranial suture in an *in vivo* model. A biologically derived cranial suture may prove to reduce surgical trauma from craniotomy in craniostyostosis patients to localized osteotomy. Additionally, a biologically derived cranial suture may accommodate subsequent craniofacial growth, in contrast to clinically observed secondary synostosis after the resection of synostosed cranial sutures in some patients.

4. Temporomandibular joints (TMJ)

Disorders of the temporomandibular joint (TMJ) affect over 10 million Americans by some estimates (NIH, 2006), with approximately 3–4% of the population seeking treatment [128]. The TMJ connects the mandible to the temporal bone of the skull. As a diarthrotic joint, layers of cartilage are present on both the condyle of the mandible and the glenoid fossa and articular eminence of the temporal bone [128]. The TMJ disc is a fibrocartilaginous disc that sits between the two cartilage layers, connected to the joint capsule around its periphery near its attachment to the condylar neck [128].

Many skeletal and cartilage factors have been implicated in the growth of the TMJ cartilage layers and fibrocartilage disk. Effects on cells isolated from the TMJ disk have been documented for PDGF, IGF-1, bFGF, and TGF β 1 [128–133]. Each growth factor displayed distinctive effects on cellular proliferation and extracellular matrix production, suggesting that a combination cocktail of growth factors would be more beneficial [129]. In mouse explants of the mandibular condyle, the growth factors TGF β 1, IGF-1, and growth hormone stimulated proteoglycan synthesis in cartilage layers, while interleukin-1 α (IL-1 α) exerted an inhibitory effect [134]. Using a collagen carrier, BMP-2 was demonstrated to increase cartilage regeneration in internally deranged rabbit TMJ [135].

Efforts are currently underway in tissue engineering the different structures of the TMJ. Toward engineering a TMJ disc, TMJ disc cells were isolated and the growth factors necessary to maintain the cells *in vitro* were established. The TMJ disc cells were seeded onto non-woven poly-glycolic acid (PGA) scaffolds and could be cultured up to 6 weeks [136]. Use of the growth factors bFGF, IGF-1, and TGF β 1 either alone [136] or in combination [129] modulated cellular proliferation and matrix biosynthesis. The use of a rotating bioreactor for TMJ disc culture was also explored, with no clear beneficial results [133]. In these studies, the need for high levels of growth factors (100 ng/mL for IGF-1 and bFGF and 30 ng/mL TGF β 1) suggests that a controlled drug delivery system may potentially be advantageous, especially for maintenance of the disc when implanted *in vivo*. Tissue engineering of the condyle portion of the TMJ requires the fabrication of an osteochondral construct containing both a cartilage layer and underlying subchondral bone. Toward this end, the large amount of work into osteochondral constructs for appendicular skeletal joints can

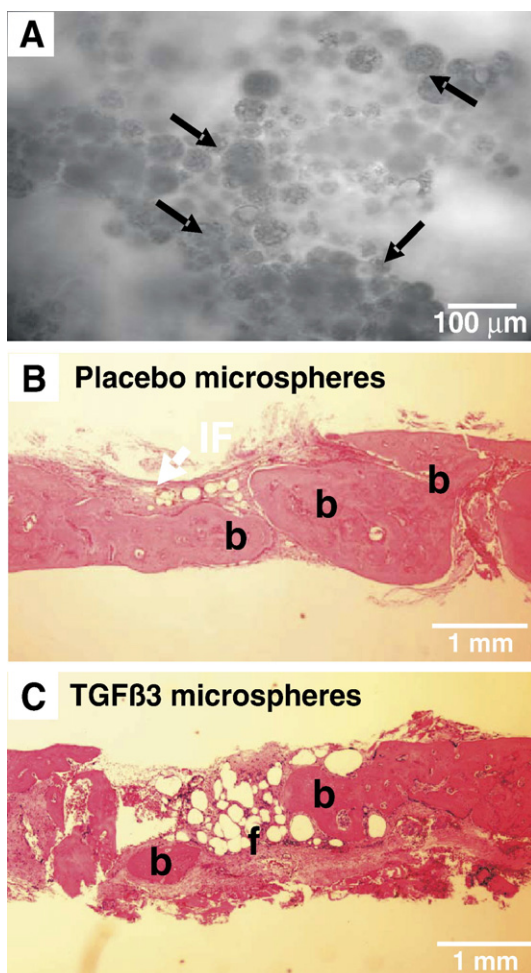


Fig. 3. Engineered cranial suture implants. A. TGF- β 3 loaded poly-lactic-co-glycolic acid (PLGA) microspheres (arrows) in gelatin sponge as a pre-sized engineered implant. B. Osteogenic healing of osteotomized region of the fused rat cranial suture after implantation of placebo loaded collagen sponge implants (4 weeks). C. Formation of a bone–fibrous tissue–bone interface and regulation of osteogenesis in the osteotomized region of the fused rat cranial suture after implantation of TGF- β 3 treated engineered cranial suture implants (4 weeks). H&E stain. b: bone, f: fibrous tissue.

be directly applied to the articular condyle of the TMJ [137–142]. Specifically for the TMJ, polylactic/polyglycolic copolymer was used in a gelatin sponge complex (PGS) as a carrier for BMP-2 and demonstrated increased bone and cartilage-like tissue regeneration in rabbit after condylectomy [143]. Interestingly, no significant increase in growth was realized with addition of BMP-2, attributed to the osteoinductive capabilities of the PGS scaffold. Computational design and solid freeform fabrication allows layer-by-layer fabrication of complex scaffolds with defined architecture, and can even be matched to patient-specific computed tomography (CT) or magnetic resonance imaging (MRI) scans. This technology allows production of biphasic osteochondral constructs in which the subchondral portion incorporates internal architecture and biological factors ideal for bone ingrowth and the upper layer incorporates cartilage-specific parameters [144]. Non-invasive injectable systems for drug and/or cell delivery hold promise for treatment of cartilage defects, and have been accomplished using PLGA microspheres [145], thermo-reversible hydrogels [146,147], oligo(poly(ethylene glycol) fumarate) (OPF) [65,148], and alginate [149]. Similar approaches to TMJ repair may prove beneficial to regeneration of cartilage without surgery.

The authors have demonstrated the feasibility of tissue engineering a synovial joint condyle in the shape of the TMJ using hydrogel matrix loaded with both cartilaginous and osseous components from a single population of rat bone marrow-derived mesenchymal stem cells (rMSCs). Rat MSCs were isolated from femur and tibia bone marrow of adult rats and exposed separately to either chondrogenic or osteogenic supplements [3,150]. Poly(ethylene glycol) diacrylate (PEGDA) was dissolved in PBS with a biocompatible ultraviolet photoinitiator [3,150]. MSC-derived chondrogenic and osteogenic cells were suspended in PEGDA polymer/photoinitiator solution and loaded into a negative mold of a cadaver adult human mandibular condyle in two stratified and yet integrated layers. The photopolymerized osteochondral construct was removed from the mold and implanted in the dorsum of severe combined immunodeficient (SCID) mice (4–5 week old males). At both 4 and 8 weeks *in vivo* implantation, the tissue-engineered synovial joints retained the macroscopic shape and dimensions of the cell-hydrogel construct. Importantly, the constructs retained both chondrogenic and osseous layers, expressing cartilage-specific glycosaminoglycans (GAGs) in the upper chondrogenic layer and bony mineral nodules revealed by von Kossa staining in the lower osseous layer [3,150]. Using an identical approach but increasing the cell-seeding density 4-fold, further tissue maturation of both the chondrogenic and osseous layers as well as mutual infiltration forming a primitive osteochondral interface was achieved after 12-week subcutaneous implantation in SCID mice [4]. Improvements to the tissue-engineered TMJ condyle are warranted along multiple fronts including structural integrity, tissue maturation, mechanical strength, and host integration [151], and the aforementioned approaches of drug delivery from hydrogels will likely be critical in this process.

5. Drug delivery in implant dentistry

Despite the great advances in completely tissue engineered oral structures, the current gold standard for load bearing craniofacial replacements remains titanium implants. Since the initial studies establishing its use in dentistry [152], titanium's strength, durability, and biocompatibility has led to innumerable applications, including single tooth replacement to complete bridges in the mandible and maxilla and even for craniofacial reconstructions. Titanium has high success rates of initial anchorage (over 90%) and has been shown to co-exist with host bone for the life of the patient [153], but failures still occur during both initial bone integration and long-term fixation.

Critical for initial and long-term efficacy of titanium implants is establishment of adequate bone fixation. This process resembles natural fracture repair and requires the cooperation of multiple cell and tissue types resulting in bone ingrowth into the irregular surfaces of the titanium and eventually direct contact between bone and titanium (osseointegration) [152,154]. During the initial inflammatory phase, disrupted bone matrix and inflammatory cells release over 100 biologically active substances, including the skeletal growth factors platelet-derived growth factor (PDGF), transforming growth factor β (TGF β), fibroblast growth factor (FGF), and insulin-like growth factor (IGF) [155]. This initial phase is critical to set in motion the complex processes to follow, as the administration of anti-inflammatory drugs can significantly decrease bone ingrowth [156,157]. During the repair phase, proliferation and deposition of extracellular matrix by immature bone progenitors as well as establishment of a new vascular bed via angiogenesis is induced by growth factors such as TGF β , bFGF, PDGF, and vascular endothelial growth factor (VEGF). The mineralization and maturation phase is the final phase of fracture repair in which differentiation factors such as TGF β and bone morphogenetic protein 2 (BMP2) are elevated and critical extracellular matrix components such as osteocalcin and osteopontin begin to be expressed. The spatial and temporal profiles of numerous growth factors have been studied for fracture repair [158,159], and some of these growth factors such as TGF β , PDGF, and BMP have been demonstrated in oral implant sites [160,161].

Because of its similarities to fracture repair, titanium dental implant osseointegration could potentially be improved by delivery of the same growth factors. Although not a defined cocktail, platelet rich plasma contains a multitude of growth factors expressed during the inflammation phase, and has been used to increase the volume of peri-implant bone in rat tibiae [162] and increase bone ingrowth when delivered to peri-implant defects via a demineralized freeze dried bone carrier [163]. In applications where there are existing gaps, such as craniofacial reconstruction or augmentation of bone at implant sites or peri-implant defects, increased regeneration of bone, often at comparable levels to bone grafts, has been accomplished with delivery of TGF β and BMP-2 via various scaffolds including absorbable collagen sponge [164], 25% pluronic gel [165], refined collagen gelatin (Gelfoam) [166,167], titanium mesh [168,169], and beta tricalcium phosphate (β -TCP) [166].

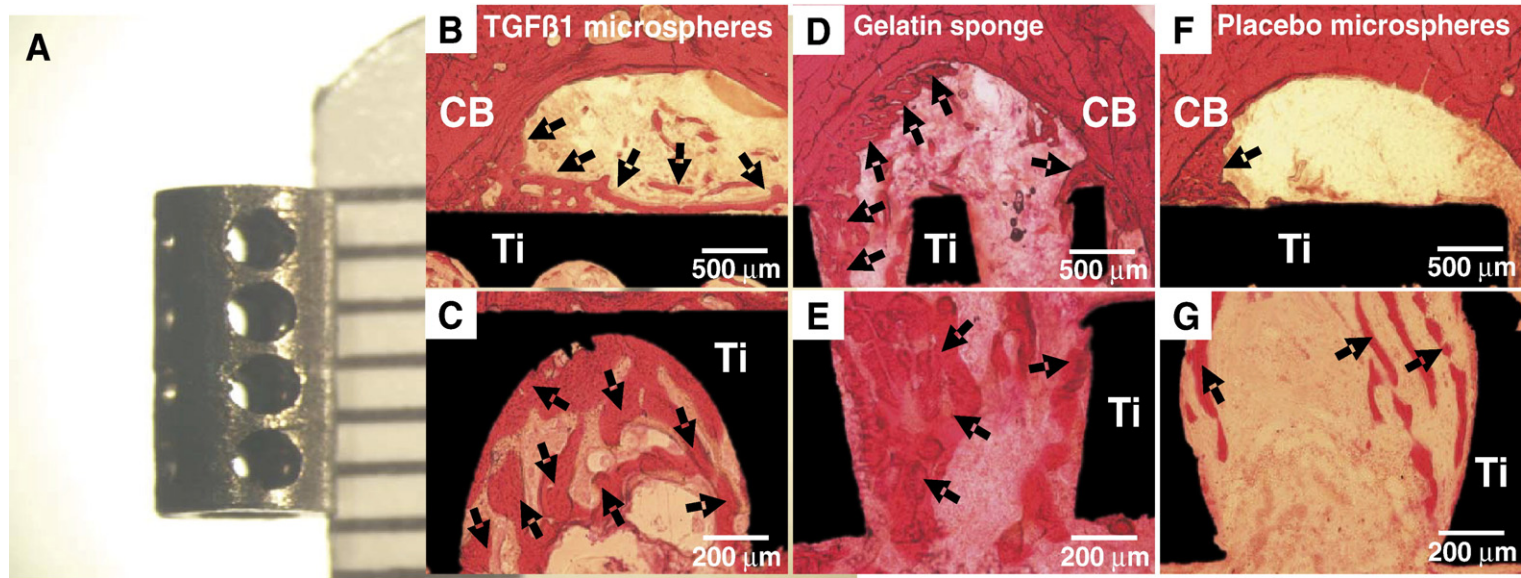


Fig. 4. Controlled release of TGF- β 1 from poly-lactic-co-glycolic acid (PLGA) microspheres increases bone ingrowth into hollow titanium implants. A. Hollow titanium implants with 1 mm macropores were custom fabricated. To compare outcome to a rapid release system, 1 μ g of TGF β 1 was also adsorbed to gelatin sponge carrier (D and E). After 4 weeks of implantation in rabbit humeri, delivery of TGF- β 1 led to increases in bone-to-implant contact (BIC) and bone volume within 1 mm macropores (BV/TV) (B–E) as compared to placebo control spheres (F and G). Approximately 10 times more TGF β 1 was required for rapid release from gelatin sponge (D and E) to obtain comparable results to controlled release using PLGA microspheres (B and C). H&E stain. CB: cortical bone, Ti: titanium implant.

However, minimizing gaps between implant and host bone is necessary, thus the application of bulky scaffolds is usually not possible and alternative delivery approaches are required. Adsorption of TGF β and/or BMP to tricalcium phosphate or hydroxyapatite coatings on titanium implants leads to enhancement of bone ingrowth in gap models [170–173]. However, adsorbed growth factor in these systems is rapidly exhausted whereas levels of TGF β and BMP-2 remain elevated throughout the fracture repair process [158]. More complex methods of growth factor adhesion to surface coatings have been explored, including fabrication of ultrahydrophilic titanium surfaces [174] and incorporation of BMP-2 directly into calcium phosphate coatings [175], showing promising results.

To mimic the temporal profile of growth factors, controlled release technology has been explored for titanium implants. Titanium and steel wires were coated with poly(D,L-lactide) incorporating TGF β or IGF-1 and demonstrated continuous release of the growth factors up to 42 days *in vitro* and *in vivo* [176]. The authors have fabricated hollow titanium implants with 1 mm macropores; these hollow implants maintain the biomechanical strength required for oral load-bearing applications but provide space for tissue engineered scaffolds delivering bioactive molecules or cells (Fig. 4A). A controlled release system for TGF β 1 using PLGA microspheres has also been developed by the authors, as well as other groups [63], that releases the growth factor in a controlled fashion up to 4 weeks. With a diameter averaging 64 μ m, the PLGA microspheres could be injected into a gelatin carrier (Gelfoam) and subsequently packed into the hollow core of the titanium implant. The hollow implants were placed uncortically into the humeri of adult New Zealand white rabbits and allowed to osseointegrate for 4 weeks. Incorporation of TGF β 1 in the hollow implant led to significantly increased osseointegration parameters such as bone-to-implant contact (BIC) and bone volume within the 1 mm macropores (Fig. 4B–E) as compared to placebo controls (Fig. 4F,G). Adsorption of TGF β 1 to gelatin sponge and controlled release via PLGA microspheres resulted in comparable outcomes, but 10 \times more TGF β 1 was required when delivered by gelatin sponge. The use of controlled release of growth factors by way of hollow implants is a novel approach to enhancing osseointegration and may be useful in implant dentistry, in which hollow implants have been in clinical use for years [177,178]. More advanced biomaterials, such as microporous titanium [179], may also potentially be used as high strength carriers.

6. Conclusions

Previous work has explored the application of biomaterial matrices and drug delivery in the regeneration and/or de novo formation of DOC structures. A wide range of biomaterials including polymeric hydrogels, porous scaffolds, nanofibers and microparticles have been utilized toward the regeneration of tooth, periodontal ligament, salivary gland, cranial sutures, and the temporomandibular joint (TMJ). Although the goal of eventually regenerating a whole tooth is admirable, whole tooth regeneration is likely one of the last technologies to be commercially realized. The

more near-term technologies include the regeneration of individual tooth structures, osteochondral grafts for the TMJ, cranial sutures and bone regeneration for periodontal defects. Integrated approaches that capture the fundamental knowledge in multiple biology and engineering disciplines are not only desirable, but necessary for proper regeneration of DOC structures. Specific structural and mechanical characteristics as outlined in the Introduction of this review, need to be considered in the design of tissue engineering or tissue regeneration approaches. It is predictable that successful approaches need to exert not only more control at micro-scale and nano-scale levels, but also rigorously test increasingly refined engineering techniques in animal models towards the regeneration of DOC structures.

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