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Leveraging Advancements in Tissue Engineering for Bioprinting Dental Tissues

Devin Grace Morrison
Thomas Jefferson University

Ryan E. Tomlinson
Thomas Jefferson University

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Devin Grace Morrison[1], Ryan E. Tomlinson[1]

[1] Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, Pennsylvania, USA

*** Correspondence should be addressed to:**
Ryan E. Tomlinson
Department of Orthopaedic Surgery
1015 Walnut Street
Curtis Building, Suite 504
Philadelphia, PA 19107
ryan.tomlinson@jefferson.edu

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ABSTRACT

3D bioprinting allows biocompatible materials and cells to be deposited in precise locations in three-dimensional space, enabling researchers to surpass the limitations of traditional 2D cell culture and to create innovative therapies. 3D bioprinting is one of the newest tools developed in the field of tissue engineering, which has traditionally utilized a paradigm revolving around scaffolds, cells, and signals. In this review, we discuss how new developments in each of these three research areas relates to bioprinting dental tissues – specifically teeth, periodontal ligament, and alveolar bone. Important considerations include how scaffold materials and geometry affect regeneration of dental tissues, the importance of using dental cells in these applications, and the role of signaling molecules for creating a clinically relevant bioengineered dental implant. We conclude with potential new directions for research that would allow the burgeoning field of regenerative dentistry to achieve its lofty goals.

1 INTRODUCTION

The loss of a permanent tooth is the most common organ failure [1]. Untreated tooth loss decreases self-esteem and quality of life by impeding vital functions, including mastication and enunciation [1,2]. For some time, dental implants have been the gold standard for treatment of non-restorable teeth in both fully and partially edentulous patients. In fact, approximately 5 million dental implants are placed in the United States each year, and as of 2015 this figure was projected to grow by 12% to 15% annually [3]. Although a recent meta-analysis noted 5-year failure rates of 4.4% and 10-year failure rates of 6.9%, surprisingly little has changed regarding dental implantation since the procedure itself was first introduced in the late 1960s [4].

In contrast to the titanium alloy used to fabricate most dental implants, the natural tooth is a complex organ with multiple distinct internal components. Furthermore, its relationship to the surrounding periodontal ligament and alveolar bone is critical to its function. In fact, the tooth root and the periodontium function as a single unit, despite being separate anatomic bodies [5]. When a tooth is lost, whether by disease, defect, or injury, the supporting alveolar bone and soft tissue lose height and thickness due to atrophy and resorption [6]. During the time that a tooth is missing, these effects of disuse continuously alter the alveolar ridge, making implant placement highly specific to the patient and his/her circumstances [6]. As such, the entire functional unit must be considered when exploring new technologies for tooth replacement. 3D bioprinting has been considered a potential tissue engineering strategy to address this complexity. Using this technology allows for the deposition of biocompatible materials and cells in precise locations in three-dimensional space, enabling the design of complex constructs made from materials optimized for specific applications. Bioprinting dental and periodontal tissues is an extremely active area of research with the potential to create the first major paradigm shift in dental restoration since implantation took off nearly 60 years ago. As the technology develops, bioprinting has the potential to produce dental implants that possess fewer points of failure, provide benefits such as osseoperception [7], and reduce the risk of infection by providing an innate immune response. Additionally, bioprinting may eventually be used to create not only implants, but patient-specific constructs to restore alveolar bone and other support tissues in the same procedure. Currently, patients who have insufficient alveolar bone to support a dental implant must undergo alveolar bone augmentation before an implant can be placed. Reducing the number of procedures that are required for tooth replacement lowers the risk for patients and saves time for oral surgeons.

Although the distinction between 3D printing and bioprinting appears to blur frequently in the literature, this review will primarily focus on bioprinting technologies. We define bioprinting, in contrast to 3D printing, as a process that incorporates living cells into the scaffold material before or during the printing process. A number of different techniques are available for bioprinting applications, including extrusion, inkjet/droplet, laser-assisted bioprinting, with new technology becoming available each year [8–10]. Nonetheless, extrusion bioprinting, in which pressure is applied to push a biomaterial through a needle or nozzle, remains the simplest, most accessible, and most popular bioprinting method for research and translation towards regenerative dentistry [8–10]. The process is identical to commonly used fused deposition modeling (FDM) 3D printers with the exception of the mechanisms that control material temperature and propel materials through the nozzle. Furthermore, materials used for extrusion bioprinting must generally have low viscosity, such as hydrogels, and/or display significant shear thinning such that they can be extruded either pneumatically or mechanically from a syringe.

While bioprinting may not entirely replace the standard techniques currently used in tissue engineering, it does provide new utility for combining scaffold materials as well as investigating biological factors. These changes allow previous research to be reimagined with a fresh perspective and enable development of entirely new approaches. To illustrate the potential bioprinting represents as an evolution of the traditional paradigm of scaffolds, cells, and signals, we have organized this review to briefly describe bioprinting research that has leveraged advances in these three arenas with a specific focus on the generation of dental and periodontal tissues.

2 SCAFFOLDS

A tissue engineered scaffold is generally designed to simulate the extracellular matrix of the desired tissue in order to harness the impact of the ECM on adhesion, migration, proliferation, and differentiation of resident cells [8]. Early efforts to regenerate dental tissues and whole teeth without bioprinting used simple scaffolds such as molded collagen gels [11–13]. 3D printing and bioprinting technologies have ushered in a new era of scaffold generation by allowing the construction of ECM that mimics the complexity of the native tissue with vastly improved spatial resolution. Successful bioprinting of dental tissues will require consideration of both the material choice as well as the spatial organization of the scaffold.

2.1 Materials

The tooth is responsible for applying occlusal forces in complex patterns and is constantly exposed to a challenging microenvironment, so tissue engineering efforts must use materials with the appropriate material and chemical properties. In the native tooth, this challenge is principally met by the combination of two mineralized tissues: dentin, which provides strength and toughness, and its overlaying enamel, which is hard and resistant to both fracture and wear [14]. In contrast, the titanium alloys used to fabricate dental implants far exceed the strength and stiffness of native dentin [15]. As a result, a significant amount of alveolar bone is resorbed following placement of an implant, regardless of the timing of loading [16,17]. In contrast, bioprinted constructs that closely match the material properties of native teeth may reduce alveolar bone resorption, improve osseointegration, and reduce implant failure. Recent bioprinting efforts have utilized naturally rigid biomaterials as well as soft biomaterials that can be stiffened before use to meet this clinical need and engineering challenge.

2.1.1 Rigid Biomaterials

Rigid synthetic polymers, ceramics, composites, and even metals have been utilized as scaffolds for dental and periodontal tissue generation [1,8,9]. Although rigid biomaterials offer significant advantages for generating load-bearing tissues, cells usually cannot be added during scaffold fabrication because the conditions required to manipulate these materials can be cytotoxic. As a result, most of these efforts are 3D printing for biological applications, rather than bioprinting as defined above. Nonetheless, polycaprolactone (PCL) is the most common rigid biomaterial used to generate mineralized engineered constructs. This popularity is due to its versatility and favorable material properties as well as FDA approval for PCL-based sutures and drug delivery devices [1,8]. Furthermore, PCL can be 3D printed (FDM) or electrospun and can also serve as a matrix for composite materials containing inorganic minerals such as hydroxyapatite (HA) or tricalcium phosphate (TCP) [1]. Two PCL composites investigated for use in 3D printing periodontal tissues are PCL/ß-TCP (80:20 wt%) and PCL/HA (90:10 wt%) [5,18]. Another synthetic polymer,

poly(lactide-co-glycolide) acid or PLGA, has been used for tissue engineering scaffolds, but this polymer degrades more quickly than PCL and, as a result, is less popular for dental applications [19].

In addition to synthetic polymers, ceramics and metals have also been investigated for use in dental applications. In particular, ceramics degrade slowly and create an ion-rich environment within the scaffold that encourages osteogenesis, making them promising materials for regenerative dentistry. Unfortunately, ceramics are also generally brittle and not ideal for load-bearing sites [9]. Nonetheless, granules of bioglass, a bioceramic consisting of amorphous silicate compounds, implanted into tooth extraction sockets consistently incorporated into newly formed bone, whereas tissue that formed around particles of synthetic and natural hydroxyapatite varied unpredictably between bone and fibrous connective tissue [20]. Of course, metals are used for dental implants, but some researchers have suggested that inert metal could be replaced with a novel biodegradable metal alloy that is able to integrate with bone [8]. In particular, magnesium alloys are mechanically similar to bone and can degrade in aqueous environments [8].

2.1.2 Soft Biomaterials

Hydrogels are highly adaptable and customizable soft biomaterials created by linking hydrophilic polymers with a cross-linking agent [8,9]. Both natural and synthetic polymers have been used to create hydrogels for dental tissue engineering [1,8,9,21]. Natural polymers include polysaccharides, such as alginate, agarose, hyaluronic acid, chitosan, gellan gum, and dextran, as well as proteins, such as collagen, gelatin, fibrin, and silk [1,8,10]. Some synthetic polymers have also been used, including polyethylene glycol (PEG) and PEG diacrylate (PEGDA) [1]. While hydrogels are ideal for extrusion bioprinting and excellent substrates for 3D cell culture, their lack of mechanical strength is a significant drawback. Indeed, hydrogel stiffness is generally several orders of magnitude below that of load-bearing craniofacial tissues [9]. Nonetheless, some approaches to make use of the advantages of these biomaterials for bioprinting dental tissues have emerged.

A common strategy is permitting cells seeded into a hydrogel to differentiate and/or proliferate *in vitro* prior to *in vivo* utilization. For example, whole-tooth regeneration was attempted by positioning embryonic epithelial and mesenchymal cells adjacent to each other at high cell densities (5×10^8 cells/mL) in a type 1 collagen gel drop [12,22]. These seeded collagen drops were cultured *in vitro* as well as in subrenal capsules to allow the cells to self-organize prior to implantation in a tooth extraction socket [12,22]. While a scaffold bioprinted entirely from a hydrogel may not be able to maintain its shape or provide structural support, the primary function of a scaffold is to simulate the native ECM. Indeed, collagen is a component of the dentin matrix and induces differentiation, organization, and adhesion of cells, thus choreographing the formation of properly organized dental tissues [23]. As a result, these early studies demonstrate that a drop of collagen-based hydrogel provides an inductive, three-dimensional environment for cells; with bioprinting, the advantages of the microenvironment can be applied to more complex forms.

Along these lines, recent work has attempted to recreate the native dental ECM microenvironment using bioprinting. Here, advanced hydrogel bioinks consisting of alginate and insoluble dentin matrix proteins (primarily type 1 collagen) are utilized [21]. In bioinks containing 1:1 and 1:2 ratios of alginate-to-dentin matrix proteins, the survival of mouse odontoblast-like cells (OD-21) after five days was improved by 25% when compared to a pure alginate bioink [21]. Similarly,

when human stem cells of the apical papilla (SCAP) were bioprinted in bioink containing 1:1 ratio of alginate-to-dentin matrix proteins, cell survival was above 90% for at least 5 days after printing [21]. These successes are credited in part to the availability of natural cell-specific binding sites available on the dentin matrix proteins [21]. In the same study, the addition of soluble ECM components to the dentin-alginate bioinks, including proteoglycans, glycosaminoglycans, cytokines, and growth factors, resulted in significantly higher ALP and RUNX2 gene expression, indicating higher odontogenic potential than the dentin-alginate bioink alone [21].

Furthermore, the mechanical performance of constructs generated using dentin-alginate bioink was improved by crosslinking with calcium chloride during the extrusion bioprinting process [21]. Other crosslinking agents and additives such as hydroxyapatite (HA), ß-tricalcium phosphate (ß-TCP), glutaraldehyde, diphenylphosphoryl azide, and photoinitiators have previously been used to enhance the mechanical properties of soft biomaterials for dental bioprinting [1,10]. These additives often provide scaffolds with benefits other than mechanical stiffness. For example, incorporation of HA and ß-TCP into either soft or rigid scaffolds enhances cell adhesion and proliferation [1,8]. While calcium phosphates improve soft biomaterials' biocompatibility and structural integrity, they also are known to make the microenvironment more acidic and be resorbed by the body slowly, which extends healing time [24]. Magnesium phosphates have also been employed to regenerate bone, and amorphous magnesium phosphate (AMP) in particular may be advantageous in the regeneration of dental tissues. AMP is resorbed rapidly by the body and the availability of magnesium ions enhances cellular activities such as protein expression and mineral deposition and accelerates differentiation and mineralization [24,25]. In recent research, the addition of AMPs to an ECM-mimetic hydrogel bioink containing dental pulp stem cells (DPSC) significantly increased ALP activity and mineralization *in vitro* as well as new bone formation *in vivo* compared to constructs bioprinted without AMPs [24]. While this study utilizes AMPs for the formation of craniofacial bone, the effect of this additive on DPSC may advance bioprinting of dental tissues.

2.2 Geometric Cues

While the choice of scaffold material(s) is important, it is not the only factor of scaffold design that impacts the successful regeneration of tissue – scaffold architecture optimized to provide the appropriate geometric cues is also an important consideration. For example, a recent series of studies incrementally improved an engineered periodontal ligament replacement, starting with a biphasic scaffold generated by thermally fusing an FDM 3D printed PCL bone compartment to an electrospun PCL periodontal ligament compartment [18]. In the next iteration, the electrospinning process was altered to produce thinner fibers and larger pores in the PDL compartment, which enhanced angiogenesis, improved the connection between tissues at the bone/PDL interface, and resulted in PDL tissue with fiber orientation similar to that of the native PDL [19]. Given the substantially greater control over scaffold architecture afforded by bioprinting as compared to electrospinning, this technology is ideal for creating specific microstructures that optimize the function of the engineered replacement.

Specifically, studies have suggested that odontoblastic and osteogenic behavior can be manipulated by controlling surface tomography and pore interconnectivity in a scaffold [5,8,26]. Research has suggested that the surface texture of scaffolds can influence dental pulp-derived stem cells' osteoblastic differentiation and new bone formation, with concave surface texture resulting in greater differentiation and new bone than smooth or convex surface textures [26]. Odontoblastic

differentiation may also be directly related to the size and interconnectivity of the microchannels formed in the 3D printing process [5,26]. When a PCL scaffold was FDM 3D printed to optimize the dimensions for dentin regeneration, researchers found that microchannels 100 μm in diameter were the most effective for inducing odontoblastic differentiation, resulting in the deposition of dense mineralized tissue, whereas larger microchannels with diameters of 200 to 300 μm did not induce odontoblastic differentiation [5]. Recent research has also indicated that scaffolds containing gradient pore sizes are beneficial for bone tissue engineering because they create an oxygen gradient in the scaffold [26]. This strategy is only possible because of the control 3D printing and bioprinting allow researchers to have over scaffold architecture. As the quality and resolution of bioprinting technology improves, intentional control of scaffold geometry may enable engineered tissues that do not involve exogenous bioactive molecules, potentially reducing both costs and risks.

3 CELLS

Constructs engineered for the replacement of dental tissues have utilized a variety of exogenous and endogenous cell types to produce impressive results and fundamental insights but have yet to generate a clinically viable bioengineered tooth replacement. To move towards clinical translation and widespread adoption of tissue engineered constructs for dental applications, the limited availability of many of the cell types under investigation, potential donor site morbidity, and the high cost of isolating and culturing cells before implantation must be addressed [8]. In this section, outcomes from both dental and non-dental derived cell sources that may permit the clinical translation of bioprinting technology for use in dental applications are discussed.

3.1 Dental-Derived Cells

Dental-derived cells useful for bioprinting can be isolated from either embryonic or postnatal tissue. Since embryonic cells cannot be harvested from a current patient, research using postnatal cells is more applicable for clinical use. Nonetheless, early studies in regenerative dentistry used epithelial and mesenchymal cells derived from embryonic tooth germ to produce bioengineered teeth consisting of pulp, dentin, and enamel as well as penetrating nerve fibers and blood vessels [12,22]. Similar research that utilized postnatal epithelial and dental pulp cells formed calcified nodules, but the authors noted that these features were not consistent with native dental tissue [13]. Although this study suggests that cells sourced from postnatal tissues are inferior to embryonic cells, some evidence indicates that cells isolated from dental pulp or apical papilla of wisdom teeth may be viable, particularly if bolstered with signaling molecules such as recombinant human amelogenin and bone morphogenetic protein-2 (BMP-2) [5,11]. For example, dental pulp cells isolated from extracted wisdom teeth of adults (age 18-39) were suspended in a collagen solution and infused into the microchannels of a multiphasic, FDM 3D printed PCL scaffold [5]. When time-released amelogenin, connective tissue growth factor (CTGF), and BMP-2 were used in conjunction with dental pulp cells, primitive versions of dentin, periodontal ligament, and alveolar bone formed in the three compartments of the scaffold, while scaffolds with no signaling molecules showed suboptimal tissue development [5]. In another study, stem cells from the apical papilla (SCAP) of extracted wisdom teeth of young adults (age 18-20) were capable of odontoblastic/osteoblastic differentiation when cultured in inductive conditions [11]. In fact, when compared to dental pulp cells sourced from the same tooth, SCAP cells were more proliferative, produced more dentin, and had higher cell motility than dental pulp cells [11]. Given the recent

successes utilizing postnatal dental-derived cells, it is likely that such cells could be successfully employed for bioprinting dental tissues if sufficiently supported by appropriate scaffolds and signaling molecules.

3.2 Non-Dental Derived Cells

Although cells derived from embryonic and postnatal dental tissues show great promise for bioprinting, the inherent challenges regarding sourcing these cells may prevent clinical translation. In contrast, bone marrow-derived cells can be harvested on-demand and have a track record of success in craniofacial tissue engineering [8]. Similarly, bone-derived osteoblasts have been used in conjunction with periodontal ligament cells to form integrated bone/PDL interfaces for the repair of defects in the alveolar bone [18,19]. The drawbacks regarding the use of these cells include the low number of mesenchymal stem cells harvested per unit of bone marrow, donor site morbidity, and loss of stemness and proliferative capacity as the donor ages [8]. More recently, induced pluripotent stem cells (iPSCs) and adipose-derived stem cells (ASCs) have emerged as two non-dental derived cells for bioprinting engineered tissues for regenerative dentistry [8].

ASCs and iPSCs are both stem cells with the capability to differentiate into multiple cell types, although ASCs are more lineage restricted than iPSCs [27]. Nonetheless, on-demand harvesting of autologous ASCs can be readily accomplished in most patients and will yield a large quantity of cells suitable for bioengineering applications. One recent study directly compared rabbit ASCs to dental pulp stem cells (DPSCs) for tooth regeneration in an incisor extraction socket [28]. Each cell type was suspended at a density of 5×10^6 cells/mL and then mixed 1.43:1 with porcine type 1 collagen, resulting in a final collagen concentration of 1.1 mg/mL. The material was thermally polymerized in a 48-well plate to form a gel before implantation. In combination with BMP-2, a tooth-like structure consisting of dentin surrounded by a thin layer of cementum and anchored to bone by a PDL-like tissue was achieved in 75% (3 out of 4) of the DPSC constructs and 90% (9 out of 10) of the ASC constructs. These structures consisted primarily of dentin surrounded by a thin layer of cementum. Despite similar outcomes, the authors concluded that ASCs are a superior cell choice, owing to the ease of procurement as well as their improved proliferative speed and capacity for differentiation [28]. Furthermore, these findings strongly support the potential of non-dental cells for bioprinting structures that compare favorably with native teeth.

While the use of iPSCs in bioprinted dental constructs has yet to be investigated, research indicates that iPSCs are able to generate periodontal, enamel, dentin, and dental pulp tissues and can play a role in whole tooth regeneration when applied in combination with mesenchymal and epithelial cells [29]. The interaction between epithelial and mesenchymal cells is an important part of tooth organogenesis, during which dental epithelial cells give rise to ameloblasts, which in turn form enamel [30]. To address the inaccessibility of dental epithelial cells sourced from embryonic tooth germ, one recent study has induced the differentiation of mouse iPSCs (miPSCs, iPS-MEF-Ng-20D-17) into dental epithelial-like cells [30]. Embryonic bodies formed from miPSCs that were cultured with neutrophin-4, a neurotropic factor important in tooth development, in serum-free conditions strongly expressed epithelial progenitor markers p63 and CK14, suggesting that this microenvironment induced the miPSCs to differentiate preferentially into dental epithelial-like cells. These differentiated cells expressed ameloblast specific markers at both the mRNA and protein levels, suggesting potential to differentiate into ameloblasts. Continued research into iPSCs as an alternative source of dental epithelial cells and other cell types could be important in providing an accessible cell source for the future clinical use of bioprinted dental implants.

4 SIGNALS

In this final section, recent experiments that incorporated biological signals into bioprinted dental constructs are reviewed, including both established and emerging technology. Bioactive cues are utilized in bioprinted constructs to enhance cell function, promote proliferation, induce differentiation, or act on the host tissue. However, the use of growth factors does increase the risk of potential adverse side effects, such as those documented for the use of exogenous BMP-2 in spinal fusion [31]. Nonetheless, studies of otherwise promising tissue engineered dental constructs without bioactive cues resulted in poorly formed or disorganized tissues [5,28,32]. As a result, the development of a clinically useful bioprinted dental constructs may depend upon prudent use of bioactive signals.

4.1 Bone Morphogenetic Proteins

Since both BMP-2 and BMP-7 are FDA approved and rapidly induce the production of new mineralized tissue [33], it is no surprise that these factors have been targeted for use in bioprinting dental tissues. In one study described previously, time-released BMP-2 was used to promote the development of alveolar bone by DPSCs in one section of a multiphasic periodontal scaffold while human amelogenin was utilized with the same cell type to produce a different mineralized tissue similar to dentin in a different section of the scaffold [5]. Similarly, BMP-2 increased calcification and odontoblastic differentiation in both monolayer and 3D cell cultures of human stem cells of the apical papilla [34] and increased generation of both dentin and cementum when included in molded type 1 collagen gel containing either DPSCs or ASCs [28]. These studies confirm that the use of BMP-2 may be warranted in bioprinted dental tissues, but the high cost and rapid degradation of rhBMP-2 led some researchers to pioneer the use of a synthetic peptide designed to mimic a fragment of the native BMP-2 protein for bioprinting tissues for dental applications [35]. In this study, the researchers utilized a methacylated gelatin (GelMA)-based bioink formulation seeded with hDPSCs, resulting in greater than 90% cell viability following bioprinting. Similar to BMP-2, the novel BMP-mimetic peptide increased alizarin red staining and expression of the differentiation markers DSPP and OCN. Moreover, by tethering the synthetic peptide to GelMA, over 50% of the peptide remained in the bioprinted construct following 3 weeks of *in vitro* cell culture. In contrast, BMP-2 delivered using an absorbable collagen sponge only remains in the implantation site for eight days [36]. As a result, this clever approach may be able to provide a long-lasting boost to osteogenic potential of bioprinted dental constructs at a low cost.

4.2 Innervation to promote health of bioengineered teeth

The dental pulp of native teeth is densely innervated [37], but tissue engineered dental constructs are generally aneural. Since nerve fibers in the dental pulp transmit sensory information and may be responsible for regulating the inflammatory response to dental disease, the incorporation of neurogenic factors into bioprinted dental tissues may improve the long-term performance of these constructs [37]. One recent study presented an effective approach to promote innervation of bioengineered teeth by suppressing the inhibitory effect of Semaphorin 3A (Sema3A) on axon growth [38]. Tooth germs from Sema3A-deficient embryonic mice (Sema3A $^{-/-}$), wild-type embryonic mice (Sema3A $^{+/+}$), and heterozygotes (Sema3A $^{+/-}$) were associated with dissected trigeminal ganglia and implanted subcutaneously in ICR mice. The researchers observed innervation of the tooth germ developed after two weeks in 100% of the Sema3A-deficient

implants, 50% in the heterozygote implants, and only 13% of the wild-type implants. These findings indicate that activities of Sema3A do inhibit innervation of the dental pulp. To discover if innervation could be achieved in non-immunosuppressive conditions, reassociations of dissociated ICR dental epithelial and mesenchymal cells were implanted with trigeminal ganglia subcutaneously in ICR mice for two weeks. During that time, local injections were administered to the site every two days containing either Membrane Targeting Peptide NRP1 (MTP-NRP1), which suppresses the inhibitory activity of Sema3A, or a vehicle (LDS). After two weeks, 90% of the bioengineered teeth that had received injections of MTP-NRP1 were innervated and contained axons that extended to the odontoblast layer; conversely, none of the controls were innervated. Successful innervation of bioengineered teeth is challenging, and application of this inhibitory peptide may help to remediate this shortcoming. However, the efficacy of this strategy in combination with adult cells and non-dental cells has yet to be determined. Furthermore, the inclusion of MTP-NRP1 into bioprinted constructs has not yet been examined.

4.3 Signals that Induce Cell Homing

Cell homing, also known as revitalization, is a strategy to mobilize endogenous cells from surrounding host tissues towards the implanted scaffold using signaling molecules. Cell homing may increase the potential for clinical translation of bioprinted dental constructs due to the limited availability of autologous stem cells, the cost of cell isolation, processing, and storage, and the hurdles in obtaining regulatory approval [1,39]. Cell homing has been investigated as a technique to regenerate dental pulp for many years, but explorations of its potential for whole-tooth regeneration are less common [1,39]. However, one group reported some success in whole-tooth regeneration via cell homing by attracting endogenous cells to a 3D printed PCL and hydroxyapatite composite scaffold using a cocktail of stromal-derived factor-1 (SDF-1) and BMP-7 [32]. Here, a scaffold in the shape of a rat incisor tooth was coated in a collagen solution containing SDF-1 and BMP-7 before being implanted in an incisor extraction socket and allowed to heal for nine weeks. When harvested, the scaffolds' microchannels had been populated by cells from the surrounding tissues and both new bone and a fibrous tissue similar to the PDL had formed around the scaffold [32]. However, it is unclear how deeply the host cells penetrated into the scaffold or if mineralized tissue formed beyond the scaffold edges. In other bioengineered bone-PDL interfaces, fibrous tissue was anchored to the adjacent bone with Sharpey's fibers (Figure 1, A-L), which is not evident in the fibrous tissue developed in this study (Figure 1, M-N) [5,11,28,32]. This anchoring of the implant to the native bone shows an important distinction between true regeneration of PDL tissue rather than fibrous tissue, as fibrous encapsulation can cause poor osseointegration in traditional dental implants.

5 DISCUSSION

The gold standard for the restoration of lost teeth has remained the same for nearly 60 years. Nonetheless, the adoption of 3D bioprinting as well as ground-breaking research in scaffold optimization, validation of appropriate cell sources, and identification of critical growth factors has resulted in a field poised for a rapid transition away from traditional restoration techniques towards bioengineered solutions. However, clinical translation and widespread adoption of bioprinted dental tissues will be challenging. Bioprinted constructs containing human cells or tissues will be subjected to the same FDA regulations that normally apply to devices and biological products [40]. Despite rigorous regulatory demands, the bioprinting revolution will eventually yield greatly improved patient outcomes for previously intractable clinical scenarios.

Some exciting studies have directly tackled the issue of bioengineering dental tissues with the goal of artificially inducing tooth organogenesis. Although this work has generated stunning results [12,22], it is unlikely this approach can be translated for use in the clinic in the short-term, owing to the complexity, expense, and required reagents for the labor-intensive technique as well as the potentially onerous regulatory requirements. However, it may be possible to partner a bioengineered component with traditional dental implant components to create an implant system that is integrated with the alveolar bone but avoids the complication, time, and labor of growing a complete tooth. Over time, we propose that the traditional implant components, such as the implant, abutment, and crown, could transition from 0% bioengineered to 100% bioengineered (Figure 2). As demonstrated by the studies cited in this paper, the production of bioprinted dental tissues is being explored in many ways, but an incremental approach such as the trajectory shown in Figure 2 could bring the benefits of bioengineered dental implants to patients more quickly. Based on the current standing of the field, the generation of a bioengineered dental implant or defect-specific base that could be fitted with a traditional abutment and crown after healing is the step closest to clinical realization. Considering the history of bone repair, throughout which defects have been patched with precious metals, plant materials, animal bone and horn, and acrylic, the transition from foreign materials to biomaterials and autologous cells has already begun with the successful practice of bone grafting [41]. Bioengineered crowns and 100% bioengineered teeth are feasible as this trajectory continues but will require more time and research to reach clinical application. In particular, whole-tooth regeneration has been achieved in the laboratory by leveraging the interaction of embryonic epithelial and mesenchymal cells. Continued research on the use of iPSCs for whole-tooth regeneration could enable the replication of embryonic organogenesis with non-embryonic cells.

In the short term, safety concerns may be avoided by limiting the use of exogenous signaling molecules, such as BMP-2. Instead, bioprinting could be used for careful optimization of scaffold parameters, such as material composition, microchannel size and interconnectivity, surface tomography, and stiffness [5,8,42], permitting the generation of readily mineralizable bioengineered constructs with fewer safety and regulatory issues. Future studies may test this hypothesis by directly comparing scaffold optimization with exogenous signaling molecules in bioprinted constructs. Furthermore, the development of odontogenic, growth-factor-free bioinks – such as commercially available bioinks supplemented with amorphous magnesium phosphate (AMP) – may be a key step towards reducing the cost and risks associated with bioengineered dental implants. On the other hand, additional research and development of more complex bioinks, such as the BMP-mimetic peptide tethering bioink discussed above, may improve the clinical feasibility of bioengineered dental implants by enabling the use of signaling molecules and growth factors at a lower cost with greater effects and fewer safety concerns. Constructs utilizing synthetic peptides derived from signaling molecules, rather than the native molecules themselves, may be more simple, affordable, stable, and long-lasting. In total, additional investigation is warranted for the development of such bioinks and similar novel technologies.

While advances in scaffold and signal development are vital to future clinical applications, the most significant concern for translation of bioprinted dental tissues is sourcing the appropriate type and sufficient number of human cells that can be used to populate the construct. In this regard, emphasis on the routine use of adipose-derived stem cells (ASCs) in dental bioprinting would aid in future translational efforts. ASCs can differentiate and self-organize sufficiently to create anatomically correct dental structures, including dental pulp, dentin, cementum, and periodontal

ligament, and these structures are identical to those generated using dental pulp cells [28]. Furthermore, ASCs can be harvested on-demand in abundant numbers from adults using mildly invasive techniques. Even so, the use of dental pulp cells is far more frequent in the literature, with discussion typically focused on how such cells might be harvested and saved for future autologous use. Considering the logistical concerns of storing shed deciduous teeth or extracted wisdom teeth for decades, this strategy would be challenging to extend to the general patient population. Induced pluripotent stem cells (iPSCs) are also a promising area for future research in bioprinted dental implants, but the expense of their development may reduce their potential for clinical applications. In total, ASCs appear to be the most promising autologous cell source for the translation of bioprinting technology to the dental clinic.

When considering the research discussed in this review, it is important to remember that the tooth, periodontal ligament, and supporting alveolar bone function as a complete unit [5]. Successful placement of a dental implant requires existing, robust alveolar bone to support the implant [6,11]. Today, a patient with insufficient alveolar bone to support dental implants can undergo a variety of procedures to reinforce and replenish the bone including bone grafts, guided bone regeneration, hydroxyapatite augmentation, or the sinus lift procedure [6]. For patients with minor bone loss, guided bone regeneration and placement of a dental implant may be able to be performed in a single procedure, but more often alveolar bone augmentation requires multiple procedures with up to six months between augmentation and implant placement [43]. Bioengineered solutions that only regenerate dental tissues and not the entire periodontal complex will provide improvements to today's technology but will not address some of the biggest challenges. Some researchers have proposed constructs in which multiple tissues can be regenerated simultaneously with proper tissue integration [5,18,19]. Other research has aimed to repair periodontal defects by 3D printing defect-specific scaffolds to generate new alveolar bone in a specific form around a patient's existing teeth [44]. Bioprinting patient-specific, multiphasic constructs designed to generate dentin, PDL, and alveolar bone in defect-specific forms may reduce bone augmentation and implant placement to a single procedure, even in cases with more severe bone loss.

In summary, leveraging progress made in the three traditional tissue engineering components – scaffolds, cells, and signals – is critical for new advances in bioprinting dental and periodontal tissues. Bioprinting provides the benefits of tailored geometry, diverse bioactive materials, and precise placement of cells and signals to facilitate sophisticated bioengineered constructs with the potential to facilitate improved solutions for tooth loss in the near future as well as experimental models that will push the field forward. A strategic choice of material and scaffold design paired with readily available cells will enable bioprinting to meet the existing clinical need for reliable dental implants. Furthermore, the possibilities that bioprinting offers will only grow as the technology matures, including new solutions for dental and periodontal regeneration yet to be imagined.

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FIGURES

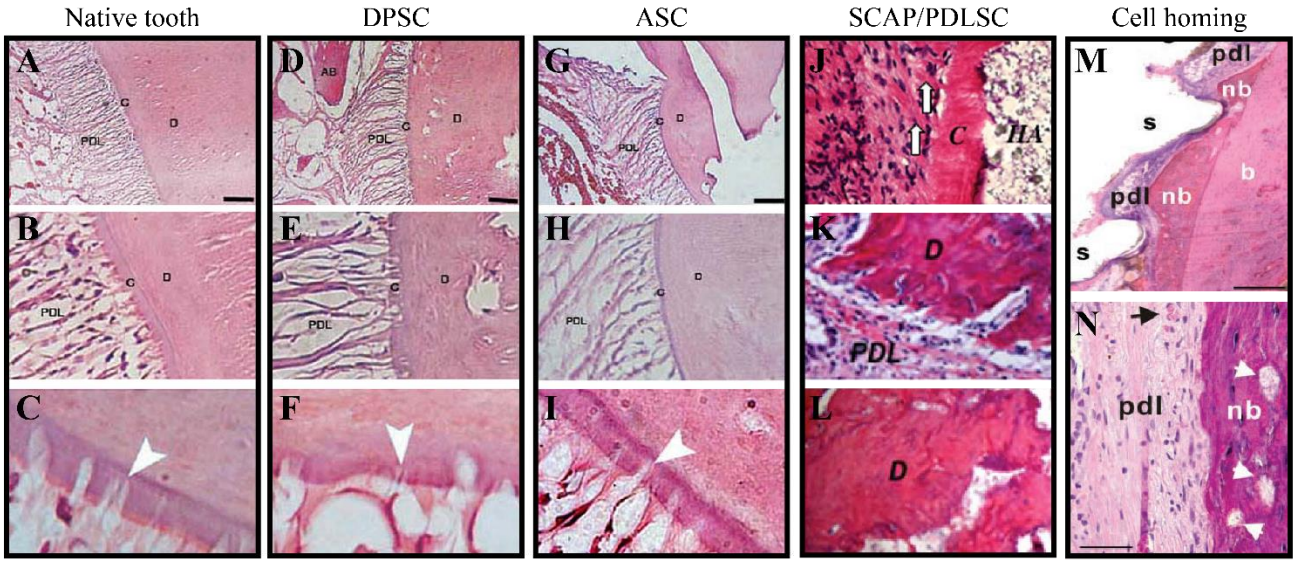

Figure 1: Comparison of interfaces between PDL adjacent tissues. A-C) Native tooth, D-F) bioengineered tissue generated using dental pulp stem cells (DPSC), and G-I) bioengineered tissue generated using adipose-derived stem cells (ASC) with labels for dentin (D), cementum (C), periodontal ligament (PDL), and Sharpey's fibers (white arrow). Adapted from *[28]*. J-L) A HA/TCP block scaffold containing stem cells of the apical papilla (SCAP) was wrapped in Gelfoam loaded with periodontal ligament stem cells (PDLSC) and implanted for J) 8 weeks or K-L) 3 months, with labels for cementum (C), PDL, and Sharpey's fibers (white arrows). Adapted from *[11]*. M-N) 3D printed PCL/HA scaffold containing SDF-1 and BMP-7, with labels for scaffold (s), old bone (b), new bone (nb), and periodontal ligament (pdl). Adapted from *[32]*.

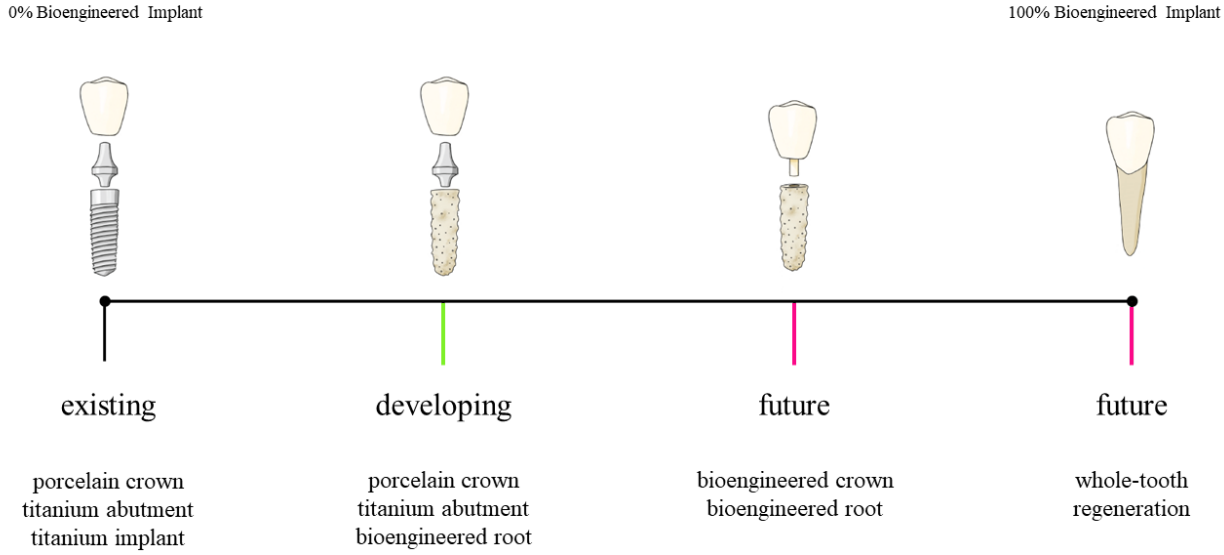

Figure 2. Potential trajectory for the development of bioengineered dental implants. Bioprinting allows for the development of an array of hybrid solutions for tooth loss that can meet the current needs and the long-term goal of whole tooth regeneration.

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