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Development and application prospect of stem cell combined with 3D printing technology for oral disease

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Abstract: With organ transplantation facing many dilemmas, tissue and organ regeneration as an alternative has bright prospects. In regenerative medicine, Three-dimensional (3D) printing technology and stem cells has been widely applied to the treatment of diseases related to tissue or organ replacement in dentistry, respectively. However, there are very few studies on the combination of the two, and even fewer clinical studies have been reported in dentistry. In this review, the current oral tissue engineering *in vivo* and *in vitro* based on 3D printing and stem cell technology will be summarized, and the discussion on the development prospects of this research direction will be given. Besides, the working principles and advantages & disadvantages of several types of 3D printers, as well as the mechanism of stem cells in tissue engineering will be elucidated. This review provides clinicians and researchers with the current state of research and trends in the combination of stem cells and 3D printing technology to treat oral-related diseases. In the future, 3D bioprinters are poised for ongoing innovation with the advancement of relevant technologies, catalyzing an increase in clinical studies focused on treating oral diseases using stem cells and 3D scaffolds. Consequently, these developments will further advance the field of oral tissue engineering.

List of Abbreviations

3D	Three-dimensional
MSCs	Mesenchymal stem cells
CAD-CAM	Computer-aided manufacturing technology
NNT	Neural network tissue
TrkC	Tropomyosin receptor kinase C
NSCs	Neural stem cells
ESCs	Embryonic stem cells
iPSCs	Induced pluripotent stem cells
ALP	Alkaline phosphatase
hDPSCs	Human dental pulp stem cells
pTDM	Porcine treated dentin matrix
xTDM/PCL	Xenogeneic TDM-polycaprolactone
CNPs	Ceria nanoparticles
ICAM-1	Intercellular cell adhesion molecule-1
ECM	Extracellular matrix
IGF-1	Insulin-like growth factor 1
TNF-α	Tumor necrosis factor-α
SDF-1	Stromal cell-derived factor
SHED	Stem cells from human exfoliated deciduous teeth
FBR	Foreign body reaction
HA	Hyaluronic acid
ITOP	Integrated tissue–organ printer
HUVECs	Human umbilical vein endothelial cells
KC	Keratinocytes
FB	Fibroblasts
PCL	Polycaprolactone
Cs	Carbon steel
CP	Calcium phosphate ceramic
IPCABs	Inkjet-printed custom-made artificial bones
TCP	Tribasic calcium phosphate

| BMSCs	Bone marrow mesenchymal stem cells
ADMSCs	Adipose-derived mesenchymal stem cells
SLS	Selective laser sintering
PDLSCs	Periodontal ligament stem/progenitor cells
ABSCs	Alveolar bone stem/progenitor cells
hAFSCs	Human amniotic fluid–derived stem cells
ASCs	Adipose-derived stem cells
VML	Volumetric muscle loss
GMSCs	Gingiva-derived MSCs
BMP	Bone morphogenetic protein
PDGF-AB	Platelet-derived growth factor-AB
Tregs	Regulatory T cell
IL	Interleukin
VEGF	Vascular endothelial growth factor
ISSCR	International society for stem cell research

Introduction

Organ and tissue loss or severe dysfunction caused by disease and(or) injury usually need transplantations or regenerations [1]. While organ transplantation is a well-established treatment for organ and tissue failure, significant challenges such as donor shortages, immune rejection, high costs, and societal issues persist and need to be addressed [2,3]. In response, regenerative medicine has emerged as a promising alternative [4], with tissue engineering pioneering the field since the 1980s [5]. Advances in technologies like stem cells, additive manufacturing, biomaterials, and microfluidics have enabled significant progress in reconstructing damaged tissues and organs such as bone [6], skeletal muscle [7], blood vessels [8], etc. These advancements have laid a solid foundation and made critical strides in regenerating oral tissues [9,10]. However, clear and important challenges remain in the development of reproducible and clinically safe methods for tissue repair and regeneration [11]. In recent years, stem cells have been frequently used in tissue engineering studies. Different stem cells have a variety of differentiation potentials, such as bone tissue and adipose tissue [12]. With biological characteristics, including secreting growth factors and cytokines, immunoregulation,

anti-inflammation, anti-apoptosis, promoting angiogenesis, tissue repair, and other regulatory functions. Among the various stem cells, mesenchymal stem cells (MSCs) are among the most frequently used in tissue engineering due to their easy acquisition and the absence of significant ethical concerns. The mechanism of regenerating tissues of MSCs may be related to intercellular interactions and immunoregulation [13,14].

Furthermore, the successive development of biomaterials has also injected new impetus into tissue engineering: newly developed biomaterials not only have good biocompatibility but also can interact with cells. However, traditional methods of manufacturing scaffolds, such as freeze-drying, solvent casting and particle leaching, gas foaming, etc., cannot be customized for complex tissues, which limits the application of biomaterials in tissue engineering [15].

Tissue engineering based on stem cell technology and three-dimensional (3D) printing technology can easily solve that problem, which combines high precision and high-resolution printing equipment with stem cells for refined and customized tissue repair and regeneration [16]. In dentistry, 3D bioprinting offers advantages over conventional techniques by better simulating natural tissue's 3D structure and cellular interactions [17,18]. Coupled with mathematical modeling and computer-aided technologies (CAD-CAM), this approach enables more accurate tissue repairs [16]. Currently, 3D bioprinting is widely used in other biomedical fields [19]. For a example, Li et al. [20] transplanted neural network tissue (NNT) derived from tropomyosin receptor kinase C (TrkC)-modified neural stem cells (NSCs) into rats after seeding of NSCs on the 3D engineering scaffold, and the results showed the NNT survived for up to 8 weeks and significant recovery of paralyzed limb motor function. In addition, many studies have used stem cells combined with 3D printing technology to produce heart patches, which have been shown to promote cardiomyocyte proliferation, angiogenesis, and improvement of cardiac function in rat models of myocardial infarction [21,22]. Likewise, 3D bioprinting using stem cells has already been used to treat oral diseases, current applications include regeneration of various oral tissues (Fig. 1). However, the technology remains stuck in
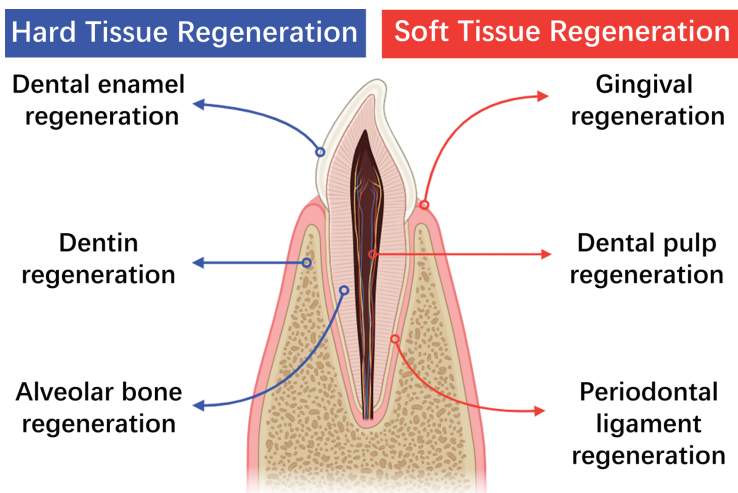
FIGURE 1. Current applications of 3D-bioprinting in oral diseases (created by adobe photoshop).

experimental research, and it is rarely used in clinical trials. This review highlights recent research progress and existing challenges in utilizing 3D printing and stem cell engineering for oral diseases. By summarizing current advancements and obstacles, it aims to provide a theoretical framework for advancing basic research and facilitating future clinical applications in this evolving field.

3D Bioprinter-Based Tissue Engineering

The advent and dissemination of stereolithography apparatus, commonly referred to as 3D bioprinters, catalyzed a transformative epoch in the domain of tissue engineering during the late 20th century [16]. This technological innovation, grounded in the principles of additive manufacturing, enables the precise deposition and assembly of biomaterials or cellular constructs, guided by a digital 3D blueprint. The resultant constructs span a spectrum from medical devices to tissue-engineered scaffolds and organs, heralding a new era of personalized medicine and regenerative therapy. Typically, a 3D bioprinter comprises a printhead, a construction platform, control systems, material supply systems, cooling systems, a computer and relevant software, working together for fabricating complex biological structures. Bioprinters print tissues or organs with bioink made from biomaterials, cells, growth factors, and other materials. The process hinges on the use of bioinks, a composite of biomaterials, cellular components, growth factors, and ancillary substances. These bioinks are meticulously layered to construct a computer-assisted biological engineering structure, leveraging the additive manufacturing process [23,24]. In the realm of tissue engineering, stem cells form the cornerstone of bioinks. Pluripotent stem cells, notably embryonic stem cells (ESCs), MSCs, dental pulp stem cells (DPSCs), stem cells from apical papilla (SCAPs) [25] and Periodontal ligament stem cells (PDLSCs) [26] are pivotal due to their inherent capacity for differentiation and regenerative potential [27,28]. Stem cells from human exfoliated deciduous teeth (SHEDs), with high proliferation rate and multilineage differentiation potential, the noninvasive harvesting procedure, also have a promising prospect [29]. Bioinks, typically hydrogel-based, encapsulate these cellular elements within a supportive matrix, facilitating their viability and functionality during the printing process [30–32]. Contrary to conventional biomaterials, bioinks are uniquely characterized by their filamentous deposition during additive manufacturing [33]. Yet, this deposition necessitates a delicate balance of conditions, particularly in terms of temperature, to preserve the biological integrity of the cellular components. The bio-3D printed microenvironment is closely related to the processes of cell proliferation, differentiation adhesion and so on. Temperature is one of the factors that affect the printing material. Suitable temperature helps to improve the stability of the printing material for *in vitro* preforming, and it can also serve as part of energy and plays an essential role in the formation of cell-laden materials. 34℃ is similar to the ambient temperature in homeostasis and is favorable in regenerative medicine [34]. High-temperature intolerance of living cells underscores the requirement for mild printing environments, ensuring the bioink maintains its biological attributes throughout the additive manufacturing process [30]. Liquid environment also plays an important role as liquid itself singly stimulates tissues or organs to manufacture or transformation. Lipid-responsive materials transform in spatial and temporal manner thus they probably mimic the normal hormone-like effects [34].

Three principal methodologies underpin the construction of 3D bioprinter constructs capable of emulating the complexity of living tissue or organs, namely, biomimicry, autonomous self-assembly, and mini-tissue building blocks [16]. The application of 3D bioprinting in basic biological research and biomedical science should meet the following requirements: the cells in the printed structure must represent the cell types in the real tissue, and the cell structure of the artificial tissue should best reproduce the physiological tissue [35]. Diverse 3D printing techniques exist, with laser-assisted bioprinting, inkjet printing, and extrusion-based bioprinting standing as the most prevalent applications. Laser-assisted bioprinting harnesses laser energy to precisely target and manipulate a layer of material containing a laser absorption layer. The energy imparted by the laser pulse causes the targeted material, embedded in matrices such as collagen or plasma, to be propelled forward without compromising cellular integrity [27,36]. This technique has been successfully employed in various bioprinting applications, encompassing skin and bone tissue regeneration [36,37]. Inkjet bioprinting operates through dual methodologies: direct printing and powder-based printing. Direct printing involves direct printing, where materials are gathered at the nozzle and the ink or material is maintained in a liquid state and held within the nozzle. When the pressure within the nozzle changes, it triggers the ejection of droplets, which are then deposited onto the surface in a precise and controlled manner. Powder-based printing utilizes a powdered form of the target material, which is positioned under the nozzle. This process relies on an adhesive or binder that is dispensed from the nozzle. Upon mix with the powdered material, the adhesive causes the particles to coalesce and form a solid structure. The polymerization or solidification of the material is achieved through the interaction between the adhesive and the powder, resulting in a strong and cohesive structure. There are two factors that can promote the change in nozzle pressure: piezoelectric induction and thermal induction [38,39]. This technology can be used to customize an artificial bone of a special size and shape to repair bone defects [40]. Extrusion-based bioprinting, distinguished by its efficiency in material delivery and parallel printing capabilities, is particularly adept at rapidly printing large constructs [39]. However, its utility is somewhat constrained by the requirement for thermoplastic materials, which may not be entirely conducive to cellular viability [27]. Notwithstanding, recent advancements have demonstrated the successful survival of human dental pulp stem cells (hDPSCs) in bioinks printed using this technology [41]. A comparative overview of the salient features of these three printing methodologies is delineated in Table 1, elucidating their respective advantages, limitations, and applications in the burgeoning field of 3D bioprinting.

TABLE 1

The characteristics of 3 modalities of 3D printing technology

Type of 3D bioprinter	Stem cell type	Materials	Advantages	Disadvantages	Applications	References
Laser-assisted printing	MSCs HUVECs	PCL; Cs; **Hydroxyapatite**; metal; composites	High resolution and precision; high viscosity bio-ink usable; high cell viability (>95%); non-contact mode of printing	Time consuming; high cost; low stability and scalability	Bone tissue regeneration; vascular engineering	[27,37,42]
Ink-jet printing	hMSCs; Neuronal cells and Schwann cells; KC and FB	Binders; CP; **Hydroxyapatite**; bioactive glass	Wide availability; high speed, lower cost; cell viability	Limitation of ink's viscosity; Limitations of vertical printing	Bone tissue engineering; IPCABs; skin tissue repair	[40,43,44]
Extrusion based printing	Chondrocytes; MSCs	TCP; PCL; Hydroxyapatite; Alginate; Bioactive glass	Avoiding high temperature processing; high speed; ability to print with very high cell densities	Low resolution; solvent and polymer concentration selection; lower cell survivability	Bone and cartilage tissue engineering; drug delivery or dosage	[45–47]

Note: MSCs: mesenchymal stem cells; HUVECs: human umbilical vein endothelial cells; KC: Keratinocytes; FB: fibroblasts; PCL: polycaprolactone; Cs: Carbon Steel; hMSCs: human mesenchymal stem cells; CP: calcium phosphate ceramic; IPCABs: Inkjet-printed custom-made artificial bones; TCP: tribasic calcium phosphate.

Practical Applications of 3D Bioprinting Using Stem Cells in Oral Diseases

At present, there are two primary approaches to leveraging stem cells within the context of 3D bioprinting for medical applications, distinguished by the inclusion or exclusion of supportive biomaterials that act as scaffolds. Alveolar ridge defects are often caused by bone loss, trauma, infection or severe periodontitis [48], hard tissues such as alveolar bone and teeth usually have complex morphology and structure, which brings great difficulties to autologous bone transplantation, and 3D bioprinting has unique advantages in this respect. These scaffolds are crucial as they offer the necessary physical structure and living conditions for the stem cells. An overview of how 3D printing technology, combined with stem cells, is being applied in the treatment of oral diseases will be provided in Table 2.

Scaffolds based on 3D bioprinting using stem cells in oral diseases
In previous research, a prevalent approach in integrating 3D printing with stem cells involves embedding the stem cells

TABLE 2

Summary of Scaffolds and Scaffolds-free type of bioprinting technology

	Author	Country	Type of 3D bioprinter	Stem cell type	Model	Applications	Reference
Scaffolds	Lee, CH et al.	USA	Inkjet bioprinting	PDLSCs	*In vitro*	Periodontium complex regeneration	[49]
	Morrison, RJ et al.	USA	Laser assisted bioprinting	ABSCs	Rats	Craniofacial cartilage engineering	[50]
	Jensen, MB et al.	Denmark	Extrusion based bioprinting	MSCs	Mice	Bone regeneration	[47]
	Moncal, KK et al.	USA	Extrusion based bioprinting	rBMSCs	Rats	Bone regeneration	[51]
	Kuss, MA et al.	USA	Extrusion based bioprinting	ADMSCs, HUVECs	Nude mice	Vascularization for large bone construct	[52]
	Tu, C et al.	China	Extrusion based bioprinting	BMSCs	Rats	Bone regeneration	[53]
	Roskies, M et al.	CA	SLS	BMSCs, ADSCs	*In vitro*	Bone regeneration	[54]

(Continued)

Table 2 (continued)

|	Author	Country	Type of 3D bioprinter	Stem cell type	Model	Applications	Reference
Scaffolds-free	Kang, HW et al.	USA	Extrusion based bioprinting	hAFSCs	Rats	Bone reconstruction	[55]
	Park, JH et al.	USA	Extrusion based bioprinting	hDPSCs	*In vitro*	Improving odontogenic differentiation	[56]
	Han, J et al.	South Korea	Extrusion based bioprinting	hDPSCs	*In vitro*	Dentin-pulp complex regeneration	[41]
	Skardal, A et al.	USA	Laser assisted bioprinting	AFSC	Mice	Healing of skin wound	[57]
	Kim, BS et al.	South Korea	Inkjet and extrusion based bioprinting	ASCs	Mice	Accelerate wound closure, re-epithelization, and neovascularization	[58]

Note: USA: United States of America; CA: Canada; SLS: selective laser sintering; PDLSCs: periodontal ligament stem/progenitor cells; ABSCs: alveolar bone stem/progenitor cells. rBMSCs: rat bone marrow stem cells; ADMSCs: adipose-derived mesenchymal stem cells; HUVECs: human umbilical vein endothelial cells; hAFSCs: human amniotic fluid–derived stem cells; hDPSCs: human dental pulp stem/progenitor cells; ASCs: adipose-derived stem cells.

with a 3D-printed scaffold to establish a conducive milieu for cellular proliferation [6,59]. In these studies, researchers are apt to immobilize stem cells on scaffolds using the gel method (stem cells attached to 3D printed scaffolds using gel as a medium) [60–62] rather than incubating them directly [63], as considerations include biocompatibility and precise control of cell distribution. Within stomatology, this methodology parallels scaffold-based tissue regeneration applications for oral diseases. Lee et al. [49] conducted studies cultivating periodontal complexes, encompassing dentin/cementum, periodontal ligament, alveolar bone, and other tissues *in vitro* using multiphase region-specific microscaffolds. These scaffolds incorporated spatiotemporal delivery of bioactive cues in conjunction with DPSCs, effectively mitigating issues such as delamination commonly associated with traditional methods. In another *in vitro* study, Han et al. [41] employed hDPSCs, a fibrin-based bioink, and polycaprolactone (PCL) to successfully fabricate a dentin-pulp complex via 3D printing. This approach not only maintained structural integrity over an extended period but also exhibited robust cellular adhesion, optimal oxygenation, and efficient fluid transport capabilities. Furthermore, the versatility of customization according to patient-specific requirements was demonstrated.

Although significant advancements have been achieved in bone regeneration through 3D bioprinting using stem cells, controlling the directional differentiation of these cells remains a formidable challenge, often resulting in suboptimal tissue regeneration outcomes. Consequently, various strategies, such as scaffold modifications and material enhancements, have been devised to address these limitations [51–53]. Likewise in oral diseases, researchers have improved the performance of scaffolds by changing the raw materials and modifying scaffolds, and these studies have shown that the scaffolds made progress in osteogenic differentiation [28,64,65], porosity of bone [66], cell viability [28], angiogenesis [67], cementogenesis [68]. These investigations underscore ongoing advancements in the application of 3D-printed scaffolds and stem cells in stomatology. While numerous *in vitro* and animal studies have been conducted, clinical trials remain largely unexplored. To date, limited clinical study has been documented involving surgical treatment of alveolar clefts using 3D-printed scaffolds seeded with autologous bone marrow mesenchymal stem cells (BMSCs) [69]. Employing PCL scaffolds fabricated via micro-extrusion 3D printing, previously reported for periodontal repair in 2015 [70], this study [69] capitalized on PCL's biocompatibility and the participant-derived nature of the stem cells to avoid immune rejection and ensure favorable postoperative healing outcomes. The process of treatment is illustrated by Fig. 2. It is noteworthy, however, that the average bone mineral density of the regenerated teeth did not match that of the subjects' normal teeth, suggesting room for optimization in bone regeneration protocols.

This clinical case underscores the promising future of *in situ* stem cell engineering utilizing minimally manipulated stem cells (cell populations obtained and used with minimal alteration of their natural state [71,72]. The autologous stem cells not only exhibit no immune rejection but also eliminate the concerns of ethical and moral issues. In addition, the stem cells isolated during the surgical procedure maintain high viability and a stable phenotype because of the minimal intervention [71]. Bajuri, MY and co-workers [73] reported on the application of autologous stem cells combined with 3D bioprinted scaffolds for the treatment of diabetic foot ulcers. Owing to the benefits mentioned above, their novel therapeutics enhanced efficacy in treating diabetic foot ulcers, accelerating wound healing and significantly reducing the size of the ulcers. During a typical treatment plan, stem cells are extracted and subsequently combined with a 3D bioprinted scaffold. The scaffold provides both physical support for the stem cells and a microenvironment conducive to their survival, growth, and differentiation, closely resembling physiological conditions *in vivo*. This microenvironment simulates the natural niche of stem cells in the body to ensure their survival, proliferation and differentiation [74]. After seeding on the scaffold, the entirety is surgically implanted into the body. Within the host environment, these stem cells engage with other cell types and the extracellular matrix through signaling molecules, facilitating proliferation and
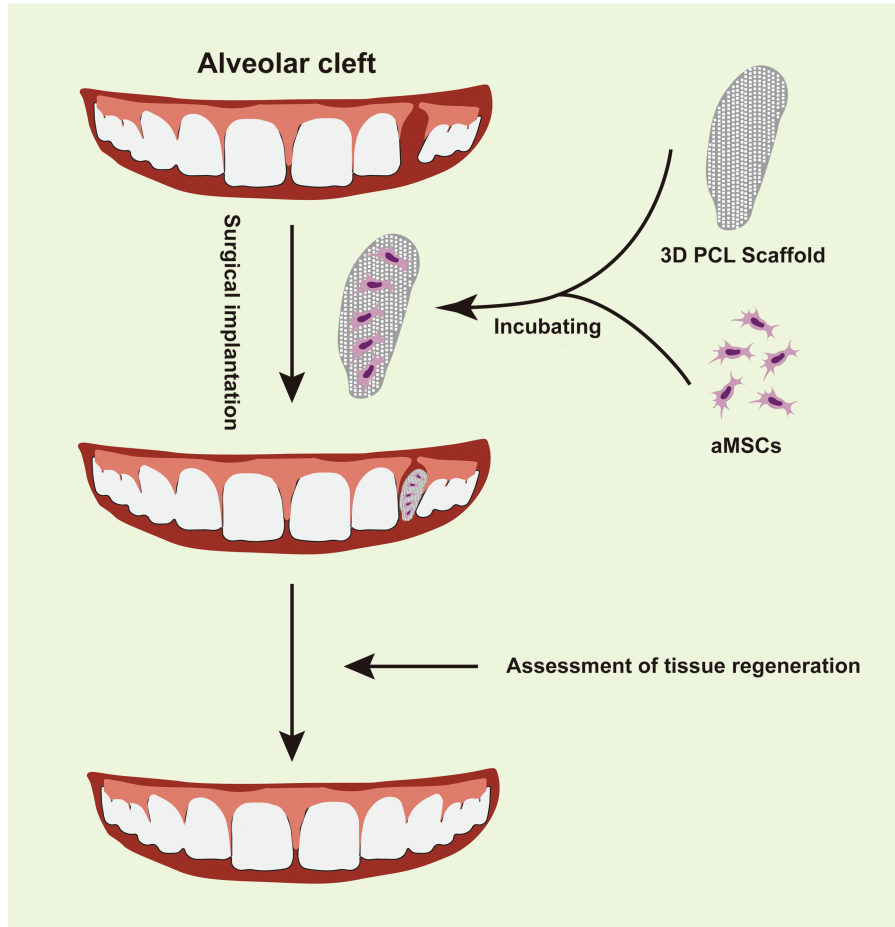
FIGURE 2. Illustration of surgical treatment of alveolar cleft with the mesenchymal stem cells-seeded 3D printed scaffold [69]. 3D: Three-dimensional; PCL: Polycaprolactone; aMSCs: Autologous mesenchymal stem cells (created by adobe illustrator).

differentiation *in vivo* supported by an adequate blood supply. This interaction activates or represses specific gene expressions responsible for various functions related to directional differentiation [75–77] of stem cells and tissue formation for clinical treatment. While *in situ* tissue engineering technology offers significant advantages, it is not without its drawbacks. Autologous stem cells are typically obtained in smaller quantities [72], as they undergo minimal (20 min incubation [69]) *in vitro* manipulation for proliferation. This limited proliferation is due to the inherent risks associated with *in vitro* expansion, which include unsuitable culture conditions, compromised stem cell proliferation, unpredictable differentiation outcomes, and complications arising from labeling procedures. All these factors significantly contribute to the phenomenon of "senescent drift" in stem cells during *in vitro* cell culture. In other words, uncontrollable factors and non-standardized *in vitro* culture of stem cells on scaffolds may lead to genotype-phenotype relationship changes in stem cells cultivation.

In 3D-bioprinted scaffolds for stem cells cultivation, there are two primary modes: synchronized printing of stem cells with scaffolds and sequential printing of scaffolds followed by implantation of stem cells [78]. When stem cells are cultivated on 3D bioprinted scaffolds *in vitro* over extended periods, they undergo a complex and transformative process marked by a series of intricate

phenotypic changes. This metamorphic process encompasses the adaptation of stem cells to their surrounding microenvironment, their subsequent proliferation and differentiation, culminating in the functional maturation that is pivotal for tissue regeneration. Within this microenvironment, 3D scaffolds [79,80], enriched with bioactive media, serve as the physical substratum for stem cell anchorage. These cells secrete adhesion molecules, such as ICAM-1 and integrins, enabling their adhesion to the scaffold's surface. Simultaneously, they are influenced by a myriad of factors such as growth factors, oxygen tension, intercellular signaling, and the extracellular matrix (ECM) [81], which collectively orchestrate the migration, proliferation, and differentiation of stem cells within the scaffold (details described in the next part). Eventually, the stem cells differentiate into target cells and form tissue, which is able to replace the diseased tissue normally and perform its normal function.

However, the *in vitro* culture of stem cells has its inherent limitations. For instance, "adherent wall" culture methods can lead to the emergence of heterogeneous stem cell populations [82]. The proliferation rate of stem cells is highly sensitive to changes in environmental oxygen concentration [83]. Inadequately managed freezing and thawing processes may adversely affect the phenotype and functionality of stem cells [84]. Additionally, certain components present in the serum of cultured cells can influence the proliferation of

stem cells. Smith et al. reported that certain platelet concentrations can enhance stem cell proliferation and eliminate the xenogeneic serum components [85]. When considering stem cell differentiation, challenges such as uncontrolled differentiation pathways and the complete loss of differentiation potential arise. Besides, there exists a risk of senescence and transformation among stem cells cultured *in vitro*. Numerous reports highlight that prolonged expansion of MSCs can induce cellular senescence [86]. Finally, it is noteworthy that for stem cells subjected to labeling, the efficiency of proliferation [87] and even cell viability may be compromised [88], as many exogenous tracers used for labeling are not optimal for stem cell health. It is believed that these problems are caused by the absence of standardization. Therefore, it is essential to establish a standardized protocol for culture of stem cells on 3D bioprinted scaffolds designed for culturing in clinical treatments.

Most current studies involving 3D-printed scaffolds and stem cells are primarily conducted *in vitro* and in animal models, each with inherent limitations. For instance, while successful bone tissue culturing using scaffolds has been achieved *in vitro*, further investigations are necessary to assess the scaffolds' capability for craniofacial applications [54]. In addition, Rodriguez et al. [89] utilized tissue engineering techniques, excluding 3D printing, to address craniofacial volumetric muscle loss (VML) in a large animal model (sheep), revealing comparable functional outcomes between treated and untreated groups, unlike previous successes in repairing VML in sheep hindlimbs. This disparity may stem from severe craniofacial denervation and ischemia, intensifying repair challenges. Applying the same experimental methods used for repairing cranial and facial bone defects in rats to larger animals or humans may lead to issues such as inadequate oxygen supply during bone repair [90]. Consequently, the nutritional needs of cells must be considered when regenerating larger tissues or organs, necessitating further investigation into vascular and neural regeneration [91,92]. Induction of osteogenesis, angiogenesis and inhibition of inflammatory response is another difficulty in tissue engineering of oral diseases, as well as a research hotspot. Emerging new materials and sights has brought new hope to overcome these problems. The layered bioceramic scaffold can simulate the structure and biological characteristics of normal bone tissue. Bredigite (BRT, Ca7MgSi4O16) is a ceramic biomaterial that can induce bone and angiogenesis and can be manufactured by 3D printing technology. Its combination with 3D printing technology has great application prospects in onlay graft [93].

Furthermore, the immune response of the implanted scaffold to the participant should also be taken into account. Because implantation of poor biocompatible biomaterials can trigger severe foreign body reaction (FBR) [94] in the body. For 3D printed scaffolds, specifically, different components within 3D printed scaffolds can trigger various immune responses. Studies have shown the surface properties of biomaterials, their physicochemical properties, and their interactions with cells affect the behavior of immune cells. Natural polymers, such as gelatin, collagen [95] and highly purified alginate [96], have the characteristics of low immunogenicity. However, chitosan and hyaluronic acid (HA) are the exceptions. The former leads to activation of macrophages and dendritic cells [97] and aggregation of neutrophils [98], which is related to the physicochemical properties of chitosan, and its immunogenicity can be mitigated by changing the deacetylation degree, molecular weight and positive charge [99]. The latter's fragment (<200 kDa) can recognize HA-binding receptors TLR-2 and TLR-4 and triggers pro-inflammatory responses, while high molecular (>1000 kDa) weight HA does not cause immune response [100].

Scaffold-free based 3D bioprinting using stem cells in oral diseases

Scaffold-free bioprinting is a technology that circumvents the use of artificial scaffolds to support biological tissue or organ structures during the 3D bioprinting process. This approach has garnered considerable interest in recent years. Scaffold-free bioprinting offers potential advantages over scaffold-based methods, including mitigating issues such as rejection and tissue failure associated with scaffolds. It also promotes enhanced biocompatibility, facilitates intercellular interactions, supports long-term functionality, and improves cell viability [101–103].

For repairing cranial and maxillofacial bones, Kang et al. [55] utilized integrated tissue–organ printer (ITOP) to fabricate the defected mandible using human amniotic fluid-derived stem cells (hAFSCs) as cell sources. Their study demonstrated osteogenic differentiation and stimulated calcium deposition within the printed structure. Moreover, hAFSCs were employed to print a skull structure and implanted into rat cranial defects, resulting in the formation of vascularized bone tissue surpassing scaffold-only treatments. While inkjet, laser, and extrusion-based bioprinters can transport living cells and biological materials to fabricate 3D structures, achieving surgical implants with precise size, shape, and tissue structure remains challenging. ITOP technology offers a promising solution to this issue.

The maxillofacial nerve may be injured due to trauma, surgery, or other reasons. Zhang et al. [104] simulated fundamental development processes via tissue self-assembly. Human gingiva-derived mesenchymal stem cells (GMSCs), a subgroup of MSCs isolated from gingiva, have been employed for 3D printing and culture. Implantation of GMSC spheroids has demonstrated efficacy in promoting facial nerve repair and regeneration.

Tooth loss, stemming from dental caries, periodontitis, or trauma, has spurred interest in whole-tooth regeneration within oral regenerative medicine. Park et al. [56] successfully utilized hDPSCs and a bone morphogenetic protein (BMP) mimetic peptide tethering bioink to bioprint a structurally sound tooth substitute. However, these studies remain predominantly confined to *in vitro* settings and primarily focus on partial tooth tissue regeneration, highlighting significant avenues for further research in this domain.

Despite the advantages of scaffold-free bioprinting, such as avoiding scaffold-related biocompatibility concerns, it faces inherent limitations. Achieving tissue maturation and the

transition from liquid to solid phases rapidly is crucial to maintaining product composition and integrity [105]. Furthermore, scaffold-free bioprinted constructs may exhibit inferior mechanical properties compared to scaffold-based methods [106]. Additionally, the unpredictable behavior of cells and the extended preparation time required for stable assembly pose significant challenges [35]. Recent efforts have explored mathematical modeling to predict and optimize scaffold-free constructs, aiming to mitigate these limitations [101].

The Mechanism of Stem Cells in Tissue Engineering

The application of stem cells in 3D bioprinting for oral diseases shares similarities with their application in other diseases. In stem cell bioengineering, the primary goal is to achieve precise and strategic modulation of stem cell populations, ensuring consistent and predictable outcomes. This objective is heavily influenced by microenvironmental cues perceived by stem cells, which encompass both biochemical and biophysical signals. Biophysical signals include intercellular interactions, as well as the shape, topology, compliance, and interactions of extracellular matrix proteins [107].

Stem cells are known to secrete various cytokines and growth factors [60,108]. Previous research has demonstrated that adult BMSCs can effectively repair many tissues and migrate in response to chemokines and growth factors secreted by cells. Among these factors, platelet-derived growth factor-AB (PDGF-AB) and insulin-like growth factor 1 (IGF-1) are particularly potent. Chemokines have also shown effectiveness on tumor necrosis factor-α (TNF-α)-primed cells, suggesting that MSC mobilization and subsequent homing (incorporation into damaged tissue via the extracellular matrix) are linked to both systemic and local inflammation [108]. Cytokines also play an important role in osteogenic differentiation, Kim et al. constructed a scaffold composed of PCL and HA with a combination of stromal cell-derived factor 1 (SDF-1) and BMP-7. Their results suggest that SDF-1 and BMP-7 are critical in attracting endogenous cells, including MSCs and endothelial cells, to promote vascularization [109].

In addition to cytokines and growth factors, exosomes secreted by stem cells have garnered increasing attention in recent years [110,111]. Numerous studies have indicated that MSC-secreted exosomes possess therapeutic effects akin to those of MSCs themselves. The primary mechanism is thought to involve the horizontal transfer of proteins, mRNAs, and regulatory microRNAs that alter the activity of target cells to exert their effects. MSC-derived exosomes are also believed to modulate the activity of immune cells such as B cells, T cells, natural killer cells, dendritic cells, and macrophages, mimicking the effects of MSCs [112]. In one study involving THP-1 cells treated with MSC exosomes, pro-inflammatory cytokine responses induced by these exosomes were reduced, while anti-inflammatory interleukin (IL)-10 expression was significantly enhanced. Furthermore, treatment with activated CD4$^+$ T cells enabled THP-1 cells to induce regulatory T cell (Treg) polarization, confirming that MSC-derived exosomes can attenuate immune activity

in vivo by promoting IL-10 production and Treg differentiation [113].

Angiogenesis is a major topic in tissue engineering, with poor angiogenesis, inadequate oxygen transport will lead to the death of cells in the central area. This is due to the lack of oxygen in the center of the tissue, and if they are far from the blood vessel >200 μm, they will begin to necrosis [114]. In the realm of stem cell therapy, significant attention has been directed towards angiogenesis. For instance, Schlosser et al. [115] conducted studies where BMSCs were transplanted into ischemic mice tails, resulting in notable enhancements in functional capillary density and blood flow at the ischemic loci. This improvement is attributed to the secretion of proangiogenic cytokines by bone marrow-derived circulating MSCs, particularly vascular endothelial growth factor (VEGF), recognized as pivotal in angiogenesis [116]. VEGF also serves as a prominent therapeutic target for anti-angiogenic treatments in various diseases, including tumors, underscoring its crucial role in vascular development.

Among the mechanisms exploited by stem cells in tissue engineering, immunoregulation, anti-inflammation, and anti-apoptosis are paramount (Fig. 3). MSCs exemplify these functions by secreting diverse soluble factors that suppress the activation, proliferation, and differentiation of immune cell subsets such as T cells, B cells, and macrophages [117]. MSCs also interact with regulatory T cells (Tregs) and monocytes, pivotal in their immune-modulatory capacities [118]. Notably, the specific immunoregulatory roles of distinct stem cell types, such as dental pulp stem cells (DPSCs) *vs.* BMSCs, warrant further investigation [117]. Bone immune regulation and microenvironment are the focus of research. Macrophage polarization can promote bone regeneration [93,119]. The biological scaffold material can influence the microenvironment, thus inducing osteogenesis and soft tissue differentiation. For instance, Chen et al. fabricated a xenogeneic hard tissue scaffold based on Porcine treated dentin matrix (pTDM) xenogeneic TDM-polycaprolactone (xTDM/PCL) and modified the scaffolds by Ceria nanoparticles (CNPs) (xTDM/PCL/CNPs). Regeneration of teeth evaluated on beagles demonstrated that xTDM/PCL/CNPs scaffold expedited the calcification inside the scaffolds and helped form periodontal ligament-like tissues surrounding the scaffolds [119].

Dentin is rich in calcium structure, so ossification and stem osteogenic differentiation are important for dentin regeneration. During the process of dentine regeneration, new bone tissue invades the dentine tubules and genes associated with odontogenesis would be upregulated.

In Macalester's work, a hMSC-laden hydrogel-human dentine interface model is developed using extrusion 3D bioprinting, they found that dentine surface could generate changes in cell morphology and to induce osteogenic differentiation of hMSCs. They also noted that dentine surface stimulates ECM production and tissue maturation, as well as collagenous structures [120].

Levels of components in bio-ink also influence the effect of osteogenesis. Wang et al. [64] used gelatin methacryloyl of different concentrations as the carrier of DPSC and founded that DPSCs in 10% GelMA bioprinted structures showed
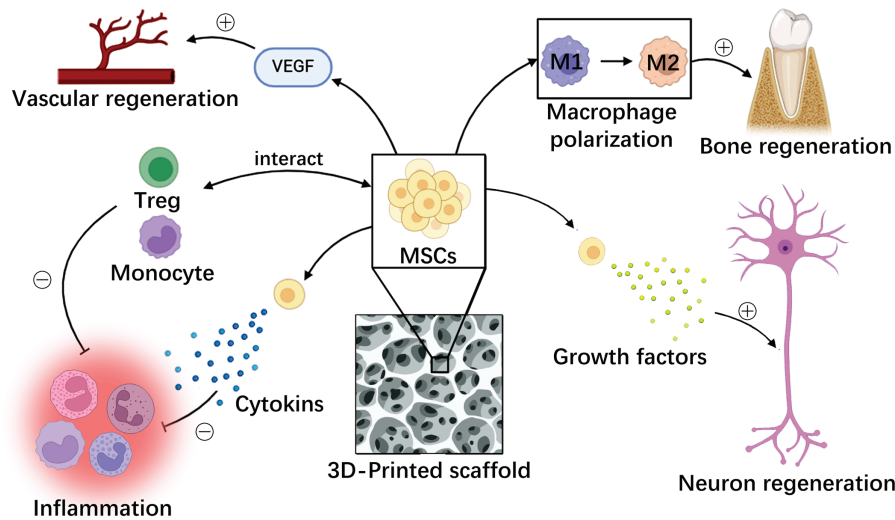
FIGURE 3. MSCs function in various aspects of tissue regeneration. MSCs promote multiple tissue regeneration and inhibit local inflammatory responses through different mechanisms. MSCs: mesenchymal stem cells, VEGF: vascular endothelial growth factor, Treg: regulatory T cells (created by adobe photoshop).

better osteogenic differentiation potential, which was accompanied by higher ephrinB2/EphB4 signal expression levels. Incorporating bioactive glass microsphere (BGM) into hydrogel could improve its bioactivity, and could promote the formation of mineralized nodules and osteogenic differentiation of mBMSCs. The incorporation of BGM into the scaffold resulted in enhanced cell adhesion, proliferation, alkaline phosphatase (ALP) activity, calcium nodule formation and the promotion of osteogenesis-related gene expression (OPN and Runx2). *In vivo* periodontal repair experiment revealed that mBMSCs and growth factor-laden 3D-bioprinted scaffolds promoted the reconstruction of periodontal tissue significantly [121].

MSCs additionally exhibit anti-inflammatory effects by releasing IL-1, TNF-α, and interferon-γ, thereby mitigating inflammatory responses in target areas [13,60]. In studies on myocardial infarction, BMSCs have been observed to upregulate the cell survival gene Akt during ischemia, particularly potent following transient hypoxia, and confer protective effects via paracrine mediators, including inhibition of left ventricular remodeling [122].

In addition, stem cells contribute to tissue repair by secreting growth factors like glia-derived neurotrophic factor, brain-derived neurotrophic factor, neurotrophin-3, and nerve growth factor b, promoting neurite outgrowth and neuronal survival *in vitro* [123]. This capability underscores their potential in facilitating tissue regeneration.

The application of stem cells in tissue engineering may undergo modifications when employed in 3D bioprinting. While the self-assembling process shares foundational developmental pathways akin to natural biological processes [124]. Recent studies have highlighted distinct outcomes. For instance, research has demonstrated that MSCs often aggregate into 3D spheres within bioprinted constructs, thereby exhibiting heightened differentiation capabilities [104]. Meanwhile, the utilization of BMP-mimetic peptide-tethered bioinks has been shown to significantly bolster the viability, proliferation, and odontogenic differentiation of hDPSCs [56]. The above studies show that 3D bioprinting

may enhance the occurrence of the above mechanisms in oral diseases.

Summary and Perspective

In the field of stomatology, the regeneration of human teeth and oral tissues poses significant challenges due to their complex organization and specialized environment. The application of 3D bioprinting utilizing stem cells offers promising therapeutic avenues for patient treatment. In this research direction, preliminary successes have been noted in addressing craniofacial bone defects and regenerating periodontal tissues. Regarding craniofacial bone defects, 3D bioprinting leverages materials conducive to cellular growth and tissue formation, thereby promoting effective tissue regeneration while meeting the mechanical demands of hard bone tissues [125]. Various innovative materials have emerged in this context. Furthermore, enhancing angiogenesis is crucial, particularly in treating large bone defects. Consequently, research efforts have focused on technologies to stimulate angiogenesis and vascular anastomosis [52]. In terms of the application of periodontal tissue regeneration, although it is difficult to cultivate a variety of tissues with correct structures at the same time, successful examples exist where scaffolds have facilitated the cultivation of the periodontium complex, encompassing alveolar bone, periodontal membrane, and periodontal ligament [49]. These reports indicate that 3D bioprinting using stem cells also has good application prospects in the field of stomatology. Beyond oral diseases, Liu et al. [126] demonstrated the feasibility of *in situ* 3D bioprinting using BMSCs and methacrylated hyaluronic acid/polycaprolactone incorporating kartogenin/β-TCP for osteochondral defect repair in rats osteoarthritis.

The utilization of bioink in 3D bioprinting plays a pivotal role in enhancing cell differentiation and tissue formation, thereby significantly augmenting the efficacy of tissue repair. Notably, the hydrogel component within bioink serves dual purposes: it acts as a scaffold matrix for structural support
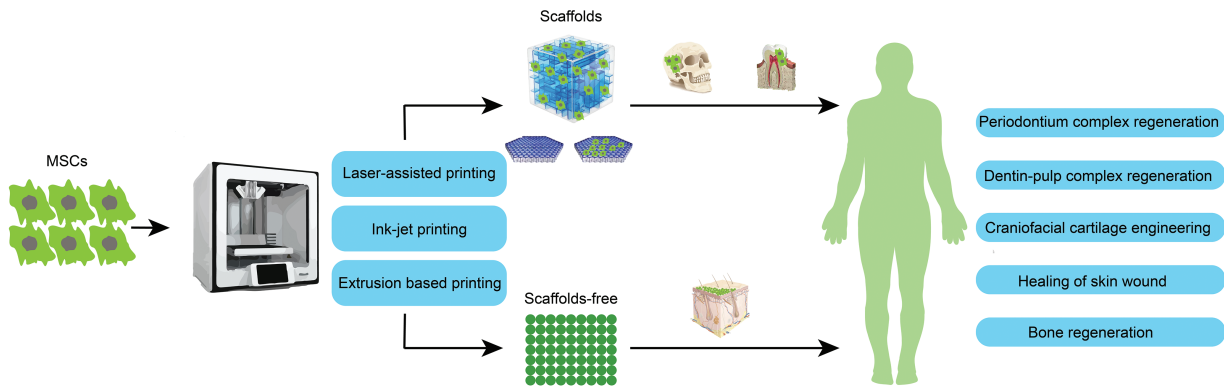
FIGURE 4. Overview of 3D bioprinting mainstream methods and applications in oral diseases (created by adobe illustrator).

and provides vital protection to living cells throughout the printing process, distinguishing 3D bioprinting technology in its capability. By integrating stem cells and biomaterials, such as hydrogels, with precision, this technology can more accurately replicate designed tissues and organs, thereby enhancing the functionality of stem cells and advancing tissue repair outcomes beyond those achievable through traditional bioengineering methods.

Presently, limitations in stem cell application primarily revolve around ethical concerns, cell quality control, specific usage standards, and associated controversies. The International Society for Stem Cell Research (ISSCR) guidelines serve as critical references, offering researchers guidance on ethical considerations and other pertinent issues in stem cell research [127]. Additionally, the adoption of induced pluripotent stem cells (iPSCs) presents a promising avenue. iPSCs, derived without ethical quandaries, possess self-renewal capabilities and the potential to differentiate into all three germ layers (ectoderm, mesoderm, and endoderm) [128]. Efforts towards iPSC banking and rigorous quality control initiatives in many countries ensure a stable and accessible source for research. The emergence of iPSC banks also makes their source more stable and easier to obtain than other stem cells [129]. Nonetheless, due to unique cellular attributes, iPSCs present vast opportunities for advancement in their application within 3D bioprinting compared to other stem cell types [35].

Finally, the current studies on 3D bioprinting using stem cells are almost all *in vitro* and in animal experiments. Rare studies and clinical studies are needed to confirm the effectiveness of such therapies in the future. However, a large number of studies have confirmed that stem cells have the ability to promote tissue repair and have important application prospects in tissue repair. In addition, the emergence of new materials opens up the prospect of treating more oral diseases, for example, Xin et al. reported a photoactive nanomaterial that can be applied to the surface of tooth enamel and can eliminate bacteria and biofilms, which may play a role in the treatment of dental plaques, enamel demineralization, dental caries, and periodontitis [130]. However, at present, the application of 3D bioprinting technology in nanomaterials is limited, and new breakthroughs are still needed [131]. With the deepening of research, the ethical problems of cells, the

quality control of cells, and the continuous improvement of printing technology, 3D bioprinting using stem cells technology will have good application prospects in human oral diseases (Fig. 4).

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