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Quintessential commence of three-dimensional printing in periodontal regeneration-A review.

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Abstract The prime focus of regenerative periodontal therapy is to reconstruct or regenerate the lost periodontium, including both hard and soft tissues. Over the years, periodontics has witnessed different regenerative modalities, such as bone grafts, guided tissue membranes, growth factors, stem cell technology, 3D printing, etc. 3D printing is a newly emerging manufacturing technology that finds applications in diverse fields, including aerospace, defense, art and design, medical and dental field. Originally developed for non-biological applications, 3D printing has undergone modifications to print biocompatible materials and living cells to minimize any potential compromise on cell viability. Thus, the utilisation of 3D printing in the regeneration of lost periodontal tissues represents a novel approach that facilitates optimal cell interactions and promotes the successful regeneration of biological tissues.

1. Introduction

Periodontitis is a disease that gradually affects the periodontium, leading to severe attachment loss and bone loss. In addition, the supporting tissues are highly destructed, altering the integrity of the periodontium. Thus, if left untreated, periodontitis may result in impaired functional integration of the periodontium, eventually contributing to teeth loss. The primary objective of periodontal therapy is to reconstruct or regenerate the damaged periodontium, ensuring its health and stability. Over the last several decades, various treatment modalities have been suggested and implemented for the restoration of the lost periodontal tissue complex. These approaches include the use of techniques, such as bone grafts, guided tissue membranes, growth factors, and stem cells (Lakkaraju et al., 2017). A cutting-edge technology that has emerged in the field of periodontal regenerative therapy is 3D printing, also referred to as additive manufacturing. It involves constructing an object in multilayers (Ma et al., 2019). 3D printing technology finds extensive applications across various fields, such as art and design, aerospace, defense, as well as in the medical and dental sectors. Meanwhile, in the realm of periodontal regenerative therapy, 3D printing holds immense promise for the regeneration and functional integration of the destructed periodontal tissue complex.

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This review article highlights the fundamentals of 3D printing and its pivotal role in advancing periodontal regeneration.

2. Definition of 3D printing

The term 3D printing refers to a manufacturing approach that builds objects by sequentially adding layers by layers to form the final product. This process is commonly described as additive manufacturing or rapid prototyping (Carter et al., 2017).

3. History of 3D printing

3D printing technology was first introduced in 1986 by its inventor Charles W. Hull and Raymond S. Freed. This led to the establishment of DTM Corporation in 1987, aimed at commercialising selective laser sintering (SLS) technology (Oberoi et al., 2018). Another pioneering system, known as the stereolithography apparatus, emerged in 1988 as the first commercial 3D printing system available in the market. Further, Stratasys Inc. was founded in 1989 and has developed fused deposition modelling technology. This method was initially developed by Scott Cramp in 1988, and the patent for it was awarded in the U.S. in 1992. Meanwhile, the first commercially available inkjet printing technology was developed in the year 1993, marking a significant milestone in the advancement of 3D printing (Oberoi et al., 2018).

4. Types of 3D printers

3D printers can be broadly classified into three major categories: inkjet printing, extrusion printing, fused deposition modeling, light-assisted printing, and electrospinning Table 1.

In inkjet printing, a controlled flow of biological fluid, typically polymer, is allowed to pass through an orifice on the printer head using acoustic, thermal, or electromagnetic force. This process facilitates the binding of the polymers to form a multilayered object with the help of a binder (Asa'ad et al., 2016).

Extrusion printing, a pressure-driven technology, utilizes an extruder that operates through either pneumatic or mechanical pressure to continuously deposit the biomaterial. The method involves a temperature-controlled system for material handling, a dispenser, and the stage (Asa'ad et al., 2016).

The fused deposition modeling (FDM) technique employs a mechanical extruder to continuously deposit the materials. It uses a heated thermoplastic semiliquid substance to create 3D objects with multiple layers. This type of printer is widely used in the fabrication of multiphasic scaffolds for periodontal regeneration (Asa'ad et al., 2016).

Light-assisted printing is a category of 3D printing that relies on the polymerisation of biomaterials. It is further subdivided into various types, including laser-based printers, stereolithography techniques, and direct light processing (DLP). The stereolithography technique involves the use of a perforated platform, liquid polymer, and either UV or laser light. In this process, a beam of laser is used to cure the first layer of liquid resin, forming the first layer of the object on a perforated platform. Subsequently, additional layers are added in sequence, continuing this process until a complete 3D-printed object is formed. Meanwhile, DLP is based on the optical principle, utilising a light projector emitting light at different wavelengths to cure and solidify a material, resulting in the formation of a 3D product (Asa'ad et al., 2016).

Electrospinning has become one of the most prevalent techniques, alongside FDM technology, to fabricate scaffolds in the field of regenerative dentistry. This method involves the use of components such as a syringe, needle, and collector plate. A polymer solution is condensed to form the fibrous scaffold under a high-voltage electric supply. The obtained scaffold is then collected on the collector plate for further use (Ma et al., 2019).

5. Types of 3D printing

5.1. Direct 3D printing

Direct 3D printing is a method that enables the direct construction of objects. The technique allows for the deposition of cells, extracellular matrix, and bioactive molecules directly during the printing process. Furthermore, it offers the capability to fabricate 3D scaffolds with extracellular matrix and cells (Carter et al., 2017).

5.2. Indirect 3D printing

Indirect 3D printing involves a two-step process where a wax mold is initially printed and later used to cast with the final polymer. In this approach, a computed tomography (CT)

Table 1 Comparison of different 3D printers: (Ma et al., 2019).

Contents	Inkjet printer	Extrusion printer	Light-assisted
Gelation methods	Chemical, Photo cross-linking	Chemical, Photo cross-linking, shear thinning,	Photo cross-linking
Print speed	Medium (50–60 mm/s)	Slow (40–150 mm/s)	Fast Up to 550 mm/s
Droplet size	50 µm wide	5 µm–1 µm wide	1 µm wide
Mechanical integrity	Poor	Poor	Excellent
Cell viability	$>85\%$	40–80%	85–95%
Cell densities	Low $< 10^6$	High	Medium
Material viscosity	3.5–12 mPa/s	30 mPa/s	1–300 mPa/s
Printer cost	Low	Medium	High

image of the patient's defect can be used to fabricate a wax mold, which is subsequently cast to form a scaffold. For instance, a 3D wax mold can be prepared to fabricate a fiber-guiding scaffold for preserving the alveolar ridge in the extraction sockets (Park et al., 2014a, 2014b).

5.3. *3D printing with live cells*

This method allows for the 3D printing of living cells. Cell aggregates can be incorporated into the 3D-printed scaffolds. Additionally, these scaffolds have the potential to promote cell signaling and tissue formation (Kačarević et al., 2018).

6. 3D printing in periodontal regeneration

Tissue engineering is an approach aimed at regenerating lost or damaged tissues and organs. In 2008, Mason and Dunnill defined tissue engineering or regenerative medicine as a therapeutic strategy for replacing or regenerating human cells, tissues, or organs with the goal of restoring or establishing their normal function. In addition, Brower et al. (2015) defined tissue engineering as the process to stimulate the regeneration of tissues and organs by either implanting biomaterial for in vivo regeneration or by constructing substitutes in vitro (Asa'ad et al., 2016).

6.1. *3D scaffold design in periodontal regeneration*

A scaffolding design that closely resembles the complex shape of periodontal tissues poses a notable challenge in regenerative periodontal therapy. Ceramic biomaterials and synthetic polymers are intensively employed in periodontal regeneration applications. Among the polymers used in 3D printing, polycaprolactone (PCL) blended with either polymers or ceramics (calcium phosphate, CaP and bioactive glasses) stands as a commonly utilised option.

6.1.1. *Ideal requirements of scaffolds:*

- o Biocompatible and biodegradable
- o Promotion of cell growth and maturation
- o Porous 3D framework, facilitating cell attachment, migration, proliferation, and differentiation. The pore sizes of the scaffold play a major role in nutrient diffusion and vascularisation, with a minimum recommended pore size of 100 μm
- o Adequate degree of hydrophilicity
- o Surface roughness and specific topography
- o Mechanical strength
- o Bone and periodontal attachment formation and integration
- o Facilitation of cementum formation onto the root surface
- o Orientation and attachment of periodontal ligament (PDL) fibers into the regenerated bone and cementum (Dawood et al., 2015)

6.2. *Monophasic Scaffolds*

Scaffolds containing a single compartment are said to be monophasic scaffolds. These can be loaded with cells or growth factors to promote the regeneration of the periodontal tissues. In a study conducted by Kim et al., in 2010), 3D-printed anatomically shaped scaffolds for human molars and rat incisors were fabricated via extrusion printing using a combination of polycaprolactone and hydroxyapatite. The fabricated scaffolds were loaded with SDF-1 and BMP-7, and implanted orthotopically and ectopically in the extracted incisors and dorsum of the rats, respectively. The findings indicated that the use of these loaded scaffolds resulted in improved angiogenesis and regeneration of periodontal tissues.

In addition, Baba et al., (2011) fabricated a 3D-printed woven fabric scaffold using polylactic acid and injected it with bone marrow-derived mesenchymal cells and platelet-rich plasma. The scaffold was then implanted into the experimentally created mandibular bone defects in dogs. The study found improved regeneration of the periodontal tissues within the created defects.

6.3. *Biphasic scaffolds*

Biphasic scaffolds are designed to have distinct compartments for the PDL and bone, facilitating the formation of human tooth periodontal ligament-bone complexes. These scaffolds are typically fabricated via indirect 3D printing techniques (Park et al., 2018). The process involves creating wax molds, which are later cast to produce biphasic scaffolds with the desired architecture. The characteristics of the wax molds differ in terms of pore size, the orientation of the channels, and tissue-specific compartments. In a study by Park et al., (2018), a biphasic scaffold comprising both bone and PDL compartments was developed for the treatment of fenestration defects in rats, showing controlled and predictable periodontal regeneration.

6.4. *Cell sheet technology in combination with 3D printing*

Cell sheet technology can be integrated into 3D-printed scaffolds. In this direction, Vaquette et al., (2012) introduced this technology in combination with 3D printing, where they fabricated a biphasic scaffold consisting of bone and periodontal compartments. The bone compartment was formed by PCL containing beta-tricalcium phosphate (β-TCP), while the micro-fibrous membrane was used to deliver PDL cell sheets. Further, the scaffolds underwent heat treatment to achieve fusion. The process resulted in the notable deposition of the extracellular matrix and increased mechanical stability of the cell sheets. However, the bone compartment did not exhibit ectopic bone growth.

In order to enhance the osteoconductive properties, Costa et al. (2014) applied a CaP coating on the bone compartment of the scaffold. Additionally, they used a melt electrospun membrane with a large pore size to enhance the communication between the PDL and alveolar bone.

6.5. *Triphasic scaffolds*

Triphasic scaffolds represent an extension of biphasic scaffolds and were created by Lee et al. (2014) using the FDM technique. The scaffold design includes specific compartments for the cementum/dentin interface, PDL, and alveolar bone, allowing the controlled delivery of recombinant human amelogenesis, connective tissue growth factor, and bone morphogenetic protein 2 (BMP-2). The scaffolds promoted the successful regeneration of periodontal tissues; however, their inherent stiffness hindered their optimal adaptation to the complex anatomy of periodontal osseous defects.

7. Steps involved in 3D printing of Scaffolds

1. 1 Data acquisition of periodontal osseous defects: It involves the scanning of the periodontal defects using CT or cone beam computed tomography (CBCT) to obtain the necessary data.
2. Scaffold designing in Stereolithography (STL) file format: Once the data are acquired, it is transferred to STL file format, and the designing process of the scaffolds is initiated based on the specific presence of defects. Depending on the requirements, either biphasic or multiphasic scaffolds are designed.
3. 3D printing of scaffolds by 3D printers: The designed scaffold is then transferred to the 3D printers for the fabrication process. The choice of 3D printing technology depends on the biomaterials used for periodontal regeneration. The 3D printing process involves building the scaffold layer-by-layer, gradually forming the desired 3D structure.
4. Post-processing: Once the predetermined 3D printing is completed, post-processing of the printed scaffolds is performed, which includes removal of the support material, sandblasting, and grinding. In the case of metallic 3d objects, heat treatment may be required to optimize their properties. (Dawood et al., 2015) Fig. 1.

8. Applications of 3D printing in periodontal regeneration

8.1. Education of patient and dental students in periodontal regenerative Procedures:

Utilising 3D-printed models (such as VANPERIO), patients can be effectively educated about the intricacies of their treatment plan. These 3D models enable patients to grasp the complex treatment procedures involved in achieving successful regeneration of periodontal tissues. The VANPERIO model can also be utilised to carry out regenerative procedures using bone grafts and membranes. This can assist clinicians to understand the complexity of the periodontal defect, enabling them to formulate an appropriate treatment plan that attain the comprehensive regeneration of the periodontal tissues (Prasad and Vandana, 2019).

8.2. Socket preservation

3D-printed scaffolds have shown potential in socket preservation of the alveolar ridge following tooth extraction. A study conducted by Goh et al. (2015) evaluated the efficacy of a prefabricated 3D PCL scaffold, printed using FDM, in socket preservation. The scaffold demonstrated the ability to maintain the structure and integrity of the alveolar ridge over a period of 6 months. Besides, custom-made 3D scaffolds exhibit high potency in comparison to prefabricated scaffolds. However, these scaffolds have been observed to cause soft tissue dehiscence with minimal bone repair. This could be probably

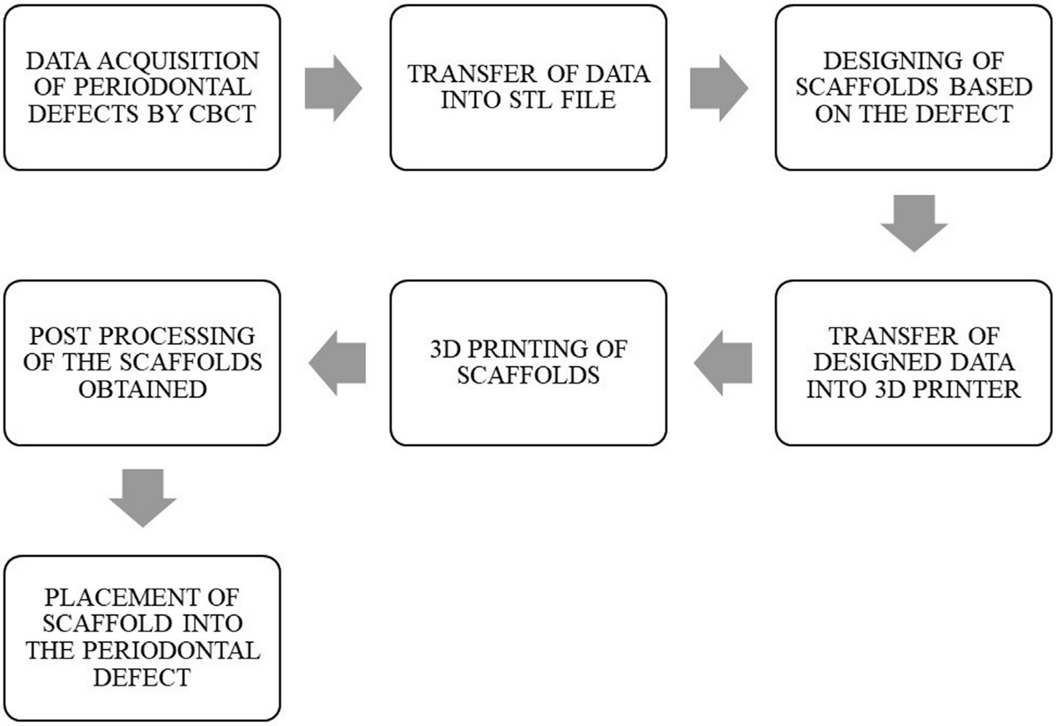

Fig. 1 Schematic illustration of 3D printing of scaffolds in periodontal regeneration.

attributed to the slower degradation of the PCL scaffolds, weak osteoconductive effect, and limited cell affinity. Further, Kijartorn et al. (2022) assessed and compared the efficacy of a 3D-printed nanoporous hydroxyapatite bone graft with a nanocrystalline bone graft for alveolar ridge preservation. The findings revealed that the former exhibited reduced dimensional changes in soft tissue surface resorption when compared with the latter.

8.3. Fenestration defects

The use of 3D-printed scaffolds extend to the treatment of fenestration defects as well. In this direction, Park and Coworkers (2014a, 2014b) evaluated the effectiveness of the 3D-printed amorphous and fiber-guiding PCL scaffolds in the regeneration of surgically created fenestration defects on the buccal side of the mandible in rats. After the surgical placement of the 3D-printed scaffolds, the adaptation ratio and quantity of mineralised bone formation in the fiber guiding scaffolds were also analysed through micro-CT examination. Notably, the fiber-guided scaffolds showed the regeneration of the mineralised tooth structures in the vicinity of the tooth defect. Based on these observations, the authors concluded that fiber-guided 3D-printed scaffolds promoted the successful regeneration of lost periodontal tissues.

8.4. Peri osseous defects

Furthermore, 3D-printed scaffolds can also be utilised in the management of *peri*-osseous defects. These scaffolds help in the fabrication of customised structures that precisely match the size, configuration, and architecture of the *peri*-osseous defects. Along these lines, Rasperini et al. (2015) examined the impact of a 3D-printed PCL scaffold produced via SLS in the management of *peri*-osseous defects. The scaffold featured extended pegs to guide PDL formation, along with an internal port for the delivery of human recombinant platelet-derived growth factor. The scaffolds contributed to improvement in clinical attachment level and partial root coverage of up to 12 months. Nevertheless, the long-term assessment at 13 months revealed the instability of the scaffold, resulting in dehiscence and impaired wound healing. Consequently, this study did not demonstrate long-term success for the regeneration of osseous defects and it is imperative to conduct further clinical trials to validate the effectiveness and durability of 3D-printed scaffold designs.

Additionally, in a scoping review conducted by Mohd et al. (2022), which encompassed 10 animal studies, it was observed that 3D-printed scaffolds made of hydroxyapatite and TCP showed promising outcomes in terms of new bone formation for the treatment of intraoral bony defects.

According to Adel-Khattab et al. (2018), the 3D-printed bio ceramic-based bone graft material fabricated from silica-containing calcium alkali orthophosphate possesses mechanical and physical scaffold properties that close resemble those of in vitro autogeneous bone grafts. These findings suggest that this material holds the potential for successful in vivo regeneration of bone tissues.

In another attempt, Carlo Reis et al. (2011) determined that the bilayered scaffold consisting of the PLGA-CaP complex exhibited a strong framework suitable for addressing grade II furcation defects to promote successful periodontal regeneration.

8.5. Sinus augmentation

Utilising 3D-printed scaffolds in sinus augmentation procedures as a substitute for bone grafts has shown promise, providing a viable alternative. This approach resulted in complete bone regeneration, accompanied by a notable osteoconductive effect. In this regard, Mangano et al., (2015) evaluated the effect of a custom-made 3D synthetic substitute of bone composed of CaP ceramic, manufactured using a rapid prototyping method for monolateral sinus augmentation in a sheep model. The authors obtained block sections from the grafted area at 45 days and 90 days of postoperative healing. It was concluded that a 3D-printed CaP bone substitute resulted in complete bone regeneration from the periphery to the centre of the sinus cavity with significant osteoconductive properties. In addition, (Stoyanova et al., 2022) found that 3D surgical models created through the printing of preoperative CBCT images proved to be a highly reliable educational tool among dental students and practitioners. These models were particularly effective in simulating endoscopically navigated maxillary sinus floor augmentation procedures.

8.6. Ridge augmentation

In an in vivo study conducted by (Park et al., 2018) the effect of 3D-printed PCL in combination with β-TCP was evaluated for alveolar ridge augmentation in surgically created saddle-type bone defects following extraction of mandibular premolars in four beagle dogs. In their analysis of two different 3D PCL scaffolds, one with a 400/400 lattice and the other with a 400/1200 lattice, more new bone formation was observed adjacent to the PCL scaffolds with the 400/1200 lattice configuration compared to 400/400 lattice, although the difference was not statistically significant. Further, it was mentioned that 3D PCL scaffolds maintained the physical space and promoted new bone formation adjacent to the scaffolds.

Interestingly, (Sumida et al., 2015) determined that a custom-made titanium meshwork fabricated through selective laser melting printing is a simple and secure protocol for guided bone regeneration, offering a novel protocol to reconstruct the lost alveolar bone prior to implantation.

In their review, Yen and Stathopoulou (2018) discussed Computer aided design & Computed aided manufacturing and 3D printing in alveolar ridge augmentation for optimal implant placement. They highlighted that 3D printing enables the fabrication of block grafts in a layer-by-layer approach. One key advantage of 3D printing over CAD/CAM is the minimal wastage of bio-printing material. Moreover, it was concluded that 3D printing plays a crucial role in the production of corticocancellous allogenic bone grafts and customised titanium shells with different micro and macroporosities for effective horizontal and vertical bone augmentation around dental implants.

8.7. 3D printing in Peri-implant regeneration

Won et al. 2016 compared the extrusion-based 3D-printed PCL/PLGA/β-TCP membrane and collagen membrane in the context of bone regeneration in a beagle implant model. The authors observed an increased tensile strength in the collagen membrane, while a decreased elastic modulus in the 3D-printed PCL/PLGA/β-TCP membrane. The proliferation analysis and osteogenic differentiation analysis revealed similar results for both membranes. However, the 3D-printed PCL/PLGA/β-TCP membrane showed an increased volume and rate of new bone formation. Based on these findings, the authors concluded that the 3D-printed PCL/ PLGA/β-TCP membrane possesses comparable effects to the collagen membrane in the regeneration of the *peri*-implant defects.

9. Limitations

- High cost of 3D printers
- Need for optimising the printing process for biocompatibility
- Possesses low resolution
- Requirement for the development of suitable biomaterials
- Insufficient mechanical properties of the scaffolds
- Challenges in maintaining cell viability within the printed scaffolds

10. Conclusion

3D printing is rapidly expanding and showing promising potential in the field of regenerative medicine, including periodontal regeneration. As a result, 3D printing is slowly shifting the focus of periodontal regeneration from conventional regenerative procedures by achieving the successful and long-term regeneration of lost periodontal and *peri*-implant tissue structures. Moreover, there is currently limited scientific literature available in the field of periodontics that specifically addresses the role of 3D printing in regeneration. Animal studies fail to show significant results in human trials. Hence, further human clinical trials are necessary to provide substantial evidence and support the efficacy of 3D printing in periodontal regenerative therapy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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