



(REVIEW ARTICLE)



3D printed scaffolds in bone tissue engineering and their application in periodontology

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Abstract

Scaffolds are three-dimensional (3D) porous, fibrous, or permeable biomaterials that promote cell interaction, viability, and extracellular matrix (ECM) deposition while causing minimal inflammation and toxicity and bio-degrading at a controlled rate. This narrative review focuses on 3D scaffolds, the first key component of the tissue engineering paradigm. It describes the classification of scaffolds, their properties, biomaterials used in their fabrication, and in brief their periodontal application.

Keywords: Scaffold 3D Cell Culture; Tissue engineering; Periodontics; Dental Implants

1. Introduction

Bone is the most transplanted tissue worldwide, with at least four million operations using bone grafts and bone substitute materials each year¹; however, limitations such as:

Autografts are limited in size and carry the risk of donor site morbidity, including infection and ongoing pain after surgery.

Allograft tissue harvested from cadaveric and living sources carries the risk of disease transmission and immune response while lacking a cellular component to aid tissue regeneration.

Metalwork and bone substitutes can also be used to aid bone regeneration, but they have drawbacks such as bone thinning due to stress shielding, wear, failure over time, and the risk of revision surgery.²

Significant research is being conducted in the field of bone tissue engineering, including the fabrication of 3D-printed scaffolds, to overcome the limitations. Bone tissue engineering is a subfield of tissue engineering that focuses on improving bone regeneration and repair by developing substitutes for traditional bone grafting materials (BTE). (Proliferating cells, mechanical loading, pathogen control, biocompatible scaffolds, biological signals, and an adequate blood supply are key components of tissue engineering.)

Scaffolds are three-dimensional (3D) porous, fibrous, or permeable biomaterials that promote cell interaction, viability, and extracellular matrix (ECM) deposition while causing minimal inflammation and toxicity and bio-degrading at a controlled rate.³

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The multiphase scaffolds of the periodontium, which include both hard (bone and cementum) and soft tissues (gingiva and PDL), are not only specific for the tissue but also mechanically competent.

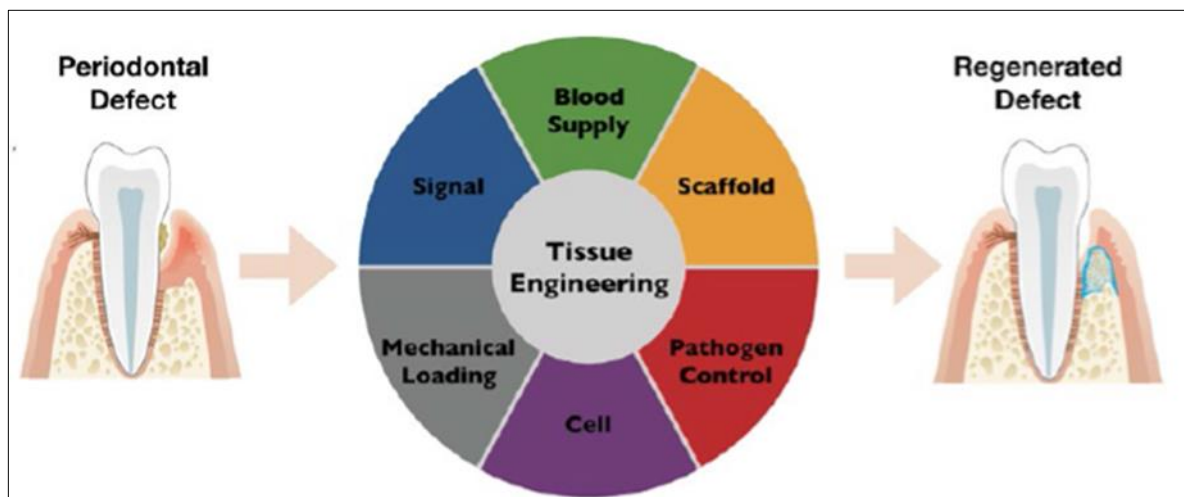


Figure 1 The components of tissue engineering

To replicate multi-phase tissue compositions, 3D printing has recently been used to fabricate scaffolds with regionally variant internal microstructures suitable for Cementum, PDL, and/or Alveolar Bone. Furthermore, various growth factors can be combined with 3D-printed scaffolds to aid in the regeneration of each tissue in the periodontium. Aside from the internal microstructure, layer-by-layer deposition 3D printing allows for the creation of a custom-designed scaffold in a specific shape and dimension that fits the anatomic shape of each periodontal defect.

This narrative review focuses on 3D scaffolds, the first key component of the tissue engineering paradigm.

2. Classification of scaffolds ³

2.1. Based on their geometry

- Porous
- Fibrous
- Microsphere/microparticle
- Solid free-form scaffolds

2.2. Based on the source material

- Alloplastic synthetic scaffolds
- Hydrogel scaffolds
- Natural tissue scaffolds

2.3. Scaffolds for hard tissue application

- Non-biodegradable and resorbable metal scaffolds
- Low degradable bioceramic, glass, and glass-ceramic scaffolds
- Polymer scaffolds
- Particle loaded polymeric and other composite scaffolds

2.4. Scaffolds for soft tissue application

- Synthetic polymer scaffolds
- Natural polymer scaffolds
- Natural-synthetic polymer blends and composite scaffolds

2.5. cell-laden scaffolds

2.6. Porous scaffolds

- The presence of interconnected pore structures aids in bone regrowth, vascularization, and ECM deposition.
- The channel network enhances gas and nutrient transport.
- For proper cell growth, in-growth, and nutrient flow Pore interconnectivity must be increased.
- Cellular penetration, ECM deposition, and neovascularization would be prevented if the pores were too small.
- Ideally, the interconnected porous structure should have at least 90% porosity, which may significantly impact the resulting mechanical properties.
- The unique properties of porous scaffolds could be obtained by employing methods that allow for pore size control.

These methods are as follows: ³

Inverse opal hydrogenation, which employs colloidal particles as templates to create ordered microporous scaffolds.

Cryogelation with frozen solvent crystals acting as interconnecting pyrogens

2.7. Fibrous Scaffolds

- Fibrous scaffolds made from biodegradable polymers such as PCL, PLA, PLGA, gelatin, cellulose, and silk fibroin have been successfully created.
- Fibers have the desired properties as scaffolds for skin, cartilage, ligament, bone, muscle, vein, drug, DNA, and protein delivery vehicles.
- Nanofiber scaffolds are found to be more promising biomaterials than microfiber scaffolds because the nanosize induces cells to acquire typical in vivo morphology.³
- These nanofibres mimic the structure and properties of the extracellular matrix, and they induce greater cellular attachment than microfibrils due to their high aspect ratio, porosity, and surface-to-volume ratio. The nanostructure could aid in the rapid diffusion of encapsulated substances as well as cell infiltration.³

2.8. Microsphere/microparticle scaffolds

These scaffolds are extensively used in advanced applications such as gene therapy, controlled drug and growth factor delivery, and site-specific targeting.

Polymers with low molecular weight exhibit rapid drug release, whereas high molecular weight microspheres exhibit slower release.

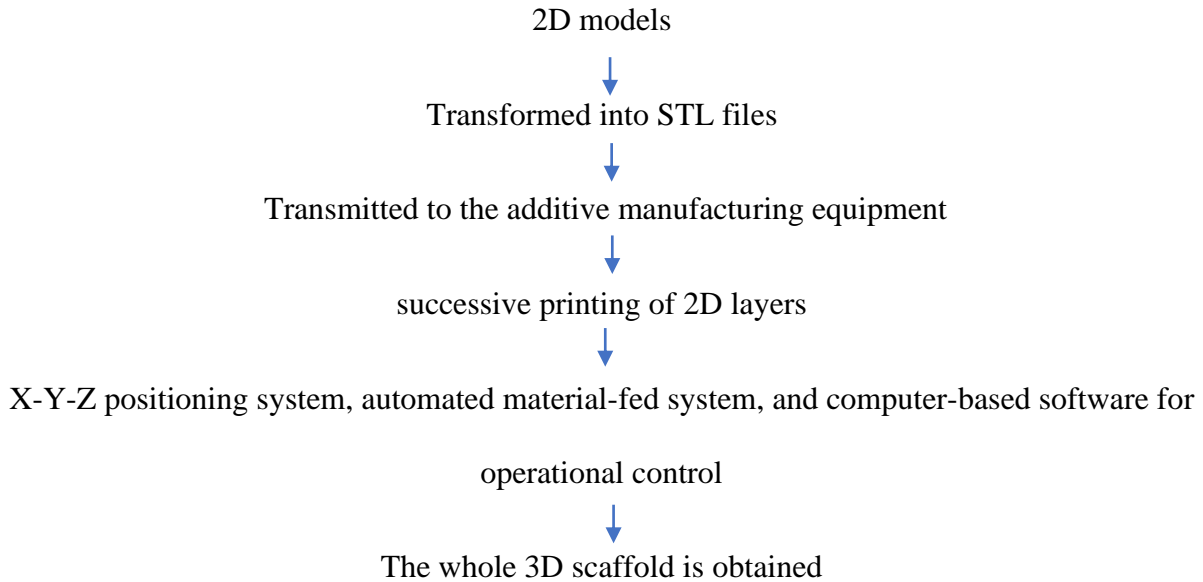
Microspheres can release encapsulated bioactive substances that are embedded as building blocks within 3D scaffolds.

Various methods, including solvent vapor treatment, solvent/non-solvent sintering, oil-in-water dispersion, and selective laser sintering (SLS), are used to create microspheres and microsphere-based 3D scaffolds.³

2.9. Solid free-form scaffolds

In general, most traditional scaffold production techniques are incapable of producing complex scaffolds with precisely controlled microarchitecture and properties.

Most conventional scaffold production techniques make it difficult to create complex scaffolds with controlled microarchitecture and properties. CAD/CAM-produced 2D shapes are stacked to create 3D scaffolds with controlled architecture and reproducible properties.³



2.10. Alloplastic synthetic scaffolds

Metals, bioceramics, glass and glass-ceramics, synthetic polymers, and a variety of composites are commonly used materials for tissue engineering because they offer nearly complete control over the mechanical properties and architecture of the construct.³

2.11. Hydrogel scaffolds

Hydrogels are made up of covalent or non-covalently bonded hydrophilic polymer chains (held by intermolecular attractions).

When natural (gelatin, fibrin, alginate, agarose, etc.) or synthetic (PEG, PAA, PEO, PVA, etc.) polymers are crosslinked via covalent or non-covalent bonds, these scaffolds form gels.³

Unlike gels, which are more solid than liquid, hydrogels absorb a large amount of water and swell without dissolving. This is due to the inherent crosslinking of hydrogels (using bifunctional monomers), which allows them to retain 3D shape and swell without dissolving.

The greater the extent of cross-linking, the lower the swelling; in the swollen state, they are soft and elastic. Hydrogels have been created using both chemical and radiation crosslinkers.

2.12. Natural tissue scaffolds

Cells, growth factors, and extracellular matrix make up natural tissues (ECM).

The extracellular matrix (ECM) is a heterogeneous hydrophilic 3D matrix that provides a proper microenvironment for cells, accumulates and presents growth factors, directs migratory cells, and participates in mechanical signaling via mechanical receptors (integrins).

The main components of ECM are collagen fibers and proteoglycan filaments (from protein and hyaluronic acid) furnishing the durability and tensile strength of ECM

To take advantage of the benefits of natural ECM, researchers are using the decellularization procedure to remove all cellular components that may cause an inflammatory response in the host. As a result, the remaining ECM retains its composition, architecture, integrity, biomechanical properties, biological activity, and hemocompatibility, and it can direct cell migration, express tissue-specific genes, and control cell fate.³

The process of eliminating cells and their sources is critical for decellularized ECM performance. The decellularized material could be used to keep the entire organ intact or refined further by cutting or digesting into the liquid to form a coat or ECM-containing hydrogel.³

2.13. Non-biodegradable and resorbable metal scaffolds

They are first-generation materials for bone substitutes, with properties such as high mechanical strength, fatigue resistance, and printing processability that make them extremely popular for load-bearing applications when compared to ceramics or polymers.

Titanium, stainless steel, cobalt-chromium (Co–Cr) based alloys, and magnesium are the most commonly used metallic biomaterials (Mg).

Titanium (Ti) is a metallic biomaterial with high biocompatibility, strength, and corrosion resistance.

3D porous Ti scaffolds benefit vascularization, nutrient and gas transport, and cell seeding.³

2.14. Low degradable bioceramic, glass, and glass-ceramic scaffolds

Inorganic calcium or phosphate salts with osteoconductive (promote new bone ingrowth) and osteoinductive (promote osteoblastic differentiation) properties are common in ceramic biomaterials.

Ceramics are divided into three types: inert (non-absorbable), semi-inert (bioactive), and non-inert (resorbable).

They are usually brittle, but they are resistant to compression and corrosion.

Hydroxyapatite (HAP, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), -tricalcium phosphate (-TCP, $\text{Ca}_3(\text{PO}_4)_2$), and bioactive glasses are the most commonly used biomaterials for 3D scaffolds in bone regeneration.

The following techniques are available for ceramics 3D printing:

- Polymer agglomeration, chemical/physical solidification, and thermal processing
- Sacrificial inverse matrix printing, ceramic slurry infiltration, and negative burning
- Traditional methods for making porous ceramic scaffolds, such as the polymer sponge method, salt leaching, dual-phase leaching, gel casting, and so on.
- Because of their similarity to the bone, bio-resorbability, and good biocompatibility, calcium phosphate biomaterials are widely used in dentistry.

2.15. Polymer scaffolds

They are widely used to renovate traumatized tissue due to their unique properties such as biocompatibility, reproducible mechanical and physical properties, workability, and low cost.

Non-biodegradable synthetic polymer scaffolds are rarely used because they require surgical removal; examples include acrylic polymers such as PHEMA, PHPMA, PMMA, and conductive polymers.³

Particle loaded polymeric and other composite scaffolds

These composite scaffolds enable the engineering of biomaterials with suitable mechanical and physiological properties by controlling the reinforcing phase's type, size, fraction, morphology, and arrangement.

The addition of a bioactive phase in the matrix can alter the degradation behavior of the scaffolds.³

2.16. Synthetic polymer scaffolds

These are simple to manufacture under controlled conditions.

These synthetic polymers' physical and mechanical properties include tunable, predictable, and reproducible properties that can be modified to produce customized scaffolds for a specific application.

The most commonly used synthetic polymer for drug, gene, and growth factor delivery is PLGA, a flexible and permeable copolymer of lactic and glycolic acid.

A flexible and permeable copolymer of lactic and glycolic acid, PLGA, is the most commonly used synthetic polymer for drug, gene, and growth factor delivery applications.³

2.17. Natural polymer scaffolds

Natural polymers are biodegradable and bioactive materials that are classified as proteins, polysaccharides, and polynucleotides.

As the main protein in collagen, 3D scaffolds are widely used in cartilage, vasculature, nerve, and muscle regeneration.

Chitosan is a polysaccharide compound derived from the partial deacetylation of chitin, which is primarily obtained from shellfish.

2.18. Alginate is a brown algae-derived polysaccharide.

Because of alginate's superior gelling properties, the polymer can be used as an injectable scaffold to manage damaged cartilage.³

2.19. Natural-synthetic polymer blends and composite scaffolds

Scaffolds made entirely of natural hydrogels fail to maintain the desired 3D shape due to poor mechanical properties. Because of their hydrophobicity, synthetic polymers have low cell affinity and a scarcity of cell recognition sites. However, they are highly adaptable in terms of modification. As a result, mixtures of natural and synthetic polymers have been tried to create scaffolds with improved biodegradability, cell attachment, and hydrophilicity.³

2.20. Cell-laden scaffolds

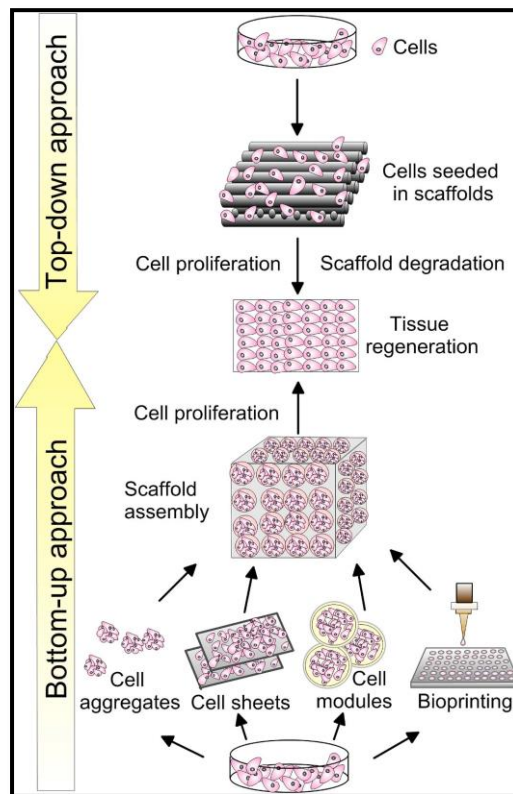
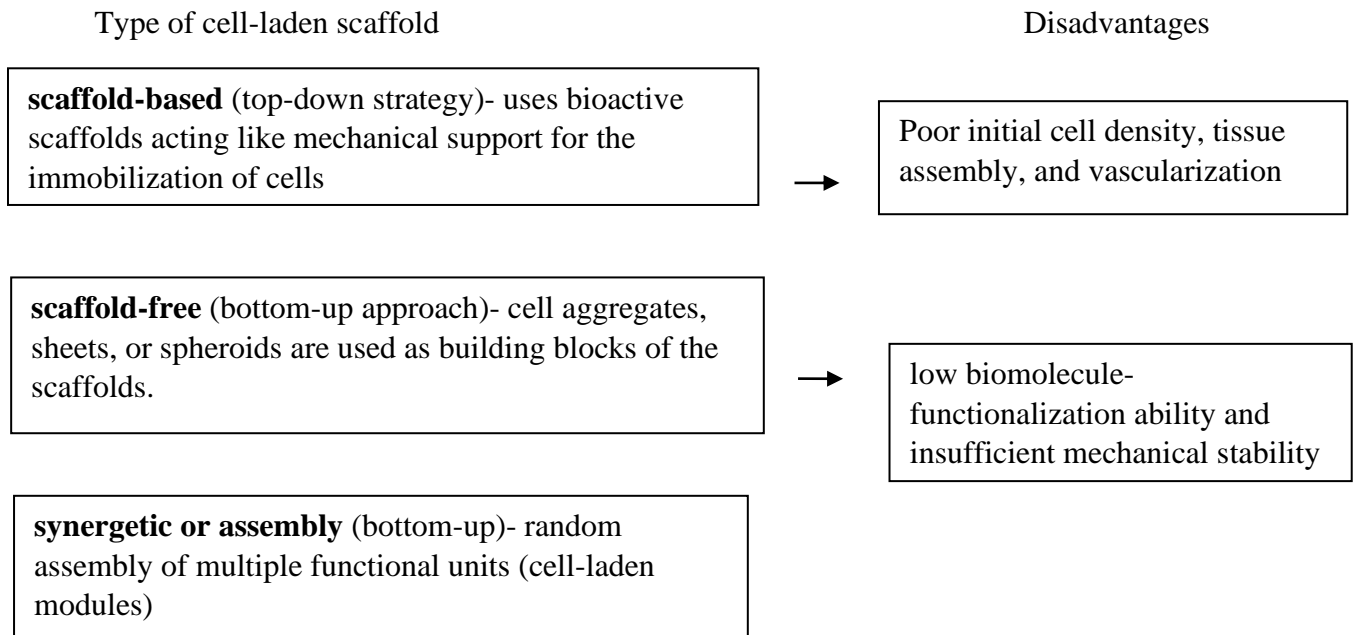


Figure 2 The methods of creating cell-laden scaffolds

Bioprinting enables the creation of pre-defined 3D architectures from living multipotent cells or ECM components that can grow for later implantation.

Additional growth and differentiation factors must be added to the design to keep the cells alive. All living tissue components (cells and ECM) will be achieved.

There are three methods for creating cell-laden scaffolds.



3. Properties of 3D scaffolds

3.1. 3D Scaffolds

Should exhibit an adequate degree of hydrophilicity, Roughness, and Specific surface topography; a topographic landscape on micro-and sub-micrometer scales must be developed to replicate the natural process of bone regeneration

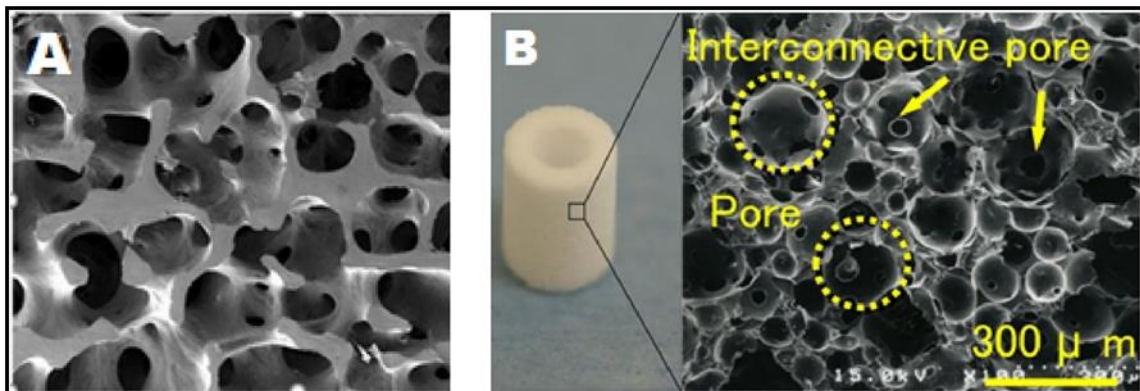


Figure 3 Properties of 3D Scaffolds (Adapted from Doi et al)¹

Must exhibit a Pore size ranging between 30% and 90%, (since human cancellous bone demonstrates a total porosity between 30% and 90%)

Should have a pore diameter ranging between 150 μm and 500 μm (facilitates vascularization and penetration of new tissues without compromising the mechanical strength of the scaffold)

Should have a degradation rate consistent with the remodeling processes of the target tissue

Should be biocompatible and bioactive (used biomaterials should not elicit any inflammatory or cytotoxic reactions) • "Compartmentalization," in the case of alveolar bone regeneration along with cementum and periodontal ligament tissues, is essential for controlling the spatiotemporal events, which could prevent tooth ankylosis.⁴

Table 1 Biomaterials used in the fabrication of 3d scaffolds for alveolar bone regeneration ¹

Biomaterials used	Properties
Biodegradable Natural Polymers	High biocompatibility Good cell recognition Enhanced cellular interactions in the surrounding environment Hydrophilicity
Collagen	The major organic component of the ECM in native bone Promote cell adhesion, proliferation, and osteogenic differentiation of bone marrow stromal cells, in vitro A denatured form of collagen termed gelatin enhances osteoblast adhesion, migration, and mineralization
Chitosan	Antibacterial and antifungal activities Rapid blood clot formation Analgesic properties
Alginate	Biocompatibility Gel forming ability non-toxicity biodegradability easy to process.
Biodegradable Synthetic Polymers.	
1. Polycaprolactone (PCL)	Biocompatibility Suitability for various scaffold fabrication techniques The remarkably slow degradation rate Mechanical stability
Bioceramics	Unlimited availability Bioactivity Excellent biocompatibility Hydrophilicity Osteoconductivity Osteoinductivity
1. Calcium phosphate bioceramics a. Hydroxyapatite b. β -tricalcium phosphate (β -TCP)	a. Hydroxyapatite shares the same chemical composition as native bone minerals, which positively influences the adhesion and proliferation of osteoblasts. b. β -tricalcium phosphate (β -TCP) forms a strong bone-calcium phosphate bond faster degradation rate
2. Bioactive glass (BG)	The very slow degradation rate Brittle and difficult to shape into the desired Structures because of their stiffness and low flexibility and moldability. Weak mechanical strength and fracture toughness

Metals	high strength, toughness, and hardness, in comparison to polymers and ceramics, enhance the mechanical properties of a scaffold by decreasing the pore size titanium-based 3D scaffolds display good hydrophilicity lack of biodegradability (Titanium-based)
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4. Applications of 3d-printed and/or compartmentalized scaffolds in alveolar bone and periodontal tissue regeneration ⁵

The applications are as follows:

- Guided bone regeneration (GBR)
- Guided tissue regeneration (GTR)
- Vertical bone augmentation
- Sinus augmentation
- Guided bone regeneration (GBR)

The majority of preclinical, in vivo, in vitro, and case report studies in the literature have revealed excellent results in periodontal regeneration.

Rasperini et al. reported the use of a 3Dprinted scaffold for the first time in human periodontal defects (labial soft and hard tissue dehiscence). His case report revealed excellent results for the first 12 months but failed after that.

Lei et al reported a significant reduction in pocket depth and bony fill in a 15month follow-up case of guided tissue regeneration using a 3Dprinted scaffold and platelet-rich fibrin for the management of bony defect around maxillary lateral incisor.

Sumida et al reported a shorter procedure time and the need for fewer screws for retention than the commercial mesh group by using a 3Dprinted custom-made device for the bone defect.

There is currently a scarcity of randomized control trials and clinical studies with long-term follow-ups.

4.1. Socket preservation

According to a systematic review, tooth extraction results in alveolar ridge loss of 3.87 mm and 1.67 mm, respectively, but this loss can be avoided by recent technological advances, such as 3Dprinted scaffolds to preserve the socket and maintain the dimension of the extraction socket.

In a study on beagle dogs, Park et al reported a predictable outcome with the use of 3Dprinted polycaprolactone in socket preservation.

Goh et al. used a 3dprinted bioresorbable scaffold in socket preservation in a pilot study and reported normal bone healing and comparatively better alveolar ridge preservation than extraction sockets without scaffold after 6 months.

Kijartorn et al. also reported in a prospective cohort that 3Dprinted hydroxyapatite has potential benefits when used as bone graft material in socket preservation. There is a need for consideration due to the scarcity of clinical studies with long-term follow-ups.

4.2. Sinus and bone augmentation

The following are the benefits of 3D printing that have led to its use in sinus and bone augmentation:

- The additive manufacturing technique allows for the replication of bony architecture and the formation of the macroporous internal structure of the graft with minimal material waste.
- No ethical issues

- Increased availability due to alloplastic material
- Reduced risk of infection transfer
- Reduced surgery chair time

Although there are no randomized controlled trials, multiple case reports and in vivo studies have reported successful outcomes following the use of a 3D scaffold for sinus and bone augmentation.

4.3. Three-dimensional printing for implants placement

Implant placement is a routine procedure to replace missing teeth that, if done incorrectly, can result in a variety of complications such as poor aesthetics, damage to anatomically important structures, infections, and implant failure.

These complications can be avoided with the development of Guided implants, which use 3D printing to fabricate surgical guides. It aids in the accurate 3D placement of implants, protecting anatomic structures, and saving time.

The following are some of the advantages of guided implants:

- Even with the flapless approach, accurate implant placement is possible in partially and completely edentulous patients.
- Reduction in chairside surgical time
- post-surgery patient comfort
- Enables simultaneous implant placement in difficult cases.

Use of three-dimensional printing for peri-implant maintenance

Implant surfaces are unique and necessitate special care when cleaning and maintaining. 3Dprinted implant models can be used to teach patients about implant maintenance.

Use of three-dimensional printing for implant education

3D printing can be used for education purposes, such as ensuring that the patient understands the procedure before consenting to implant placement on a 3Dprinted model. It aids in reducing the patient's anxiety. These models also aid in the education of undergraduate and postgraduate students on implant treatment planning and implant placement without affecting nearby anatomic structures.

5. Conclusion

Personalized or customized regenerative scaffold technologies for periodontal and alveolar ridge regeneration are becoming a reality in clinical practice. Because 3D, high-resolution imaging of both soft and hard tissues is becoming more common, integration with system software for reconstruction at bone-tooth, bone-implant interface, and larger osseous defects of the alveolar ridge and craniofacial complex is becoming more feasible. Bioprinting advances now allow cells, extracellular matrices, genes, and/or biological agents to be delivered onto polymeric, ceramic, and natural biomaterials. These advancements in regenerative medicine technology provide exciting solutions for surgeons and restorative dentists seeking predictable regenerative therapies to improve patient care.

Future perspective

As scalability and manufacturing methods continue to develop, it is hoped that treatment tailored to the individual patient can be produced in an increasingly cost-effective, efficient, and reproducible manner in the future.

Compliance with ethical standards

Disclosure of conflict of interest

No conflicts of interest.

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