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Review

Focus on periodontal engineering by 3D printing technology – A systematic review.

Enfoque en la ingeniería periodontal mediante la tecnología de impresión 3D: Una revisión sistemática.

Manchala Sesha Reddy.[1]
Shishir Ram Shetty.[2]
Raghavendra Manjunath Shetty.[3]
Venkataramana Vannala.[1]
Shakeel Sk.[4]
Rajasekar S.[5]

Affiliations:
[1]Department of Preventive Dental Sciences, College of Dentistry, Gulf Medical University, United Arab.
[2]Department of Oral and Craniofacial Health Sciences, College of Dental Medicine University of Sharjah, University City Sharjah, United Arab Emirates.
[3]Department of Clinical Sciences, College of Dentistry, Ajman University, UAE.
[4]Department of Restorative Dental Sciences. College of Dentistry, Gulf Medical University, Ajman. United Arab Emirates.
[5]Department of Periodontics, Rajah Muthiah Dental College & Hospital, Annamalai Nagar, C. Kothangudi, India.

Corresponding author: Manchala Sesha Reddy. College of Dentistry, Gulf Medical University, Al Jurf, 4184, Ajman. United Arab Emirates. **Phone:** (0971) 561073878. **E-mail:** seshaperio5@gmail.com

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Abstract: Three-dimensional (3D) bioprinting of cells is an emerging area of research but has not been explored yet in the context of periodontal tissue engineering. **Objective:** This study reports on the optimization of the 3D bioprinting scaffolds and tissues used that could be applied clinically to seniors for the regenerative purpose to meet individual patient treatment needs. **Material and Methods:** We methodically explored the printability of various tissues (dentin pulp stem/progenitor cells, periodontal ligament stem/progenitor cells, alveolar bone stem/progenitor cells, advanced platelet-rich fibrin and injected platelet-rich fibrin) and scaffolds using 3D printers pertaining only to periodontal defects. The influence of different printing parameters with the help of scaffold to promote periodontal regeneration and to replace the lost structure has been evaluated. **Results:** This systematic evaluation enabled the selection of the most suited printing conditions for achieving high printing resolution, dimensional stability, and cell viability for 3D bioprinting of periodontal ligament cells. **Conclusion:** The optimized bioprinting system is the first step towards the reproducible manufacturing of cell laden, space maintaining scaffolds for the treatment of periodontal lesions.

Keywords: Printing; three-dimensional; periodontium; regeneration; tissue engineering; bioprinting; stem cells.

Resumen: La bioimpresión tridimensional (3D) de células es un área emergente de investigación, pero aún no se ha explorado en el contexto de la ingeniería de tejidos periodontales. **Objetivo:** Este estudio informa sobre la optimización de los tejidos y andamios de bioimpresión 3D utilizados que podrían aplicarse a personas mayores en el entorno clínico con fines regenerativos para satisfacer las necesidades de tratamiento de cada paciente. **Material y Métodos:** Exploramos metódicamente la capacidad de impresión de varios tejidos (células madre / progenitoras de la pulpa de dentina, células madre / progenitoras del ligamento periodontal, células madre / progenitoras de hueso alveolar, fibrina rica en plaquetas avanzada y fibrina rica en plaquetas inyectada) y andamios utilizando impresoras 3D que pertenecen solo a defectos periodontales. Se ha evaluado la influencia de diferentes parámetros de impresión con la ayuda de andamios para promover la regeneración periodontal y reemplazar la estructura perdida. **Resultados:** Esta evaluación sistemática permitió la selección de las condiciones de impresión más adecuadas para lograr una alta resolución de impresión, estabilidad dimensional y viabilidad celular para la bioimpresión 3D de células del ligamento periodontal. **Conclusión:** El sistema de bioimpresión optimizado es el primer paso hacia la fabricación reproducible de andamios de mantenimiento de espacio cargados de células para el tratamiento de lesiones periodontales.

Palabra Clave: *Impresión tridimensional; periodoncio; regeneración; ingeniería de tejidos; bioimpresión; células madre.*

INTRODUCTION.

The great attention that three-dimensional (3D) printing currently receives around the world cannot go unnoticed by dentists. Just as technology has made its way rapidly but gradually in all fields, it has also done so in dentistry. Revolutions in technology like computers, cone beam computed tomography (CBCT) along with computer aided design software (CAD-CAM) paved the way for the evolution of 3D printing technology. The term "3D Printing" was coined at MIT in 1995.[1]

Since then, 3D printing has become one of the most important innovative technologies. It enables the digital fabrication of tangible substances necessary and adjusted for individual needs. Various methods are used to fabricate 3D scaffolds. With advances in techniques, it is now feasible to design scaffolds with a clear cut form, internal morphology, and a "reproducible" three-dimensional structure, despite their complexity.[2]

It is also known as additive manufacturing, which builds the ideal structure layer by layer through 3D printing.[3] This has great potential for rapid use in periodontics.[4-6] The additive manufacturing model is the most widely used technique to achieve periodontally essential bioprinting materials.[5] Alongside with other techniques fused deposition modelling (FDM), selective laser sintering (SLS), stereolithography (SLA) and polyjet printing are the frequently used techniques in bioprinting by additive manufacturing technique.[5,7,8] (Table 1).

Developmental research in 3D printing and its implications in periodontics can be appreciated by an increase in the growth of scholarly articles related to this area of interest. The subject of interest in the context of the field of periodontics is its application in regenerative medicine and tissue engineering. This technique uses cell "ink" based bioprinters or spheroid/microtissue based systems to create artificial materials.

Periodontitis, a chronic inflammatory disease, occurs when bacteria-stimulated inflammation or infection of the gingival tissue progressively destroys the periodontium. Global epidemiology of the periodontal disease is 20%-50% in both developing and developed countries, culminating in tooth loss.

The National Health and Nutrition Examination Survey (NHNES) reported that 42% of dentate adults in the USA aged 30 years or above had periodontitis, of which 7.8% had severe periodontitis.[9] The aim of any surgical periodontal therapy is to attain regeneration that cannot be attained by conventional methods. Innovative bioengineering has become an area of interest in dentistry, as various approaches aim to regenerate attachment apparatus around diseased teeth with the use of barriers, scaffolds, bone grafts, or biologics.[10,11]

Previous concepts attempted to improve the predictability of periodontal regeneration, but failed due to critical collaboration issues.[12] Many attempts, with varying levels of clinical success has been reported in past. Multiple commendable papers and reviews have been published on the topic of periodontal regeneration. These include applications of stem cells, guided tissue regeneration membranes with or without bone grafts, and various types of bone grafts.[13-15]

New techniques such as 3D bioprinting that has revolutionized its application in the medical field will soon be a springboard for its growth in dentistry, especially in periodontics. Bone tissue engineering (BTE) techniques have opened the doors to the forefront for periodontal regeneration with the advent of scaffolds displaying 3D designs that diligently mimic the native extracellular matrix (ECM).[16]

During the last decades a rapid development of innovative methods and products for the correction of periodontal defects has been reported. In the field of periodontics, 3D printing scaffolds are currently being explored in techniques such as guided bone regeneration (GBR), guided tissue regeneration (GTR), vertical bone augmentation, sinus augmentation and preservation of the cavity, showing tortuous results regarding its success.[17] This article emphasizes recent findings in the fields of 3D printing and innovative scaffold designs for future applications in clinical care and their implication in the field of periodontics.

MATERIALS AND METHODS.

This systematic review was carried out according to the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines. An internet based search was conducted using *PUBMED*, and *Google Scholar* databases. The keywords used for the search are listed in Table 2.

The end search date was 2nd June 2020 across the databases. The selection process was conducted in two phases. In the first phase, titles and abstracts from the selected electronic databases were reviewed by two authors. *In vivo*, *in vitro* and animal studies were included.

Articles consisting of printability of various tissues and scaffold using three-dimensional (3D) printing for periodontal defects were analysed for this review. Studies published in English was one of the criteria for the inclusion in this systematic review. Methodology of the systematic review is illustrated in Figure 1.

Reviews, library dissertations, conference papers, letters to the editor and personal opinions pertaining to the topic were excluded from the systematic review. Studies that used 3D printing scaffold for guided bone regeneration, socket preservation, sinus lifting and bone augmentation for implant placement were excluded. 3D printing technological studies in other specialities of dentistry apart from Periodontics were excluded.

Studies that used 3D printing scaffolds for periodontal regeneration, such as bony defects and guided tissue regeneration were included. In phase 2, the full text articles obtained after filtering during phase 1 were evaluated by the same reviewers. In case of a disagreement in the selection process between the two reviewers, a third author was called in to reach a consensus. Details regarding authors, year of publication, type of evaluation method, type of periodontal parameters were evaluated.

Figure 1. Flow chart of the methodology used in this systematic review.

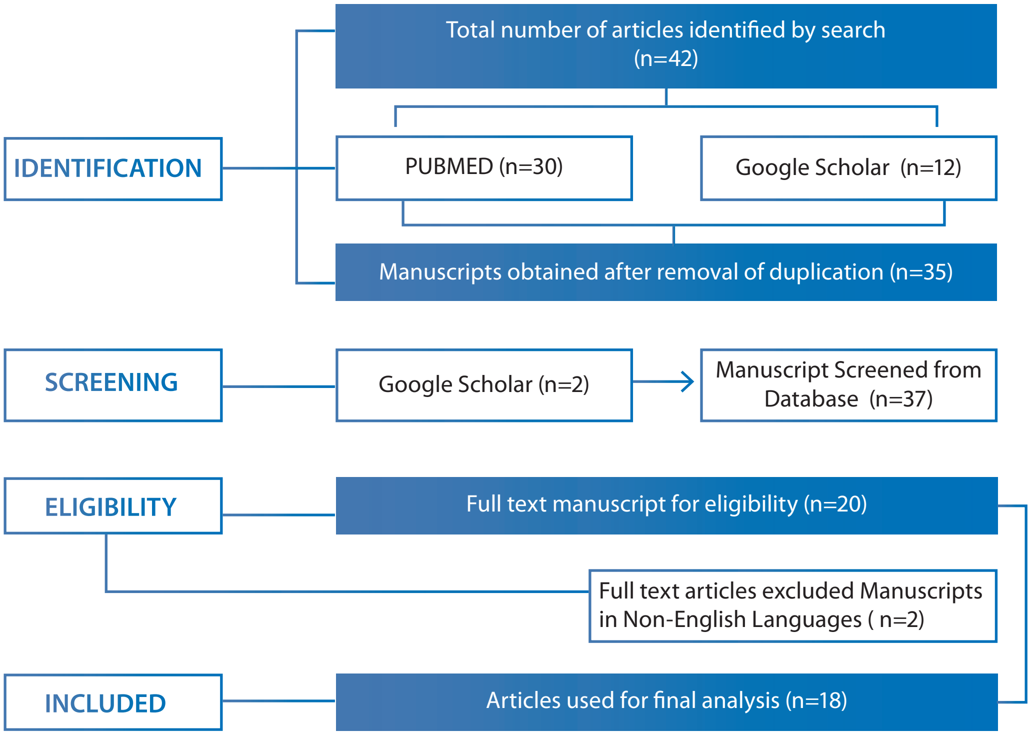

Figure 2. Flow chart for 3D scaffolds for periodontal regeneration.

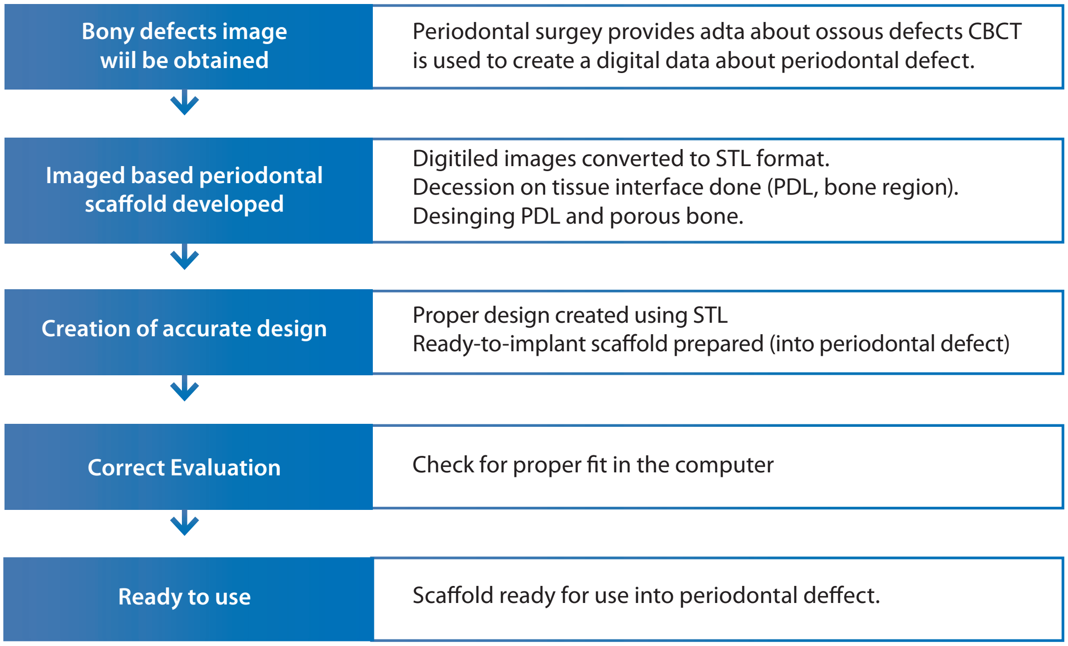

Tabla 1. Types of 3D Printing Technological Models

used deposition modelling (FDM)
Stereolithography (SLA)
Digital Light Projector (DLP) 3D Printing
Photopolymer Phase Change Inkjets (PolyJet)
Selective laser sintering (SLS) - Direct metal laser sintering (DMLS)
Plaster-based 3D printing (PP) - Powder bed and inkjet head 3D printing
Thermal Phase Change Inkjets
Laminated object manufacturing (LOM)

Tabla 2. List of keywords used for search in databases.

Databases	Keywords used
Pubmed and Google Scholar	3D printing, Fused Deposition Modeling (FDM), periodontal regeneration, perio bone grafting, guided tissue regeneration, periodontal scaffold, periodontal tissue engineering.

Tabla 3. 3D printing and periodontal regeneration – *in vitro* studies.

Article authors			3D printing Technique	
	Year	Tissue used	Scaffold fabricated by 3D printing	Conclusion
Lee CH *et al.,*[18]	2014	Dentin pulp stem/ progenitor cells (DPSCs), Periodontal ligament stem/progenitor cells (PDLSCs), or alveolar bone stem/progenitor cells (ABSCs)	Poly-caprolactone (PCL) and 10% hydroxyapatite (HA) in combination with amelogenin, connective tissue growth factor, bone morphogenetic protein (BMP) 2.	Authors reported the formation of a putative periodontium.
Ma Y *et al.,*[19]	2015	Periodontal ligament stem/progenitor cells (PDLSCs)	Nano gelatin methacrylate (GelMA) and poly (ethylene glycol) (PEG) dimethacrylate was used.	The current study reported that behavior of human PDLSCs in GelMA /PEG array was found to be dependent on the volume ratios of GelMA/ PEG. With an additional proportion of PEG, cell viability and spread out reduced.
Pilipchuk SP *et al.,*[20]	2016	Periodontium	PCL was used mixed with 5% hydroxyapatite (HA).	Researchers concluded that using this design different tissues and structures can be regenerated.
Peng C *et al.,*[21]	2018	Human periodontal ligament stem cells (hPDLSCs)	Scaffolds made of 50:50 poly (ε-caprolactone) (PCL) and poly-lactic-co-glycolic acid (PLGA)	Authors concluded that scaffold blended PCL/PLGA, displayed favorable characters such as degradation and cellular activity for further alveolar bone deposition.

Tabla 4. Human studies on 3D printing, and periodontal regeneration.

Article authors			3D printing Technique	
	Year	Tissue used	Scaffold fabricated by 3D printing	Conclusion
Park CH *et al.,*[22]	2014	Human PDL	Gelatin scaffolds	Authors concluded that periodontal regeneration could be attained by scaffolds laid with dental stem cells as it was ideal for its application.
Goh BT *et al.,*[23]	2015	PCL scaffold	PCL scaffold	Authors conducted a study to evaluate alveolar bone ridge height with and without PCL scaffold and they reported improved healing of alveolar bone with the application of PCL scaffold alongside with enhanced ridge height even 6 months later.
Rasperini G *et al.,*[5]	2015	Human PDL	PCL scaffold used	Authors reported that, in the long run, the treatment was unsuccessful.
Tayebi L *et al.,*[24]	2018	8% gelatin, 2% elastin and 0.5% sodium hyaluronate.	GTR membrane	GTR served the function as a barrier by preventing oral epithelium from the underlying tissues.
Venet L *et al.,*[25]	2017	freeze-dried cortico-cancellous blocks	Nylon polyamide	Authors suggest that horizontal ridge reconstruction in the maxillary anterior region is possible with customized bone allografts. Clinical findings such as reduced morbidity and surgical time were also reported.
Lei L *et al.,*[26]	2019	Advanced platelet-rich fibrin (A-PRF) + injected platelet-rich fibrin (I-PRF), mixed with Bio-Oss.	Photosensitive resin bony anatomy replica	Authors concluded that clinical and radiographic follow up disclosed pocket depths reduction and significant 3D alveolar bone fill at the treatment sites.

Tabla 5. 3D printing and regenerative approaches in periodontal regeneration – Animal studies.

Article authors	Year	Tissue used	3D printing Technique Scaffold fabricated by 3D printing	Conclusion
Park CH *et al.*,[27]	2010	Periodontal ligament cell	Hybrid scaffold made of 25% poly (glycolic acid) (PGA) and 15% PCL	Authors reported the development of new PDL in the correct orientation.
Park CH *et al.*,[28]	2012	Human PDL cell culture	PCL fiber-guiding scaffolds	Authors reported that biomechanical loading was well tolerated by these structures to withhold large osseous defects. They concluded that in clinical settings scaffolds lead to neogenesis of triphasic bone-ligament integration.
Pati F *et al.*,[29]	2015	mesenchymal stromal cells (hTMSCs)	PCL, PLGA, and β-tricalcium phosphate (β-TCP) and mineralized ECM	Authors reported that in comparison to bare scaffolds ECM laid scaffolds elicited better bone formation.
Park CH *et al.*,[30]	2014	humanPeriodontal ligament cells	hybrid Fiber-guiding PCL scaffold	Authors reported that hybrid PCL scaffold expedited regeneration, arrangement, and organization of multiple periodontal tissues. They concluded that this scaffold oriented the integration of PDL and mineralized tissue structures, resulting in functional tissue interfaces that mimic natural anatomy.
Inzana J *et al.*,[31]	2014	phosphoric acid-based -8.75 wt%	CPS scaffolds	Authors reported CPS scaffold facilitated new bone formation as they were resorbed or incorporated into the newly forming bone. This study demonstrates the feasibility of these processes, mechanical and cellular benefits of volumetric collagen inclusion *in vitro*.
Carrel JP *et al.*,[32]	2016	OsteoFlux(®) (OF) tricalcium phosphate (TCP) and hydroxyapatite.	Tricalcium phosphate (TCP) + hydroxyapatite.	Authors reported a controlled porous structure translated in a high osteoconductivity and resulted in a bone mass 3 mm above the bony bed that was four times greater than that obtained with standard substitutes.
Lee JW *et al.*,[33]	2017	Adipose-derived stem cells (ADSCs) and bone marrow stem cells (BMSCs)	PCL/TCP + bone grafting.	Authors reported that ADSC- and BMSC-seeded 3D-printed PCL/TCP scaffolds displayed promising enhancement of osteogenesis in a dog model of maxillary bone defects.
Li J *et al.*,[34]	2017	DPSCs	PCL scaffolds + freeze-dried and traditionally prepared PRP	Authors reported that compared with traditional PRP-PCL scaffolds or bare PCL scaffolds, the freeze-dried PRP-PCL scaffolds induced significantly greater bone formation and they concluded that PCL scaffolds with freeze-dried PRP can promote greater osteogenic differentiation of DPSCs and induce more bone formation, which may have great potential in future clinical applications
Diomede F *et al.*,[35]	2018	human gingival mesenchymal stem cells (hGMSCs), extracellular vehicles (EVs), or polyethyleneimine (PEI)-engineered Evs (PEI-Evs)	PLA scaffolds	Authors reported that Evs stimulate bone regeneration whereas PEI-Evs induce the bone apposition and emphasize the proangiogenic capacity, thus showing that scaffolds coated with PEI-Evs could represent a new tool in the critical-size bone defect.

Tabla 6. **The risk of bias in the selected studies was assessed using the Cochrane Collaboration's tool.**

Article authors	Year	Criteria	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and researchers (performance	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias) bias)	Other bias
Lee *et al.*,[18]	2014	Low	Low	Low	Unclear	Unclear	Low	Low	Unclear
Ma *et al.*,[19]	2015	Low	Low	Low	Unclear	Unclear	Low	Low	Low
Pilipchuk *et al.*,[16]	2016	Low	Low	Low	Unclear	Unclear	Low	Low	Unclear
Peng *et al.*,[21]	2018	Low	Low	Low	Unclear	Unclear	Low	Low	Unclear
Park *et al.*,[22]	2014	Low	Low	Low	Unclear	Unclear	Low	Low	Unclear
Goh *et al.*,[23]	2015	Low	Low	Low	Low	Low	Low	Low	Unclear
Tayebi *et al.*,[24]	2018	Low	Low	Low	Unclear	Unclear	Low	Low	Unclear
Venet *et al.*,[25]	2017	Low	Low	Low	Unclear	Unclear	Low	Low	Unclear
Lei *et al.*,[26]	2019	Low	Low	Low	Unclear	Unclear	Low	Low	Unclear
Park *et al.*,[27]	2010	Low	Unclear	Low	Unclear	Unclear	Low	Low	Unclear
Park *et al.*,[28]	2012	Low	Unclear	Low	Unclear	Unclear	Low	Low	Unclear
Pati *et al.*,[29]	2015	Low	Unclear	Low	Unclear	Unclear	Low	Low	Unclear
Park *et al.*,[30]	2014	Low	Unclear	Low	Unclear	Unclear	Low	Low	Unclear
Inzana *et al.*,[31]	2014	Low	Unclear	Low	Unclear	Unclear	Low	Low	Unclear
Carrel *et al.*,[32]	2016	Low	Unclear	Low	Unclear	Unclear	Low	Low	Unclear
Lee *et al.*,[33]	2017	Low	Unclear	Low	Unclear	Unclear	Low	Low	Unclear
Li *et al.*,[34]	2017	Low	Unclear	Low	Unclear	Unclear	Low	Low	Unclear
Diomede *et al.*,[35]	2018	Low	Unclear	Low	Unclear	Unclear	Low	Low	Unclear

RESULTS.

Overall, 42 articles were obtained, of which 30 articles were from *PUBMED* and 12 from *Google Scholar*. The number of eligible articles got reduced to 35 after deleting duplicate articles during the second stage of screening. Twenty full-text articles were obtained. During the third stage of determination, two non-English articles were excluded, as articles in any other language other than English were excluded from this study. Eighteen full-text English language articles were eligible for the final systematic review. (Table 3, Table 4 and Table 5); (Figure 1)

The earliest article was from the year 2010 and the most recent from the year 2019. The publishing trend reveals the growing interest in this specific area of research. There were six publications from the United States, four from China, three each from Korea and France, and one each from Singapore and Italy. (Figure 1)

Regarding type of study, nice were animal studies, four were *in vitro*, and five were human studies. (Table 3, Table 4 and Table 5) The risk of bias for the studies included in the systematic review is presented in Table 6.

DISCUSSION.

Before starting the systematic review process, we conducted a literature search to check the availability of a specific systematic review on the role of 3D printing in periodontal regeneration.

Due to the unavailability of the systematic review or meta-analysis pertaining to this research area, we have tried to fill the knowledge gap. We obtained eighteen full-text articles that were eligible for review. In our systematic review we obtained articles from *PubMed* and *Google Scholar*. Detailed analysis of the results of the eighteen eligible articles in our review is presented in three broad subtitles, such as *in vitro*, *in vivo* and animal studies. In vitro studies used 3D printable scaffolds made up of poly-lactic acid (PCL), poly L-lactic-co-glycolic acid (PLGA) or bone morphogenetic proteins (BMPs) using stem cells from pulp, periodontal ligament or alveolar bone to promote periodontal regeneration, and they reported bone-ligament-cementum formation.[18-21]

In vivo studies on 3D printing, and periodontal regeneration had mixed responses in terms of clinical success, where few authors concluded that periodontal regeneration could be attained.[22-26] In fact one study reported that in the long run the treatment was unsuccessful.5 Animal studies on 3D printing and periodontal regeneration displayed promising enhancement of osteogenesis, with authors suggesting great potential in future clinical applications.

Some authors used human periodontal ligament (PDL) with its correct orientation even in large osseous defects.[27,28,30,31,33-35] One study reported bone mass of 3mm above the bony bed which was four times greater than that obtained with standard substitutes.[32] Comparative animal studies with varies scaffolds displayed better regeneration with ECM laid scaffolds.

The new hypothesis in dentistry that applies in periodontics is a biological solution to biological problems. This leads to a shift from routine surgical techniques to the application of biological solutions for periodontal regeneration. In the current century we have a fusion of clinical problems and human genetics with other branches of biotechnology, bioengineering and bioinformatics that helps in the application of these methods for periodontal regeneration. In the past, periodontal regeneration has been tested with barrier membranes in conjunction with powerful biological mediators.

These experiments undoubtedly indicate the beginning of tissue engineering methodologies for the management of periodontal bone defects. In periodontics, the periodontium is considered a key structure for the application of these methods. Initial studies showed that the periodontal ligament and bone cells can be transferred to periodontal sites without negative immunological or inflammatory results.

The attention given to 3D printing in the last decade led to research into the use of this technology in periodontics to promote regeneration. In periodontics, 3D printing has been used to treat conditions such as gingival recession, smile design, gingivectomy, bone defects, guided tissue regeneration, guided bone regeneration.[34,37] Comparative studies on biphasic versus scaffolds reported predictable orientation, better organization of the periodontal ligament, and controlled tissue infiltration.[34]

The notion of using 3D printing for the regeneration of periodontal tissue leads cells to populate periodontal bone defects, guided tissue regeneration.[36] 3D printed scaffolds placed on cells regenerate bone defects with the help of growth factors and bioactive proteins.38 Through compartmentalisation, these scaffolds not only prevent epithelial cell migration, but also provide space for selective repopulation of periodontal ligament cells. So multiple scaffolds are designed and used that mimic the biological native periodontal complex.[39]

Regeneration with 3D scaffolding emulates native extracellular matrix. This ultimately leads to the process of tissue regeneration. Newer multiphasic 3D scaffolds could impact space-time that is essential for a predictable regeneration cascade.[20,36,40,41]

Currently 3D printed GTR membranes have shown promising results in vitro by accomplishing barrier function for which it is designed for Cell sheet engineering[24] is a more widely used method to regenerate periodontal structures with promising results compared to artificial scaffolds.[42] 3D printing technology symbolizes a hopeful paradigm shift from earlier to more precise tailor-made methods for achieving regeneration of periodontal tissue. Although exciting, due to the complexity of the periodontium, achieving ideal results is less promising in terms of clinical application.

CONCLUSION.

In the clinical setting, the implementation of 3D bioprinting for periodontal regeneration is still in the early stages, as it showed inconsistent results in clinical application. Their features are hopeful, as innovation in bioprinting could utilize cell-placed scaffolds for periodontal regeneration.

The polylactic acid (PCL) scaffold demonstrated better regenerative potentials with good reliability. These scaffolds together with other mimicking biological agents could be used to enhance periodontal regeneration in bone defects.

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