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Review Article

3D Bioprinting: Print the Future of Periodontics

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ABSTRACT

3D Bioprinting is a pioneering technology in the field of regenerative medicine that enables the fabrication of living tissues using the living cells by the printing process. Since, periodontitis has become more prevalent disease among the population; there is a need for increased periodontal regenerative procedures to restore normal healthy periodontium for the patients. Regenerative procedures require placement of tissues that are biocompatible, bioresorbabale, promotes cell growth and proliferation in restoring the defect. Such structures can be made from this 3D bioprinting technology which uses several bioinks and various bioprinting methods such as autonomous self-assembly, extrusion-based, laser bioprinting etc., to print the tissues. Every method has their own pros and cons, hence having a detailed knowledge of the method aids in improvising the techniques further. In summary, we have a detailed discussion of this 3D bioprinting technology in this review and additionally, it throws light on using various bioprinting strategies and materials to improve the field of periodontal tissue engineering further in our clinical practices.

Keywords: Bioprinting, Bioinks, Methods, Periodontitis, Regeneration, Tissue engineering

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INTRODUCTION

Technology has slowly and steadily paved its way into dentistry. Researchers are constantly working to integrate technology into dentistry. Of all the latest technological innovations in dentistry, the most talked-about innovation is three-dimensional (3D) bioprinting. 3D Bioprinting is a cutting-edge technology in the field of regeneration that facilitates the fabrication of multiscale, biomimetic, multi-cellular tissues with highly complex tissue microenvironment, intricate cytoarchitecture, structure-function hierarchy, and tissue-specific compositional and mechanical heterogenicity (Vijayavenkataraman S, *et al.*, 2018). Since, periodontitis has become more prevalent disease among the population; periodontal regenerative procedures are needed to restore a normal healthy periodontium. The 3D bioprinting technology allows the fabrication of such structures, which use several biomaterials and various bioprinting methods (Murphy SV and Atala A, 2014). This review article discusses about 3D bioprinting and provides little information about the technology behind 3D printers. It also throws light on using various bioprinting strategies and materials most often used in 3D printed scaffolds for periodontal regeneration.

LITERATURE REVIEW

History of 3D bioprinting

Bioprinting is a technique that is used to design complex biological structures using bioinks. Before gaining an insight on the 3D bioprinting of the periodontium, it is important to understand the evolution of 3D bioprinting in the medical field. After the invention of Stereolithography by Hull CW in 1983, the concept of printing human organs was developed (Hull CW, 1984).

Earlier, the machine discovered by Hull used UV lasers to engrave the layers of acrylic into shapes, which are then stacked to form objects. The major drawback was that the printer uses written codes to engrave the acrylic, so only simple shapes were created. Later in 1986, Hull discovered the 3D technology of printing and also designed the materials that go into the printers (Hull CW, 1984). In the 1990s, the 3D systems were used to fabricate dental implants and custom prosthetics using materials such as nanocomposites, blended plastics and powdered metals.

The researchers at Wake Forest Institute for Regenerative Medicine [WFIRM] made a synthetic scaffold of a human bladder using the 3D bioprinting technology in the year 2000 (Atala A, 2001). In the process of synthesis of the scaffold, they used the recipient's host cells to overcome the problem of host rejection. After 10 years of implantation, the patient had no serious complications. In 2002, again at WFIRM, a team of scientists led by Professor Anthony Atala undertook a bioprinting project of a miniature functional kidney capable of filtering blood and producing urine in an animal model. Then in 2003, Thomas Boland, a scientist from University of El Paso, invented his own designed 3D bioprinter, which uses bioinks to print live tissues (Mironov V, *et al.*, 2003). In 2004, Dr. Forgacs made his debut with his own bioprinter, which during his uprising caused a great change in the scientific community. It was the first device that allowed 3D direct biodegradation i.e., using live cells without the need to build scaffolding (Jakab K, *et al.*, 2004).

In 2006, Noble Prize winner Dr. Shinya Yamanaka discovered that mature cells acquired from cultures can be reorganized again to a stem cell state (Takahashi K and Yamanaka S, 2006). This created a revolution in the field of regenerative medicine and also in 3D bioprinting. In 2009, one of the first commercial Bioprinters from Organovo-NovoGen MMX was created. They aimed at "scaffold-free" printing process. In 2010, Organovo-the Bioprinting Company printed the first blood vessel and today the revolution continues on.

Three-dimensional bioprinting

As the term bioprinting implies, that process involves the printing of living tissues. This is done using a 3D bioprinters that uses a computer-aided design model. In this model bioinks are layered through an additive manufacturing process to create tissues that mimic the natural tissues (Murphy SV and Atala A, 2014).

Bioprinting approaches

The approaches in 3D Bioprinting are: Biomimicry, Autono-

mous self-assembly and Mini-tissue building blocks.

Biomimicry: This is one of the prime approaches in bioprinting, where the structures are created similar to the natural tissues that are found in humans. They are useful in making similar cellular as well as extracellular tissues as found in humans. It involves the synthesis of biological tissue using the synthetic materials that mimic biological functions (Atala A and Yoo J, 2015).

Autonomous self-assembly: This is the second approach in bioprinting. The basic idea of self-assembly is derived from the concept of embryogenesis and organogenesis where the cells proliferate to their tissues of interest based on signaling molecules, creating their own extracellular matrix as a foundation for the cell replication. The main advantage is that it is scaffold-free. Some of the shortcomings faced by scaffold-based systems are immunogenicity, maladaptation, etc (Atala A and Yoo J, 2015).

Mini-tissue building blocks: This is the third approach in bioprinting. This includes both the techniques of biomimicry and autonomous self-assembly, where the structures are constructed from mini functional tissue components, thereby organizing them into a larger structure of required characteristics (Thomas D and Singh D, 2019).

Types of 3D bioprinters

3D bioprinters are the machines that operate through various mechanisms such as Direct light processing, Fused deposition modeling, Inkjet printing, Extrusion based printing and Laser assisted printing were invented as depicted in *Figure 1*. In this review, we discuss only on the most widely used bioprinter technologies in current practice.

Inkjet-based bioprinting: This was the first attempt in bioprinting. In this method of bioprinting, the data from computer is fed to printer and it reproduce onto the substrate using ink drops as a non-contact technique (Murphy SV and Atala A, 2014). These printers are of three types-thermal, piezoelectric and mechanical. The cartridge is filled with bioink and during the process they are forced through microfluidic reservoir to an output nozzle. The initial problem involved during the printing process was that the cells died during printing due to immediate drying out of the substrate. This was overcome by encapsulating the cells in a highly hydrated polymers-hydrogels. In thermal inkjet printers, the printhead is heated by an electrical heat which produces pressure to force the bioink from the nozzle (Cui X, *et al.*, 2010). In piezoelectric inkjet printers, when a voltage is applied to the piezoelectric material it changes shape and produces acoustic waves to force the bioink into droplets at regular intervals (Visser J, *et al.*, 2013). In mechanical inkjet printers, application of pressure forces the bioink from the nozzle (Tekin E, *et al.*, 2008).

Table 1: Various bioinks that are used in the 3D bioprinting process (Gopinathan J and Noh I, 2018)

Bioinks	Description	Advantages	Disadvantages
Agarose	It is a marine polysaccharide taken from seaweed.	Due to its gel forming property has a wide range of use in biomedical field.	Provides limited support to cell growth.
	The disaccharides D-galactose and 3,6 anhydro L-galacto pyranose forms the backbone of this agarobiose.	Has good mechanical strength. Biocompatible.	Poor cell adhesion. Non degradable.
Alginate	It is a natural polymer derived from brown algae. Also known as algin or alginic acid. Composed of monomers such as alpha L-guluronic acid and (1-4) beta D mannuronic acid.	Has a good gel forming property and a good flexural strength. They imbibe water and other molecules by capillary forces enabling cell growth.	It has slow degradation kinetics. Poor cell adhesion.
Chitosan	It is a cationic polysaccharide which is derived from natural biomaterial chitin found in the cells of shrimp and other crustaceans.	Highly biocompatible. Anti-bacterial properties. Bio degradable. Forms stable hydrogels with good cell affinity, mechanical strength.	Has a slow gelation rate.
Collagen	It is the main structural protein component of the extracellular matrix. Found in skin and connective tissues.	Has good biomimetic property. Biocompatible. It allows cell remodeling.	Has poor mechanical property, hence need to be crosslinked with other biomaterials.
Fibrin	Fibrin is synthesized from fibrinogen by enzymatic action with thrombin. It is a protein found in blood.	Good biocompatibility. Biodegradable.	Has a weak mechanical property. Has a limited printability.
Cellulose	It is a polysachharide obtained from cellulose. CMCs are used as hydrogels by modifying their cellular properties.	Has improved cell viability. Biocompatible. Blending with bioglass it has good mechanical property.	Lack of shear thinning property and structural shrinkage on drying.

Silk	Silk fibroin is a natural biopolymer extracted from Bombyx mori.	Used in digital light processing printing method. Spider silk has a good mechanical strength. It has good printability and keeps mesenchymal stem cells viable.	Application of high shear forces causes change in conformation before the extrusion printing.
Gelatin	It is a protein based natural biomaterial obtained from partial hydrolysis of collagen.	It is biocompatible. It is water soluble. It has good flowable property when blended with other bioinks.	Poor shape fidelity. It has limited rigidity.
Graphene	Graphene is an allotrope of carbon in form of single layer of atoms in a 2D hexagonal lattice.	3D graphene composed of more than 90% graphene is flexible, conductive and a biocompatible material.	Susceptible to oxidative environments. Quite toxic in nature. Low biological relevance.
Hyaluronic acid	It is a natural biomaterial usually found in cartilages and connective tissue.	Increases the proliferation of cells. High concentration increases cell viability and stability.	Has low mechanical strength and has slow gelation property.
Decellularis-ed ecm	Decellularised ECM is obtained from native tissues by removal of cells in sequential steps leaving the ECM intact.	It keeps the cells viable and provides good functionality.	Production cost is higher as compared to the other hydrogel bioinks.
Hydroxy-apatite	Natural biomaterial found in teeth and bones.	Provides high strength and rigidity.	It offers low printability and limited tissue specificity.
Cell aggregates	Spherical cell aggregates as spheroids are used as a bioink for the 3D printing process. These cell spheroids are laid in layers to form scaffold and they fuse by self-assembling process.	They promote good cell proliferation, differentiation and cell migration. They maintain cell viability.	Low resolution and limitations in tissue thickness.
PCL/PLA/ PLGA	These are thermoplastic synthetic biomaterials. PCL-Polycaprolactone biodegradable polyester. They are hydrophobic and semi crystalline in nature. PLA-Polylactic acid is thermoplastic which is both amorphous and crystalline in nature.	PCL-it has increased flexibility in drug delivery and used as scaffold for 3D printing.	Low cell adhesion and low cell proliferation.
		PLA-it is biodegradable, biocompatible and it is easy to process. Easily metabolized in the body.	
		PLGA-being used as a effective copolymer with PLA.	

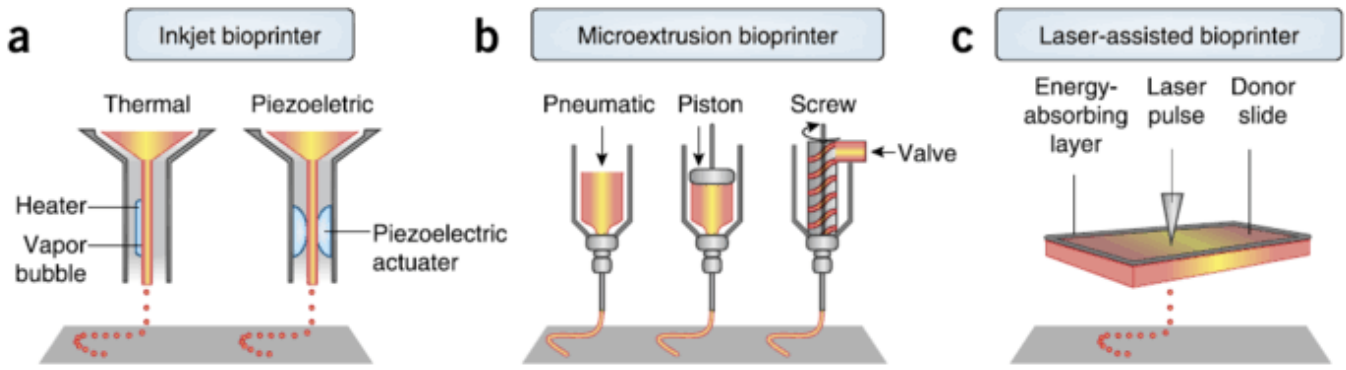

Figure 1: Methods of 3D bioprinting-a) Thermal and piezoelectric mediated inkjet printer, b) Micro-extrusion printer, c) Laser-assisted printer (Malda J, *et al.*, 2013)

Micro-extrusion bioprinting: In this method, the printer comprises of a fluid dispensing system and an automatic robotic system for the process of extruding the liquid and bioprinting the structure. This system comprises of either a pneumatic or screw-driven or piston or a solenoid-based system. The piston and the screw driven systems works mechanically to exhibit pressure necessary to eject the bioink whereas the pneumatic system employs a pressured air for the process (Visser J, *et al.*, 2013). This is a promising technique to create biomimetic structures (Chang R, *et al.*, 2008). The main advantage of this process is its ability to print using bioinks with high cell densities (Murphy SV and Atala A, 2014). The drawbacks are its limited resolution; require high pressure for extrusion of low viscous bioinks which can lead to cell death (Nair K, *et al.*, 2009).

Laser-Assisted Bioprinting (LAB): In this method a laser is used for deposition of bioink on the substrate. The laser pulses are directed through a 'ribbon' containing bioink and this ribbon is supported by titanium or gold layer which absorbs and transfers energy to ribbon (Gruene M, *et al.*, 2011). The bioink and cells are suspended on bottom of the ribbon and when vaporized by laser pulse, they create a high pressure bubble which exerts a pressure on the biomaterial thereby forcing the liquid towards the substrate. The Laser Assisted Bioprinting (LAB) is a scaffold free technique; deposits biomaterials at high resolution. Since, it is a nozzle free method; they eliminate the drawback of biomaterial clogging. It is well-suited for bioinks with varying range of viscosities. The main disadvantage of LAB is that the presence of metallic absorbing layers produces metallic residues on the structure formed; also LAB is very expensive (Murphy SV and Atala A, 2014).

Bioinks

As bioprinting is the process that involves printing of living tissues, the printer essentially requires a bioink to print the tissues. Therefore, bioinks are materials that are required to print the living tissues. The important properties of bioink should be biocompatible, non-toxic, printable, able to withstand mechanical stresses, good shape memory, ability to get nourishment from cells and enhance the metabolic activities of the cells (Gopinathan J and Noh I, 2018).The bioinks are usually comprised of natural polymers, synthetic polymers or combination of both.

The living cells used in 3D printing require specific aqueous environment to maintain the cellular functions at appropriate pH, for key nutrients and oxygen diffusion, to create an extracellular matrix, non-toxic environment and to allow printed cells to form new tissue. Such environment is provided by the materials known as hydrogels (Chimene D, *et al.*, 2020). Hydrogels are made from extracellular matrix components like collagen, hyaluronic acid that enables stem cell growth. Since, hydrogels are in liquid polymer state, they are insufficient to support successive cell layering during the printing process; to overcome these limitations, newer techniques are used to strengthen the hydrogels such as nanocomposites, supramolecular bioinks, interpenetrating networks, polymer functionalization and thermoplastic reinforcement (Shafiee A and Atala A, 2016). The detailed descriptions on various bioinks are given in *Table 1*.

Steps in 3D bioprinting

Pre-bioprinting: It is the first step in the process where the structure to be printed is designed and modeled as a 3D structure using the Computed Tomography (CT) and MRI scans. Every fine detail is recorded and tomographic reconstruction done on the images so that it is printed in a layer by layer fashion (Williams J, 2014) as shown in *Figure 2*. Later, the bioinks are prepared by isolation from living tissues and they are allowed to multiply.

Bioprinting: It is the printing process where the designed structures has to be printed using the printers. Here the bioinks are introduced to the printer cartridges and based on the digital model the cells are accumulated in a layered fashion (Ozbolat IT, 2015).

Post-bioprinting: Post-bioprinting process involves maintaining mechanical integrity and function of the 3D printed structure (Williams J, 2014). They control the remodeling and the growth of tissues by sending signals and recently, evolution of bioreactor technologies have caused rapid tissue maturation, vascularization of tissues and increased the survival rate of the transplants (Obregon F, *et al.*, 2015). Depending on the type of tissue, the bioreactors differ. The steps are summarized in *Figure 3*.

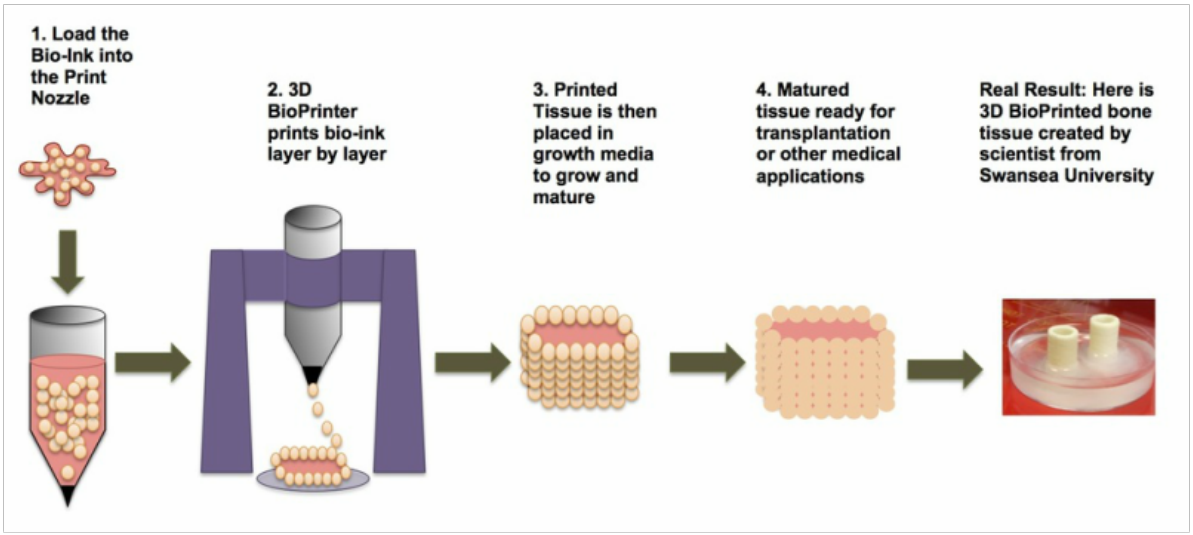

Figure 2: Represents the layering of cells during the process of bioprinting (Yeong WY and Chua CK, 2014)

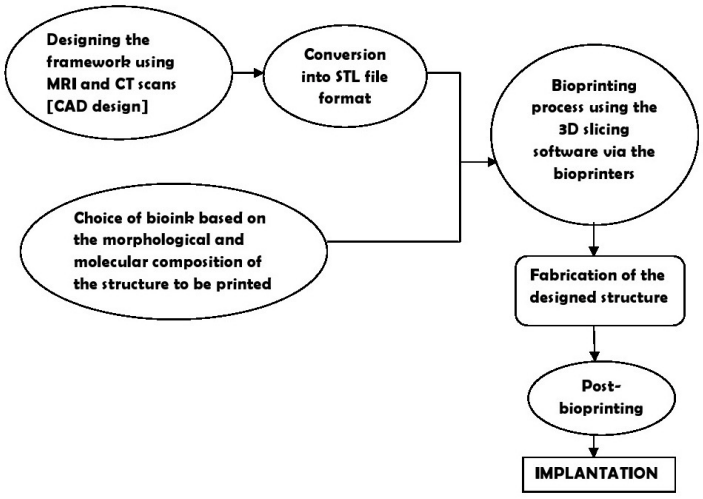

Figure 3: The flowchart depicts the individual processes that are involved in the bioprinting process

Applications of 3D bioprinting in periodontics

In dentistry, there is emerging use of this 3D bioprinting technology for its diverse applications and it proves to provide successful treatment options for the patients (Patel R, *et al.*, 2017). In this article, we briefly discuss on the periodontal complex regeneration in the field of periodontology.

In periodontology, the periodontal tissues have a complex organization which requires multilayered biomaterial constructs to restore the structural and functional integrity at the bone-ligament interface (Vaquette C, *et al.*, 2018). Periodontitis, an inflammatory disease in response to periodontal pathogens affects the periodontium causing destruction of the tissues (Gaviria L, *et al.*, 2017). Therefore, the need for periodontal regeneration procedures is increasing. Hence, many clinical researches are ongoing in the field of 3D bioprinting to restore the lost periodontal structures for the individuals suffering from periodontitis. The periodontium structures are quite complex in morphology and they require special technical knowledge in the printing process.

Since the periodontal structures exhibit different porosities and strength as shown in *Figure 4*, it requires more precise technology to bioprint the structures accurately. However, the use of additive manufacturing technology enables printing of structures with good mechanics and accurate porosities as they enable the use of line spacing, line thickness and resolution changing features (Rasperini G, *et al.*, 2015).

In a case study done by Rasperini G, et al they used a 3D printed bioresorbable scaffold in treatment of a periodontal defect and this was the first application of a personalized 3D printed scaffold in the field of periodontics (Asa'ad F, *et al.*, 2016). But to our catastrophe, this case was a failure at the end of 13th month, which led to surgical removal of the scaffold. This was because the researchers used only PCL which caused wound dehiscence due to slow tissue degradation rate and led to unsuccessful tissue regeneration due to its inferior cell affinity (Ausenda F, *et al.*, 2019). Therefore, the scientists came to a conclusion that they should use bioinks with faster resorption rate or the PCL should be incorporated with long standing devices like the titanium screws (Carrel JP, *et al.*, 2016). But this is strongly believed that this study has paved the way for further research in field of oral regenerative medicine for improved personalized 3D bioprinted structures.

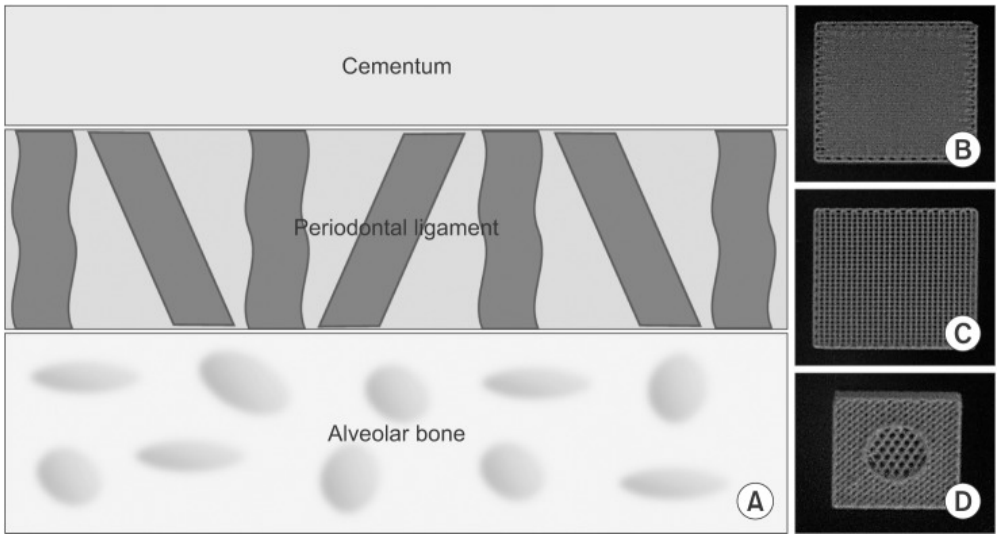

Figure 4: It represents the difference in strength and porosities of the cementum, periodontal ligament, and the alveolar bone respectively (Rasperini G, *et al.*, 2015)

The main aim of researchers lies in the production of multiphasic scaffolds for periodontal regeneration which includes the periodontal ligament, cementum and alveolar bone. After several studies, the authors advocated the use of variety of biomaterials other than PCL for periodontal regeneration using animal models. In a study done by Rasperini G, *et al.*, they suggested the use of bioceramics to be successful in sinus and bone augmentation procedures (Ausenda F, *et al.*, 2019). The research work by Carrel JP, *et al.* in a sheep animal model for vertical bone augmentation procedure used a 3D printed scaffold made of biphasic ceramic-hydroxyapatite and alpha-tricalcium phosphate and compared it to the bovine bone and particulate beta-tricalcium phosphate. The biphasic ceramic was found to be superior and they provided good mechanical integrity without the need of membranes (Sahranavard M, *et al.*, 2020). Use of bio ceramics are recommended for alveolar bone regeneration and for regeneration in non-stress bearing zones, collagen can be used as the biomaterial of choice (Ausenda F, *et al.*, 2019). Chitosan is said to one of the best bioink in regenerative procedures as they are biocompatible, biodegradable, anti-bacterial and hydrophilic in nature (Tayebi L, *et al.*, 2018). In a recent study by Tayebi L, *et al.*, they 3D printed a membrane made of gelatin, elastin and sodium hyaluronate which is found to be biocompatible and bioresorbable and also provided mechanical integrity and required surgical characteristics such as suturability for it application in guided tissue regeneration procedures (Amada P, *et al.*, 2018). Therefore, using the techniques of 3D bioprinting and the availability of wide of range of biomaterials it is more fascinating to create innovations in the periodontal regeneration procedures today.

Shortcomings of 3D bioprinting

Though 3D bioprinting technology is available for many long years, the expensiveness of the 3D bioprinters, high energy consumption, the operation and maintenance cost, clearance from ethical board as it advocates the use of cells and also the requirement of a trained operator have shown to be a barrier for its development (Kahl M, *et al.*, 2019; Rider P, *et al.*, 2018; Kačarević ŽP, *et al.*, 2018).

CONCLUSION

3D Bioprinting has caused a revolution in the field of regenerative medicine. The WHO has suggested that by 2020, 10% of the global population is affected by the periodontitis, where most of them require periodontal regeneration procedures. Hence, this use of latest 3D-bioprinting technology seems to improve the regeneration of periodontal tissues facilitating a good oral health status for the patient. Since, there are drawbacks in any technology; they have to be overcome by variety of treatment alternate methods and strategies. Further, researchers in Germany have found an ultra-low cost 3D desktop bioprinters which is easily portable and capable of printing tissues at low expenses, which creates a sense of motivation among the clinicians to use this technology in their routine practices. Many clinical researches and case studies have to be done in 3D-bioprinting of the periodontium using the available biomaterials and latest bioprinting methods to regenerate the periodontium. Being a promising technology in regenerative medicine, it will revolutionize in the field of periodontology hopefully by new researches and studies further.

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REFERENCES

1. Vijayavenkataraman S, Yan WC, Lu WF, Wang CH, Fuh JY. 3D bioprinting of tissues and organs for regenerative medicine. Adv Drug Deliv Rev. 2018; 132: 296-332.
2. Murphy SV, Atala A. 3D bioprinting of tissues and organs. Nat Biotechnol. 2014; 32(8): 773-785.
3. Hull CW. Apparatus for production of three-dimensional objects by stereolithography. United States Patent. 1984.
4. Atala A. Bladder regeneration by tissue engineering. BJU Int. 2001; 88(7): 765-770.
5. Mironov V, Boland T, Trusk T, Forgacs G, Markwald RR. Organ printing: Computer-aided jet-based 3D tissue engineering. Trends Biotechnol. 2003; 21(4): 157-161.
6. Jakab K, Neagu A, Mironov V, Forgacs G. Organ printing: Fiction or science. Biorheology. 2004; 41(3-4): 371-375.
7. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006; 126(4): 663-676.
8. Atala A, Yoo J. Bioprinting: 3D printing comes to life. Manuf Eng. 2015; 63-66.
9. Thomas D, Singh D. Novel techniques of engineering 3D vasculature tissue for surgical procedures. Am J Surg. 2019; 218(1): 235-236.
10. Cui X, Dean D, Ruggeri ZM, Boland T. Cell damage evaluation of thermal inkjet printed Chinese hamster ovary cells. Biotechnol Bioeng. 2010; 106(6): 963-969.
11. Visser J, Peters B, Burger TJ, Boomstra J, Dhert WJ, Melchels FP, *et al.* Biofabrication of multi-material anatomically shaped tissue constructs. Biofabrication. 2013; 5(3): 35007.
12. Tekin E, Smith PJ, Schubert US. Inkjet printing as a deposition and patterning tool for polymers and inorganic particles. Soft Matter. 2008; 4(4): 703-713.
13. Chang R, Nam J, Sun W. Effects of dispensing pressure and nozzle diameter on cell survival from solid freeform fabrication-based direct cell writing. Tissue Eng Part A. 2008; 14(1): 41-48.
14. Nair K, Gandhi M, Khalil S, Yan KC, Marcolongo M, Barbee K, *et al.* Characterization of cell viability during bioprinting processes. Biotechnol J. 2009; 4(8): 1168-1177.
15. Gruene M, Deiwick A, Koch L, Schlie S, Unger C, Hofmann N, *et al.* Laser printing of stem cells for biofabrication of scaffold-free autologous grafts. Tissue Eng Part C. 2011; 17(1): 79-87.
16. Malda J, Visser J, Melchels FP, Jüngst T, Hennink WE, Dhert WJ, *et al.* 25th anniversary article: Engineering hydrogels for biofabrication. Adv Mater. 2013; 25(36): 5011-5028.
17. Gopinathan J, Noh I. Recent trends in bioinks for 3D printing. Biomater Res. 2018; 22(1): 1-5.
18. Chimene D, Lennox KK, Kaunas RR, Gaharwar AK. Advanced bioinks for 3D printing: A materials science perspective. Ann Biomed Eng. 2016; 44(6): 2090-2102.
19. Chimene D, Kaunas R, Gaharwar AK. Hydrogel bioink reinforcement for additive manufacturing: A focused review of emerging strategies. Adv Mater. 2020; 32(1): 1902026.

20. Shafiee A, Atala A. Printing technologies for medical applications. Trends Mol Med. 2016; 22(3): 254-265.
21. Williams J. Research into 3D-Bioprinting may soon produce transplantable human tissues. 3D Printing Technology. 2014.
22. Yeong WY, Chua CK. Bioprinting: principles and applications. World Scientific Publishing. 2014.
23. Ozbolat IT. Bioprinting scale-up tissue and organ constructs for transplantation. Trends Biotechnol. 2015; 33(7): 395-400.
24. Obregon F, Vaquette C, Ivanovski S, Hutmacher DW, Bertassoni LE. Three-dimensional bioprinting for regenerative dentistry and craniofacial tissue engineering. J Dent Res. 2015; 94(9S): 143S-152S.
25. Patel R, Sheth T, Shah S, Shah M. A new leap in periodontics: Three-dimensional (3D) printing. J Adv Oral Res. 2017; 8(1-2): 1-7.
26. Vaquette C, Pilipchuk SP, Bartold PM, Hutmacher DW, Giannobile WV, Ivanovski S. Tissue engineered constructs for periodontal regeneration: Current status and future perspectives. Adv Healthc Mater. 2018; 7(21): 1800457.
27. Gaviria L, Pearson JJ, Montelongo SA, Guda T, Ong JL. Three-dimensional printing for craniomaxillofacial regeneration. J Korean Assoc Oral Maxillofac Surg. 2017; 43(5): 288-298.
28. Rasperini G, Pilipchuk SP, Flanagan CL, Park CH, Pagni G, Hollister SJ, *et al.* 3D-printed bioresorbable scaffold for periodontal repair. J Dent Res. 2015; 94(9S): 153S-157S.
29. Asa'ad F, Pagni G, Pilipchuk SP, Giannì AB, Giannobile WV, Rasperini G. 3D-printed scaffolds and biomaterials: review of alveolar bone augmentation and periodontal regeneration applications. Int J Dent. 2016; 16.
30. Ausenda F, Rasperini G, Acunzo R, Gorbunkova A, Pagni G. New perspectives in the use of biomaterials for periodontal regeneration. Materials. 2019; 12(13): 2197.
31. Carrel JP, Wiskott A, Moussa M, Rieder P, Scherrer S, Durual S. A 3D printed TCP/HA structure as a new osteoconductive scaffold for vertical bone augmentation. Clin Oral Implants Res. 2016; 27(1): 55-62.
32. Sahranavard M, Zamanian A, Ghorbani F, Shahrezaee MH. A critical review on three dimensional-printed chitosan hydrogels for development of tissue engineering. Bioprinting. 2020; 17: e00063.
33. Tayebi L, Rasoulianboroujeni M, Moharamzadeh K, Almela TK, Cui Z, Ye H. 3D-printed membrane for guided tissue regeneration. Mater Sci Eng C. 2018; 84: 148-158.
34. Amada P. 10 Disadvantages of 3D Printing Technology. 3D-Insider. 2018.
35. Kahl M, Gertig M, Hoyer P, Friedrich O, Gilbert DF. Ultra-low-cost 3D bioprinting: Modification and application of an off-the-shelf desktop 3D-printer for biofabrication. Front Bioeng Biotechnol. 2019; 7: 184.
36. Rider P, Kačarević ŽP, Alkildani S, Retnasingh S, Barbeck M. Bioprinting of tissue engineering scaffolds. J Tissue Eng. 2018; 9: 2041731418802090.
37. Kačarević ŽP, Rider PM, Alkildani S, Retnasingh S, Smeets R, Jung O, *et al.* An introduction to 3D bioprinting: Possibilities, challenges and future aspects. Materials. 2018; 11(11): 2199.