3D-Bioprinting a world to come for Periodontics

Review Article

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ABSTRACT

Dentistry is recently coming to the terms of use of technology and tech-savvy dentists, who nowadays use smart devices to make their life easier. Nowadays, worldwide demand for the organ replacement or tissue regeneration is increasing steadily. Periodontal Regenerative engineering is the convergence of developmental biology, stem cell science and engineering, materials science, and clinical translation to provide tissue patches or constructs for diseased or damaged organs. Various methods have been introduced to create tissue constructs with clinically relevant dimensions. Among such methods, 3D bioprinting provides the versatility, speed and control over location and dimensions of the deposited structures. 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. 3D printing was initially conceived by Charles Hull in 1986. Hull's concept was based on the idea that successive layers of a base material could be applied on top of each other to 'print' object. Uses of 3D bioprinting in periodontology include bio-resorbable scaffold for periodontal repair and regeneration, socket preservation, bone and sinus augmentations procedures, guided implant placement, peri-implant maintenance, and implant education.

Keyword

Bioprinting, scaffolds, periodontal regeneration

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.[1] 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.

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

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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 bio- printing 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. For- gacs made his debut with his own bioprinter, which during his uprising caused a great change in the scientific community.[2] 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, Orga-novo-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, Autonomous 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 bioprinting

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 . In this review, we discuss only on the most widely used bioprinter technologies in current practice. Fig1

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[3]. 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 piezoelec- tric 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).

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 sys- tem 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 struc- tures (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 for- cing 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[4].

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 insuffi- cient 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).

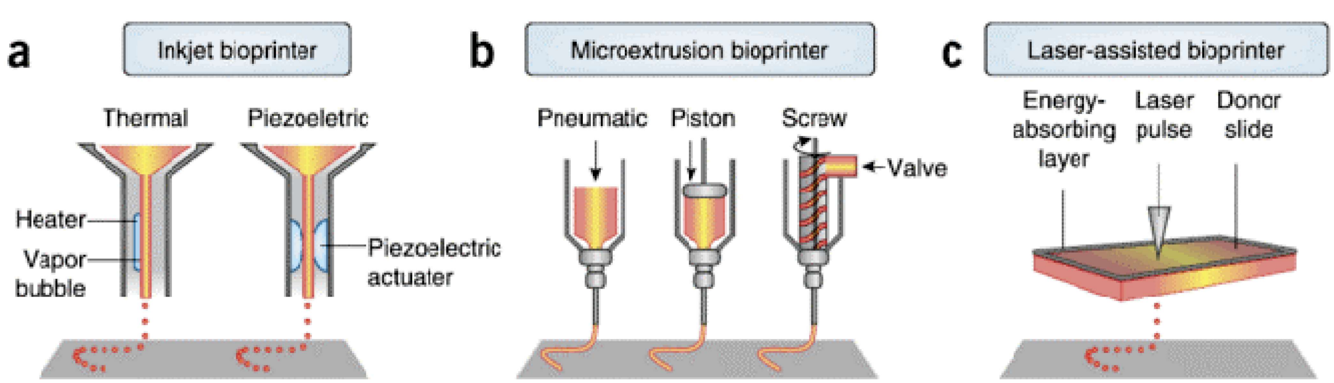

FIG 1 :Methods of 3D bioprinting-1.1) Thermal and piezoelectric mediated inkjet printer, 1.2) Micro-extrusion printer, 1.3) Laser-as- sisted printer (Malda J, *et al.*, 2013)

Steps in 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 Com- puted Tomography (CT) and MRI scans. Every fine detail is recorded and tomographic reconstruction done on the images so that it is print- ed in a layer by layer fashion (Williams J, 2014) 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).[5]

Post-bioprinting: Post-bioprinting process involves maintaining mechanical integrity and function of the 3D printed structure (Wil- liams 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 in- creased the survival rate of the transplants (Obregon F, *et al.*, 2015). Depending on the type of tissue, the bioreactors differ. Fig2,Fig3

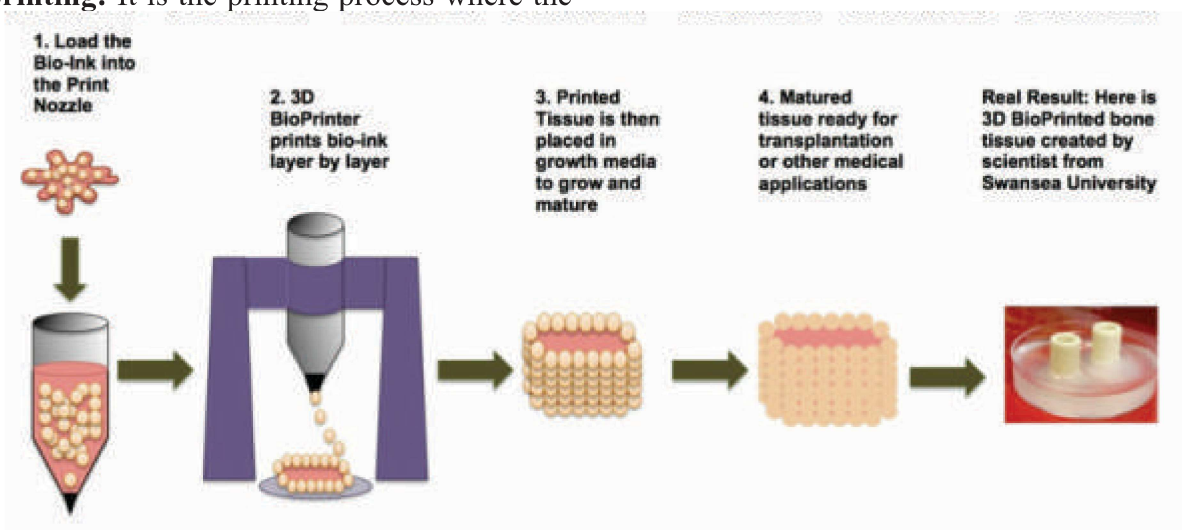

FIG 2 : Represents the layering of cells during the process of bioprinting (Yeong WY and Chua CK, 2014)

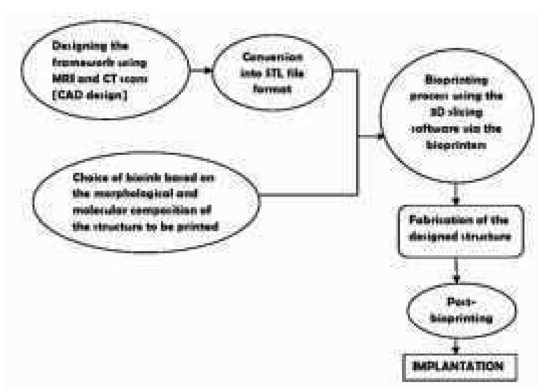

FIG3 :The flowchart depicts the individual processes that are involved in the bioprinting process

Applications of 3d Bioprinting

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.[6]

However, the use of additive manu- facturing 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 (Aus-enda F, *et al.*, 2019).[7] 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.

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 au- thors advocated the use of variety of biomaterials other than PCL for periodontal regeneration using animal models. In a study done by Ras-perini 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 ver- tical 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). [8]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).[9] 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).[10] 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.

Shortcoming 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). [11]

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-bio-printing 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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