ROLE OF 3D-BIOPRINTING TECHNOLOGY IN REGENERATIVE MEDICINE

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ABSTRACT

Regenerative medicine and tissue engineering are the two emerging fields that promotes the healing and restoration of lost function of damaged tissue or organs. Using a Novel tissue Engineering method, Bioprinting Technology Tissue mimics can be created using various living cells and Bio materials.

In this review, Printing methods such as Extrusion-based method, Robotic dispensing, Cellular Inkjet, Laser-assisted Printing and Integrated Tissue Organ Printing (ITOP) are examined. Natural and synthetic Polymers such as bio inks are discussed with emphasis on Regenerative medicine applications. Furthermore, applications of Bioprinting in Regenerative medicine are summarized.

KEYWORDS: Regenerative medicine, Tissue Engineering, Bioprinting Technology, Bio inks.

INTRODUCTION

Tissue damage and regeneration is a common phenomenon among humans(4). Recently, 3D-Bioprinting Technology has gained much development for their application in the regenerative medicine and Tissue engineering(6). The treatment for this condition is dependent upon the donor which becomes scarce and sometimes lead to graft rejection due to immune response(4). To overcome this issue, various Bio-printing technologies has been used(4). These methods involve the patient-derived autologous cells and these cells are used to develop organs/tissue for transplantation(4). Using Animal models become poor mimic of tissue in humans and also it leads to some ethical problems, therefore to overcome the issue, 3D-bioprinting has introduced(22). In this review article, different bio-printing methods were described(1). Secondly, materials such as Bio inks for developing tissue printing has been described.

Finally, role of bioprinting in regenerative medicine are summarized and also its challenges and future directions are outlined.(1)

Tissue engineering and bioprinting

The Integration of Bioengineering and Medicine results in Tissue Engineering and Regenerative Medicine (TERM)(21). Tissue Engineering and Bio printing have been used for the regeneration of various organs such as myocardium, bone, esophagus, skin etc[4]. Tissue Engineered scaffolds does not completely fulfil all the requirements that are needed for the regeneration of tissue. On other hand, Bioprinting technology (Additive Manufacturing) offer an approval that overcomes most of the issues in Tissue Engineering[4].

Bioprinting involves building a tissue or organ layer by layer by using bio ink mixed with living cells[4]. The ultimate aim of this technology is to mimic the native tissue by depositing bio-materials and cells in a particular manner, that the cells can hold together to form the required 3D Construct[4]. CAD and segmentation software were used by the 3D printing for sequential construction layer of 2D images into 3D models[21].

Conventional 3D printing uses acellular material and non-biological for the creation of 3D printing process[21]. When this type of printing uses a biological and living cells as the material the process known as Bio-printing[21]. It is the process of integration of living cells with biomaterials that allows the construction of layer-by-layer deposition of bio ink[2]. This technology was originated from the traditional 2D printing on paper and later 3D printing was developed[2].

Three central approaches were used in 3D printing technology, such as mini-tissue building blocks, autonomous self-assembly and biomimicry[7]

3D-Bioprinting involves three steps pre-process, process, post-process[8].

METHODS:

Bioprinting Technique is a complex process which depends on numerous parameters such as bio ink, humidity and temperature[1]. Micro/nano Architex, multi-cellular, 3D-structure, high cell density is essential for the replication in 3D Printing constructs[1]. Printing patterns can modify and printed using Computer Aided Software such as CAD[1].

Various methods are used in the bio-printing techniques. They are Micro-Extrusion, Inkjet bioprinting, Laser-assisted Bioprinting, Integrated Tissue Organ Printing (ITOP), Robotic Bio printing[1].

Microextrusion

Micro-Extrusion is one of the methods used for 3D-Printing[1]. It can be done by Direct ink writing (or) It is a Pressure- assisted technique[4]. In this method, the bio inks placed in a plastic/glass cartridge, are delivered through a nozzle by the pressure applied by using mechanical/pneumatic method. It is ejected in form of thin filament based on CAD design to get a desired 3D shape.

This technique is most suitable for large scale constructs. However, it has a low resolution (~100). Materials used for printing must possess specific rheological properties, that enables easy printability[4]. Applicable for the production of hard tissues[24].

It has a limitation, such when high extrusion pressure applied impose high shear stress and may lead to loss of cellular viability and distortion of the tissue structure. Comparing to other bioprinting technology, this technique has low resolution approximately 200m.

Due to continuous deposition of bio ink this technique generates good structural integrity[29]. This type of bioprinting combines both an automated robotic system and Fluid-dispensing system for extrusion and printing. Recently, developed extrusion-based bioprinter is multi-head tissue construction[10].

Advantages

Low cost of production and high density of cells that can be deposited.

Disadvantages

Slow production times Extrusion nozzle blockage due to bio inks. Very low resolution.

Inkjet bioprinting

Inkjet bioprinting is a type of bioprinting technology. Ink-jet Bioprinter also referred as a Drop-on-Demand Printers[1]. It is known as First bioprinting study that use a modified office inkjet printer for cell distribution[20]. These printers are used for many nonbiological applications[1]. It is noncontact techniques, high speed printing with multiple nozzles. it uses both thermal and piezoelectric technology[3]. These two methods are widely for jetting based printing[6]. This method uses a low viscosity bio ink which is made up of viable cells that is deposited on the bio paper[4]. By applying a local heat, it produces a bubble and ejects a small droplet[6]. Low viscosity materials such as calcium chloride, saline, thrombin and fib Rogen have been used[4]. High viscosity materials containing bio inks cannot be used to get droplets[4].

THERMAL TECHNOLOGY- Ink droplets are produced by heating, so that an inflated bubble forces the ink through the narrow nozzle and onto the substrate[2].

Advantages

It is inexpensive techniques and are using broadly.   High print speed technique.

Disadvantages

Droplets prepared are mixed, unequal in size and unorder due to repeated nozzle blockages.

Smooth printing becomes very difficult. PIEZOELECTRIC TECHNOLOGY: Drops are produced by the transient from piezoelectric actuator. Acoustic waves have been used to ejects the bio inks.

Advantages

It does not use heat. It does not cause orifice clogging. Droplets become regular and equal in size.

Disadvantage

Causes damage to cell membrane. If it is used frequently lead to cell lysis. A Combination inkjet bioprinter and electro spinning are used for the layered cartilage construction[7]. Other than these two types, pneumatic microwave-based inkjet printer are used. In this method, the bio ink is ejected under a constant pneumatic pressure[8]. One of the major drawbacks is the choice of materials (i.e) the bio ink must possess appropriate viscosity and must in a liquid state for the easy ejection of the small orifice of the nozzle[10].

Laser-assisted bioprinting

This method was originated from Laser direct-write technology and also it is a modified version of the Laser-induced forward transform technique[10]. It is also a non-contact printing technology. This method utilizes Laser-induced forward transfer for the transport of droplets[8]. It is less common than the micro-extrusion and inkjet bioprinting. It is often for tissue and organ engineering applications[7]. For the deposition of bio ink into the substrate, a pulsed laser beam is used[5]. This printer uses a ribbon coated with an absorbing layer such as gold. A Laser pulse passes through this ribbon, generates heat that induces a hydrogel droplet. It is repeated several times util a final construct in a layer-by layer created. Due to excessive heat, laser energy produced may damage the cells and affect the cellular viability in the printed tissue[3]. A Nanosecond laser with UV/ near UV wavelength as energy sources are used in this technique to print the cells, proteins, hydrogels and ceramic materials[2].

Advantages

Nozzle free. Non-contact process. High resolution Precise delivery characteristics.

Disadvantages

Bio inks of fast gelation kinetics are required. Flow rate during pumping may get hinder. Time consuming process. Containing traces of contamination. This method has been successfully used for the bone construct and skin wit cells for implantation[1]. In this process the contact between the dispersion and the bio ink avoids the cell stress therefore it results in high cell viability. It is compatible with different types of bio ink and a wide range of viscosities[10]. This printing resolution the bioprinting is dependent on speed, energy, pulse frequency of the laser and also the viscosity of bio inks[9]. LAB is an expensive process and has a limitation like it suffers from a scalability and low stability[24].

Integrated tissue organ printer (itop)

ITOP method has been used widely because of the construction of complex human tissue with good viability and vasculature[1]. This method uses a pneumatic actuated micro-extrusion method. It uses air pressure to regulate the dispensing volume and 3-axis motorized stage for 3D-patterning[1].

3D-Patterns used in this method, were produced from the magnetic resonance imaging (MRI) and Computed Tomography (CT) data of human tissue/organs by using CAD software which is finely converted into 3D Patterns[1].

Advantages

High resolution nozzles.

Better carrier materials for cell delivery.

Fabricating 3D structures with sufficient strength, size, shape is very difficult due to decreased mechanical properties and stability of Hydrogel materials[6]. To overcome this issue, a newly developed hybrid systems is developed called ITOP[6].

Robotic method

Using cell spheroids in the robotic bioprinting of 3D tissues is an emerging technique[1]. Automated robotic systems are used to obtain a pressure printing. It enables direct selfassemble of tissue spheroids for developing large scale tissue/organs. It also uses pneumatic actuated micro-extrusion printing method but differ in dispensing system. Robotic dispensing system is used for direct alignment of tissue structure on the bio papers. Recently, six-axis robotic dispensing bioprinters has developed using advanced solutions.

Advantages

TSIM-perform an MRI scan of human tissue.

This method requires a multi-phase process [(11)].Three syringe pumps were used at different speeds for measurement of their flow rates. After desired flow rate is achieved[(11)]. The components of robotic bio-printing include a multiple microfluidic pump, programmable robotic arm, an extruder for bioprinting[(12)].

Materials

During the bioprinting process, mixture of biomaterials in the hydrogel form with desired cell types (bio ink) are used for the construction of tissue/organs[(13)]. Bio inks may be natural/synthetic biomaterials alone/ in combination[(13)]. Bio inks should possess the desired temporal and spatial resolution and possess tunable gelation properties, biomimetic properties, must be biocompatible, provide mechanical and structural support. Bio ink of different viscosity are required for different bioprinting techniques[(26)]. Bio ink for Extrusion Bioprinting is divided into 3 categories[(18)]. They are support, matrix, sacrificed[(26)]. Recently developed bio ink contains helical fiber structures. Important feature of the bio inks is printability. It should be biocompatible, should provide mechanical and structural support to the growing cells for 3D microenvironment[(1)]. Bio ink solution consist of amniotic-fluid derived stem cells (AFCs) and bone-marrow derived stem cells suspended in fibrin collogen and thrombin cross linked printed directly on wound site[(4)]. Sodium alginate based smart bio inks were developed[(28)].

Natural polymers

Biological materials that naturally found in the body such as Proteoglycan’s silk, extracellular matrix, fibrin etc. are used as Natural polymers. Due to their high biological activity and compatibility these are more commonly used. They provide tissue-specific nutrients. Native extracellular matrix had been mimicked by the cell adhesion motifs present in the Natural polymers[(5)].

Synthetic polyers

They are chemically synthesized and added in the bio inks. It includes compounds such as PEG, Polystyrene, Polylactic acid, Polycaprolactone and other polymers[(5)]. They provide their own properties such as mechanical stability, photo crosslinking ability, temperature, PH responses etc[(17)]. They are highly specific. Structure and function of the polymer can be altered by Chemical modifications that has been offered by the synthetic polymers[(1)].

To improve the functionality of bio ink, Natural and Synthetic polymers are used in combination[(21)].

Ceramics

Ceramics are not used adequately. It consists of minerals such as phosphates, calcium and Hydroxyapatites[(5)]. It is mainly used to recreate the hard tissue construct (bones)[(21)].

Various plant derived biomaterials have also been used in the bioprinting techniques such as marine algae, nanocellulose, alginate[26].

Gelatin methacryloyl (gel ma)

It is used to produce crosslinked hydrogels for 3D printing and tissue engineering[1]. It is widely used in injectable tissue constructs, epidermal tissue, endothelial cell morphogenesis and cartilage regeneration. It also been used in hydrogels and microspheres for drug delivery[1].

Acellular materials

Bio ink along with acellular materials are also used in Bioprinting technology. They are porous structures that has both biochemical and mechanical properties. It also has required surface chemistry for cell proliferation, attachment differentiation[1].

EG: Hydroxyapatite (HA), Polylactic acid, Chitosan, Fibrin, Collagen.

Hydrogel-based biinks

Hydrogels are the most commonly used biomaterials for live-cell printing[3]. They are 3D networks consist of crosslinked hydrophilic polymer chains[16]. Hydrogels are biocompatible, biodegradable, some of them possess specific cell-binding sites[14]. They contain numerous attractive features[14]. Hydrogel biomaterials includes agarose, chitosan, gelatin gum, gelatin, collogen, PEG and Pluronic[14]. Upon implantation, various side effects are induced by Polymerization residues[16]. To overcome this problem, reversible deactivation radical polymerization technique was used. Hydrogels exhibit excellent mechanical properties[20]. Formulations made by bio inks reported from cell biomaterials-based bio inks to cell-based bio inks (cell aggregates and tissue spheroids) for regenerative medicine and tissue engineering[17].

Applications

By using novel bioinks and 3D printing techniques, bioprinting fabricate native organs/tissue for regenerative medicine application. Vasculature and cell viability are the important parameters that should be considered parameters that should b considered during the 3D constructs for each regenerative medicine application. It has the advantage of good resolution of the input cells. By this technology, attempts have been made to print bone, cartilage, kidneys, skin, blood vessels and nerves and other tissues. Bypass Grafting of coronary arteries, due to lack of donors for transplantation Bioprinting technique and Tissue engineering has been used to mitigate these types of issues. CARTILAGE TISSUE: By depositing PEG diacrylate and chondrocytes, Bioprinting technology serves the production of spatial patterns and different grader of cartilage tissue, that provides the relative cartilage anatomy.

SKIN TISSUE: Traditional in vitro bioprinting methods have been used to create skin tissues. Wound healing and skin endothelization have been reported successful in eight weeks of implantation. It has been used to produce both 2D and 3D structures for various purposes such as tissue constructs for tissue regeneration and fabrication of scaffolds. 3D printed human liver tissue developed by Organovo that remain fully functional and stable up to 28 days. Construction of 3D printed kidney tissue are similar to human kidney. Comparing to natural organs, 3D printed organs are low in cost, therefore it is a cost-effective approach in healthcare. Animal killing for clinical trials and studies have been reduced with the use of 3D bioprinting. 3D bioprinting is more precise and faster than the conventional systems. No adverse effects have been reported after surgery, however, Bio-printing using plastic, metallic, ceramic implants for bone, skin, tissue replacement have been implemented successfully. For the effective control of infectious diseases, 3D bioprinting has great impact on the vaccine engineering and invitro models. An important advantage of 3D bioprinting, that reduces the risk of rejection upon implantation. By using mechanical FE simulation, successfully 3D bio-printed human ear was constructed.

Limitations and future challenges

Bioprinting is one of the important tools, which is used to develop 3D constructs for clinical uses(1). There are few attempts have been made to construct a complex, multicellular and 3D native tissue however, that remains a major challenge. Bio inks with suitable biocompatibility and mechanical strength are the main technologies barriers. Bioprinting techniques developed not only for transplantations but also that serves an important purpose in analysis of chemical, drug discovery, biological, toxicological events and basic research(7). Limitations of spatial resolution in construction if capillary network can be achieved by 3D bioprinters(8). Micro-livers have been successfully fabricated by Chang, which is used for drug metabolism testing. 4D-bioprinting technique are in emerging field, to overcome some challenges in 3D bioprinting(11). A pair of robots were constructed by Zhang et al. that robots work together and they create a large 3D printed organs models by the application of cement materials. In 3D Bioprinting, no of components in the process of printing remains as a big issue. Due to scalability some of the issues may arise in the bioprinting technology(27). More strategies and novel techniques are being developed for 3D bioprinting. For deposition of a chondrocyte-laden bio ink, Bio pen from the University of Wollongong introduces a hand-held device for the treatment of cartilage defects. Bioprinting as part of the custom cell service will be offered by the Celso Biotechnologies. It is expected that in future, Bioprinting techniques has been deployed in hospitals.

CONCLUSION

3D-Bioprinting has a potential application in the tissue regeneration. Itself act as a promising innovation in tissue regeneration. This paper reviews the various bioprinting techniques using bio inks, strategies and their applications in regenerative medicine, limitation and future directions are further addressed here. Bioprinting has more advances in repeatability, precise control, individual design. When compared to manual methods of tissue culture, 3D Bioprinting techniques offer a high throughput and viable tissue printing. Overall, 3D-Bioprinting remains as the advanced technique for the 3D cell-laden fabrication.

REFERENCES

1.Sundaramurthi D, Rauf S, Hauser C. 3D bioprinting technology for regenerative medicine applications.

2.Li J, Chen M, Fan X, Zhou H. Recent advances in bioprinting techniques: approaches, applications and future prospects. Journal of translational medicine. 2016 Dec;14(1):1-5.

3.Ramadan Q, Zourob M. 3D Bioprinting at the Frontier of Regenerative Medicine, Pharmaceutical, and Food Industries. Frontiers in Medical Technology. 2021:25.

4.Agarwal S, Saha S, Balla VK, Pal A, Barui A, Bodhak S. Current Developments in 3D Bioprinting for Tissue and Organ Regeneration–a Review. Frontiers in Mechanical Engineering. 2020;6:90.

5.Saini G, Segaran N, Mayer JL, Saini A, Albadawi H, Oklu R. Applications of 3D Bioprinting in Tissue Engineering and Regenerative Medicine. Journal of Clinical Medicine. 2021 Jan;10(21):4966.

6.Seol YJ, Kang HW, Lee SJ, Atala A, Yoo JJ. Bioprinting technology and its applications. European Journal of Cardio-Thoracic Surgery. 2014 Sep 1;46(3):342-8.

7.Murphy SV, Atala A. 3D bioprinting of tissues and organs. Nature biotechnology. 2014 Aug;32(8):773-85.

8.Xia Z, Jin S, Ye K. Tissue and organ 3D bioprinting. SLAS TECHNOLOGY: Translating Life Sciences Innovation. 2018 Aug;23(4):301-14.

9.Kačarević ŽP, Rider PM, Alkildani S, Retnasingh S, Smeets R, Jung O, Ivanišević Z, Barbeck M. An introduction to 3D bioprinting: possibilities, challenges and future aspects. Materials. 2018 Nov;11(11):2199.

10.Zhang J, Wehrle E, Rubert M, Müller R. 3D Bioprinting of Human Tissues: Biofabrication, Bioinks, and Bioreactors. International Journal of Molecular Sciences. 2021 Jan;22(8):3971.

11.Kahin K, Khan Z, Albagami M, Usman S, Bahnshal S, Alwazani H, Majid MA, Rauf S, Hauser C. Development of a robotic 3D bioprinting and microfluidic pumping system for tissue and organ engineering. InMicrofluidics, BioMEMS, and Medical Microsystems XVII 2019 Mar 4 (Vol. 10875, p. 108750Q). International Society for Optics and Photonics.

12.Nguyen H, Adrian N, Yan JL, Salfity JM, Allen W, Pham QC. Development of a Robotic System for Automated Decaking of 3D-Printed Parts. In2020 IEEE International Conference on Robotics and Automation (ICRA) 2020 May 31 (pp. 8202-8208). IEEE.

13.Arslan-Yildiz A, El Assal R, Chen P, Guven S, Inci F, Demirci U. Towards artificial tissue models: past, present, and future of 3D bioprinting. Biofabrication. 2016 Mar 1;8(1):014103.

14.Gungor-Ozkerim PS, Inci I, Zhang YS, Khademhosseini A, Dokmeci MR. Bioinks for 3D bioprinting: an overview. Biomaterials science. 2018;6(5):915-46.

15.He Y, Gu Z, Xie M, Fu J, Lin H. Why choose 3D bioprinting? Part II: methods and bioprinters. Bio-Design and Manufacturing. 2020 Mar;3(1):1-4.

16.Ramiah P, du Toit LC, Choonara YE, Kondiah PP, Pillay V. Hydrogel-based bioinks for 3D bioprinting in tissue regeneration. Frontiers in Materials. 2020 Apr 30;7:76.

17.Gopinathan J, Noh I. Recent trends in bioinks for 3D printing. Biomaterials research. 2018 Dec;22(1):1-5.

18.allevi3d.com was first indexed by Google in November 2017·https://www.allevi3d.com/a-guide-to-matrix-bioinks/

19.analyticssteps.com was first indexed by Google in August 2019·

https://www.analyticssteps.com/blogs/3d-bioprinting-applications-advantages-and-disadvantages

20.Yi HG, Kim H, Kwon J, Choi YJ, Jang J, Cho DW. Application of 3D bioprinting in the prevention and the therapy for human diseases. Signal Transduction and Targeted Therapy. 2021 May 14;6(1):1-7.

21.Saini G, Segaran N, Mayer JL, Saini A, Albadawi H, Oklu R. Applications of 3D Bioprinting in Tissue Engineering and Regenerative Medicine. Journal of Clinical Medicine. 2021 Jan;10(21):4966.

22.Zhang B, Gao L, Ma L, Luo Y, Yang H, Cui Z. 3D bioprinting: a novel avenue for manufacturing tissues and organs. Engineering. 2019 Aug 1;5(4):777-94.

23.Gu BK, Choi DJ, Park SJ, Kim MS, Kang CM, Kim CH. 3-dimensional bioprinting for tissue engineering applications. Biomaterials research. 2016 Dec;20(1):1-8.

24.Kačarević ŽP, Rider PM, Alkildani S, Retnasingh S, Smeets R, Jung O, Ivanišević Z, Barbeck M. An introduction to 3D bioprinting: possibilities, challenges and future aspects. Materials. 2018 Nov;11(11):2199.

25.Chiesa I, Ligorio C, Bonatti AF, De Acutis A, Smith AM, Saiani A, Vozzi G, De Maria C. Modelling the 3D bioprinting process of β-sheet self-assembling peptide hydrogel scaffolds. Frontiers in Medical Technology. 2020;2:4.

26.Jovic TH, Kungwengwe G, Mills AC, Whitaker IS. Plant-derived biomaterials: A review of 3D bioprinting and biomedical applications. Frontiers in Mechanical Engineering. 2019 Apr 17;5:19.

27.Agarwal S, Saha S, Balla VK, Pal A, Barui A, Bodhak S. Current Developments in 3D Bioprinting for Tissue and Organ Regeneration–a Review. Frontiers in Mechanical Engineering. 2020;6:90.

28.Montero FE, Rezende RA, da Silva JV, Sabino MA. Development of a smart bioink for bioprinting applications. Frontiers in Mechanical Engineering. 2019 Sep 27;5:56.

29.Rider P, Kačarević ŽP, Alkildani S, Retnasingh S, Barbeck M. Bioprinting of tissue engineering scaffolds. Journal of tissue engineering. 2018 Sep;9:2041731418802090.

30. Skeldon G, Lucendo-Villarin B, Shu W. Three-dimensional bioprinting of stem-cell derived tissues for human regenerative medicine. Philosophical Transactions of the Royal Society B: Biological Sciences. 2018 Jul 5;373(1750):20170224.