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Review Article

3D Bioprinting: Introduction and Recent Advancement

Khalida Fakhruddin[1], Belal Yahya Hussein Al-Tam[1], Abdallah Nasser Sayed[1], Zarin Mesbah[1], Angelique Maryann Pereira Anthony Jerald Pereira[1], Al Ameerah Elza Toto Syaputri[1], Mohamad Ikhwan Jamaludin[1,2]*

[1] School of Biomedical Engineering and Health Sciences, Faculty of Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia
[2] Bioinspired Device and Tissue Engineering Research Group, School of Biomedical Engineering and Health Sciences, Faculty of Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia

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ABSTRACT

In the additive manufacturing method known as 3D bioprinting, living cells and nutrients are joined with organic and biological components to produce synthetic structures that resemble natural human tissues. To put it another way, bioprinting is a type of 3D printing that can create anything from bone tissue and blood vessels to living tissues for a range of medical purposes, including tissue engineering and drug testing and discovery. During the bioprinting process, a solution of a biomaterial or a mixture of several biomaterials in the hydrogel form, usually encapsulating the desired cell types, which are termed as bioink, is used for creating tissue constructs. This bioink can be cross-linked or stabilised during or immediately after bioprinting to generate the designed construct's final shape, structure, and architecture. This report thus offers a comprehensive review of the 3D bioprinting technology along with associated 3D bioprinting methods including ink-jet printing, extrusion printing, stereolithography, laser-assisted bioprinting and microfluidic techniques. We also focus on the types of materials, cell source, maturing, the implant of various representative tissue and organs, including blood vessels, bone and cartilage as well as recent advancements related to 3D bioprinting technology.

INTRODUCTION

Due to its tremendous potential in producing tissue-engineering chemicals, 3D bioprinting technology has received growing interest. There are a few techniques of bioprinting used and those are inkjet-based, extrusion, laser-assisted, stereolithography and microfluidic. Inkjet-based bioprinting is a sort of bioprinting technology that utilises the traditional inkjet printing method using desktop inkjet printers. It uses a non-contact technology that employs thermal, piezoelectric, or electromagnetic forces to release consecutive droplets of bioink onto a substrate, therefore printing tissue to replicate a CAD design (Li et al., 2016). Key advantages of inkjet bioprinting are speed, accessibility, and cheap prices. In comparison to other bioprinting techniques, this technique lacks accuracy in terms of droplet size and positioning (Lv et al., 2010).

Extrusion bioprinting emerged from inkjet printing technology and it is split into three systems based on their working principle, pneumatic, piston, and screw. In the extrusion bioprinting technique, constant extrusion pressure may be employed to extrude bioinks of varying viscosities into continuous fibres (Xu et al., 2020). However, since bioink is extruded utilising external mechanical forces that have the potential to harm cells, it must minimise cell damage to the greatest extent feasible (Xu et al., 2020). In laser-assisted bioprinting, biomaterials are deposited onto a substrate utilising a laser as the energy source (Papaioannou et al., 2019). A pulsed laser source, a ribbon covered with liquid biological materials placed on a metal sheet, and a receiving substrate are the typical components of this approach. The lasers illuminate the ribbon, causing the liquid biological materials to evaporate and deposit as droplets on the receiving substrate (Li et al., 2016). It is not subject to any mechanical stress, therefore its cellular activity remains normal. In contrast to inkjet bioprinting, laser bioprinting is compatible with very viscous biomaterials, hence extending the spectrum of materials that may be employed (Xu et al., 2020).

Traditionally stereolithography (SLA) was used to create cell scaffolds, but it is now employed to print bioink containing

* Mohamad Ikhwan Jamaludin
Bioinspired Device and Tissue Engineering Research Group, School of Biomedical Engineering and Health Sciences, Faculty of Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia

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living cells (Li et al., 2016). Stereolithography bioprinting employs light to crosslink bioink selectively to create three-dimensional objects. A digital micromirror device is used to selectively beam ultraviolet light onto the surface of the bioink before the components in the irradiated region begin to harden (Xu et al., 2020). In comparison to previous 3D bioprinting technologies, the equipment is straightforward and simple to use. However, it has been established that SLA-printed 3D structures are cytotoxic, lowering the viability of implanted cells.

The microfluidic system is a new technology that has been progressively used in genome sequencing, proteomics, cell biology, and medical diagnostics (Xu et al., 2020). It features micro, integration, high efficiency, high yield, and economic qualities. The microfluidic system is also known as *Lab on A Chip* due to its combination of cell or tissue culture, biochemical analysis, machine-controlled micropump and microvalve, photoelectric reading, and wireless micro-control. Therefore, reliable direct printing of biomaterial formulations on a microfluidic chip would be a game-changer for the creation of a biomimetic microenvironment (Yu et al., 2018).

Type of Materials for 3D Bioprinting

The varieties of materials for 3D bioprinting are expanding due to the development of material science. In order to choose suitable materials that fulfil the requirements in terms of functional and mechanical properties, and to achieve a successful printing procedure, some factors have to be carefully considered (Mao et al., 2020). There are some key characteristics that can be considered for the ideal materials of 3D bioprinting which are:

1)Printability, refers to the ability of materials to be accurately and controllably deposited in the specified space within a certain time (Kyle et al., 2017);

2) Biocompatibility, means that implanted materials must have positive interactions with the host tissues and/or immune systems in order to control the activity and function of host cells, tissues, and organs (Saroia et al., 2018);

3) Appropriate mechanical properties, having a specified mechanical strength that can resist external force and keep the printed initial form and structure play a crucial role in the execution of the functions of printed structures (Lv et al., 2010);

4) Biodegradability, the materials should eventually disintegrate after implantation in the body where the pace of deterioration should correspond to the rate at which cells make ECM replace implanted materials and the rate at which new tissue is formed and the breakdown products should be non-toxic, readily metabolised, and rapidly excretable (Martina et al., 2006);

6) Sterilisation stability, materials must be sterilisation-compatible and preserve its own properties or lose performance within an acceptable range (Gil et al., 2013).

The most widely used bioink formulation for 3D bioprinting of blood vessels is based on hydrogel precursors because of their superior biocompatibility, adjustable stiffness and permeability, capacity to imitate native ECM, compatibility with various bioprinting modalities, and ability to replicate native ECM (Cao et al., 2021). For the purpose of creating bioink with characteristics similar to the physicochemical composition of the ECM, a number of natural and synthetic biomaterials, either in separate or in combination, have been researched. Gelatin, collagen, elastin, fibrin, hyaluronic acid, agarose, alginate, and Matrigel are only a few examples of natural biomaterials that have been extensively employed for in vitro vascular tissue engineering (Cao et al., 2021).

The advantages of using natural biomaterial-based hydrogel are that it does not frequently result in chronic inflammation or toxicity to the host and offers an ideal microenvironment for cell adhesion, growth, and proliferation. However, the poor mechanical strength of naturally formed hydrogels prevents them from withstanding physiological pressure, which restricts their application in the engineering of vascular tissue (Boccafoschi et al., 2007). Thus, the uses of synthetic polymers in combination with natural biomaterials have been studied due to their finely regulated mechanical properties, porosity, repeatability, structural diversity, stiffness, and biodegradability. However, the use of synthetic polymers is constrained considering their poor cell adherence, lack of biocompatibility, release of toxic byproducts, and degradation-related loss of mechanical capabilities.

The addition of methacryloyl groups to polymers including gelatin, collagen, and hyaluronic acid is one of the most widely used functionalization techniques to get around these limitations (Cao et al., 2021). This process produces photopolymerizable polymers that, when necessary, can create structures with mechanical stability. The amount of methacryloyl modification and the length of exposure to light have a significant impact on the mechanical strength of these polymers; more methacryloyl modification and longer exposure to light result in stiffer constructs with less breakdown (Jia et al., 2016). For instance, the degree of crosslinking and consequently the mechanical characteristics of gelatin methacryloyl (GelMA) were boosted by the addition of gelatin. Additionally, the addition of other polymers, such as methylcellulose, 4-arm PEG-tetra-acrylate (PEGTA), 8-arm PEG-acrylate with a tripentaerythritol core (PEGOA), hyaluronic acid, PVA, or hydroxyapatite, can increase shape fidelity after bioprinting and/or improve printability (Bae et al., 2009).

Additionally, a number of bioactive substances, including polylysine and fibrin (or fibrinogen and thrombin), have been incorporated into bioinks to induce or improve their bioactivity. Bioink's bio adhesiveness and bioactivity have been demonstrated to be enhanced by fibrin, a polymer of fibrinogen that produces fibrous, viscoelastic, and porous hydrogels in the presence of thrombin (Keating et al., 2019). By strengthening the electrostatic interactions with negatively charged cell membranes, positively charged polylysine has also been utilised to improve cell adhesion.

Numerous growth factors have been identified as the inducers of angiogenesis, including transforming growth factor-α (TGF-α), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF) (Cao et al., 2021). For instance, VEGF and FGF-2 are important blood vessel formation mediators that promote EC proliferation, motility, and differentiation. Studies conducted both in vitro and in vivo revealed that VEGF was physically trapped within PEG hydrogels awaiting release in response to proteases generated by the cells leads to formation of vessels (Cao et al., 2021)

It was shown that cells behaved differently in elastic and viscoelastic hydrogels, and that the biomaterial's relaxation

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behaviors varied in response to the contact of cells, changing the spread, proliferation, and differentiation of the cells (Chaudhuri et al., 2015). In 3D, it was discovered that MSC differentiation was reliant on the hydrogel's initial elastic modulus, however in elastic hydrogels, it lost sensitivity to hydrogel stiffness, highlighting the importance of stress relaxation in cells in relation to mechanical cues in the ECM (Khetan et al., 2013). These understandings may be useful in developing vascular bioink designs for enhancing cell and vessel behaviors.

Cell Source for 3D Bioprinting

Bioprinting requires the right cell choice for efficacious print of functional tissue. Parenchymal and nonparenchymal cells whereby cells with structural, supportive, or barrier functions have to be included to generate bioprinted vascular (Hauser et al., 2021). Cells incorporated should maintain cellular homeostasis and the potential for self-renewal while delivering their assigned functions after printing to recapitulate the entire tissue physiology (Hauser et al., 2021). Besides that, the cells used should exhibit robustness to support the printing process and have control over the cells to avoid excessive proliferation of hyperplasia or apoptosis (Hauser et al., 2021).

Generally, stem cells and specialised cells are the two main cell sources that may be employed for tissue engineering. Dissociating specialised cells from a tissue donation allows for their acquisition. Analogous and patient-specific cell components are needed for the transplantation of bioprinted tissue; the former can be produced by biopsy or patient-specific stem cell differentiation (Seyedmahmoud et al., 2020). Adult stem cells induced pluripotent stem cells (iPSCs), and human embryonic stem cells (hESCs) are examples of stem cells.

Although hESCs are quite commonly used, adults' own cells are reprogrammed into iPSCs for use which can overcome any problems of an immunological response to the bioprinted organ (Vermeulen et al., 2017). Pluripotent stem cells (PSCs) are the favoured cells to use given their ability to both self-renew and differentiate into any required adult cell type (Faulkner-Jones et al., 2015). These cells have the remarkable ability to develop into practically every form of cell in the human body. However, the use of fertilised embryos aroused several ethical discussions; as a result, there is little additional study employing this cell. Adult stem cells, on the other hand, are multipotent and are found in a region known as the "stem cell niche." They tend to be dormant until they are triggered to maintain healthy tissues or heal sick and damaged tissues since they are scarce and have a restricted ability to divide in vitro. Bone marrow stem cells (BMSCs) and Adipose-Derived Stem Cells (ADSCs) are two types of adult stem cells that are employed.

Post Processing of 3D Bioprinting: Maturing

According to Zhang et al. (2021), post processing of 3D bioprinting entails maturing cell-laden constructs in order to reinforce the development of desired tissue constructs. Other than that, post-processing is also crucial to maintaining cell viability and functionality. Growth and differentiation factors are frequently and carefully selected as chemical stimuli to drive specific cell responses, as cell division, matrix synthesis, and tissue differentiation all rely heavily on growth factors (Hughes et al., 2006). However, Martin et al. (2004) confirmed that most 3D cell-laden constructs are not exposed to fluid mechanical cues during the maturation process, such as fluid shear stress, tension, and compression. Hence, using complex and advanced in vitro culture systems, such as bioreactors, is one potential approach to artificially generating the chemical and mechanical demands of human tissues. Bioreactors are devices that allow biological and/or biochemical processes to occur under closely monitored and tightly controlled environmental and operating conditions such as pH, temperature, pressure, nutrient supply, and waste removal (Martin et al., 2004). Bioreactors may imitate the biological environment required for tissue development. These technologies are largely focused on nanoimprinted tissue maturation, however, they could be applied to support structures post-printing.

Recent advancements in 3D Bioprinting

Three-dimensional (3D) bioprinting has emerged as a promising approach for a variety of biomedical applications. There have been many recent developments and advances that are pushing the 3D bioprinting field forward due to the rapidly evolving industry. Alternative bioink formulations, such as the use of exosomes instead of stem cells, have been introduced as one of the recent advances in 3D bioprinting (Jamieson et al., 2021). Exosomes have shown potential in 3D bioprinting because they can influence cell growth and development as well as help correct abnormal cellular activity and influence the growth of surrounding host tissue. Exosomes were found to reduce cartilage mitochondrial dysfunction and accelerate osteochondral defect repair when printed within a 3D scaffold at the sites of osteochondral defects (Jamieson et al., 2021).

Other than that, is the application for the printing of skin for people who are suffering from burns to restore the skin defect (Javaid and Haleem, 2021). For example, a 3D bioprinter was used to create bioactive skin scaffolds using a cell-laden alginate hydrogel mixed with other biomaterials (Javaid & Haleem, 2021). In one week, a bioink-based skin tissue scaffold made of alginate, gelatin, and encapsulated hMSCs was 3D printed and the demonstration showed full attachment to the wound surface and integrated with host tissues as well as its biocompatibility for skin tissue (Zhang et al., 2019).

Furthermore, biohybrid robots (biobots) developed by integrating living cells with soft materials have gained research interest (Sun et al., 2020). According to Gao et al (2021), researchers have used biological components with contractile cells as actuators to create diverse biobots with biomimetic behaviours and functions. Biohybrid robots utilised a variety of living cells and tissues as biological actuators, including cardiomyocytes, skeletal muscle cells, and optogenetic neuromuscular tissues. Various biobots, such as crawlers, swimmers, jumpers, and rollers, have been built showing significant potential for a wide range of applications in mechanics, biomedicine, material science, chemical engineering, and many other fields (Fang et al., 2022).

CONCLUSION

In summation, it is apparent that bioprinting innovations will constantly evolve even more quickly due to the expanding market for bioprinters and the strong interest in tissue engineering and regenerative medicine. As nothing more than a technique of fabrication for generating scaffolds, cells, tissues, and organs, 3D printing in the medical field is receiving

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increasing attention. Although it has benefits in terms of precise control, repeatability, and individual design, there are still numerous obstacles to overcome when creating complex tissues with many cell types arranged spatially. More crucially, in order to use bioprinting methodology in a clinical setting, bioink components development, resolution improvement, and vascularization are required. Therefore, more research efforts should be dedicated to develop more comprehensive techniques for further clinical use.

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