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Applications and Limitations of 3D Bioprinters in Tissue Culturing: A Review

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Abstract

3D bioprinting is an advanced technology that uses different biomaterial like hydrogels and bio-inks to develop artificial tissue cells and organs. There are three types of bioprinting techniques: Jetting-based bioprinting, extrusion based bioprinting, and integrated bioprinting. Biomaterials used in 3D bioprinter should have some ideal characteristics such as they should be biocompatible, printable, and provide mechanical and structural properties. There are different types of bio-inks, hydrogels, and growth factors used to overcome the crisis of organ shortage. Bioprinting technology is essential for the development of eleven organ systems as there is a need for organ replacement and tissue regeneration. It is possible to make complex tissue culture structures by using 3D bioprinting. The mixture of biomaterial and living cells used for bioprinting is called bio-inks. Hydrogels are one of the ideal components of biomaterials as it has similar characteristics as natural extracellular matrix and provides a hydrated environment for cells to divide. Generation and transportation of many tissues, including skin, heart tissues, cartilaginous constructs, and tracheal tissues is done by 3D bioprinting. It is used for research purposes, drug testing, and drug discovery. But our focus is to highlight the applications of 3D bioprinters in tissue engineering and the development of organ systems. Skin tissues have also been engineered to overcome complex skin treatment procedures and to save time and cost.

Keywords: 3D bioprinting; Bio-inks; Biomaterials; Hydrogels; Tissue Culturing

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1. INTRODUCTION

3D printing is a source of many inventions in many fields like arts, medicines regeneration, drug screening, disease modeling manufacturing, organ and tissue development [1]. In 3D Printing, cell-laden bio-inks are used to fabricate into functional tissue and organs [2]. 3D Printing and 3D bioprinting are interchangeable terms. It is essential to understand the difference between them [3]. In the 3D bioprinting technique, biomaterial, cell-laden bio-inks, and hydrogels must be required to construct a living tissue or organ [4] whereas in 3D printers, there is no use of cells, bio-inks, hydrogels. It also has many biomedical applications such as it is used to invent surgical devices, surgical instruments, customized implants, and anatomical models [5].

The study of bioprinters is an interdisciplinary study that includes studies of different subjects/fields like genetic engineering, tissue culturing, biomaterials science, cell biology, nanotechnology, physics, and

medicines [6]. It is helpful in biomimicry of several tissues like multi-layered skin, bone vascular grafts, tracheal splints, heart valves, and cartilaginous structures [7]. It is helpful in the increasing organ shortage crisis as it has application in the development of 11 major organ systems such as urinary system, digestive system, reproductive system, lymphatic system circulatory system and other major systems of the body [1].

It also has applications in pharmaceutical research studies for testing drug efficacy, toxicity chemotherapy, or chemoresistance to reduce some of its limitations like high cost and time-consuming procedures [8].

2. HISTORY

2.5 million people in Poland suffered from diabetes and out of these 2.5 million people, 10000 were patients of type one diabetes (T1D) [9]. Ruining and deterioration of the endocrine cells and hyperglycaemic hormones were the cause of diabetes which lead to insulin deficiency [10]. There are many diseases due to vascular and nervous disorders such as neuropathy, myocarditis, end-stage kidney damage, feet ulcer, and retinopathy due to abnormal flow of blood, insulin regimen treatment, and optimal glucose level. It may also cause severe glycemia in hypoglycaemia. To achieve the function of a normal kidney, there were only two possible options in patients with type 1 Diabetes either transformation of pancreas or transformation of islet. But these transformations have some limitations such as expensive surgical procedures are required and lifelong immunosuppressants to reduce rejection [11].

To overcome these limitations, bio mimicked pancreas was used for the treatment of T1D disease. This method is an innovation and is currently in progress. 3D bioprinting of bio mimic pancreases is made of specific biomaterials, which was like the natural extracellular matrix, that is the most promising solution to the methods. The crucial properties of 3D bioprinting are; (i) This procedure is innovative and new, (ii) Bio mimicked transplants are independent of the numbers of donors; (iii) Tissues engineering techniques are used to develop pancreatic islets by using stem cells from patients and (iv) These methods can be directly adapted to the patient's needs by tissue engineering [12]. Fig 1 and Table 1 show the conversion of 2D models into 3D models and a timeline of the development of bioprinters respectively.

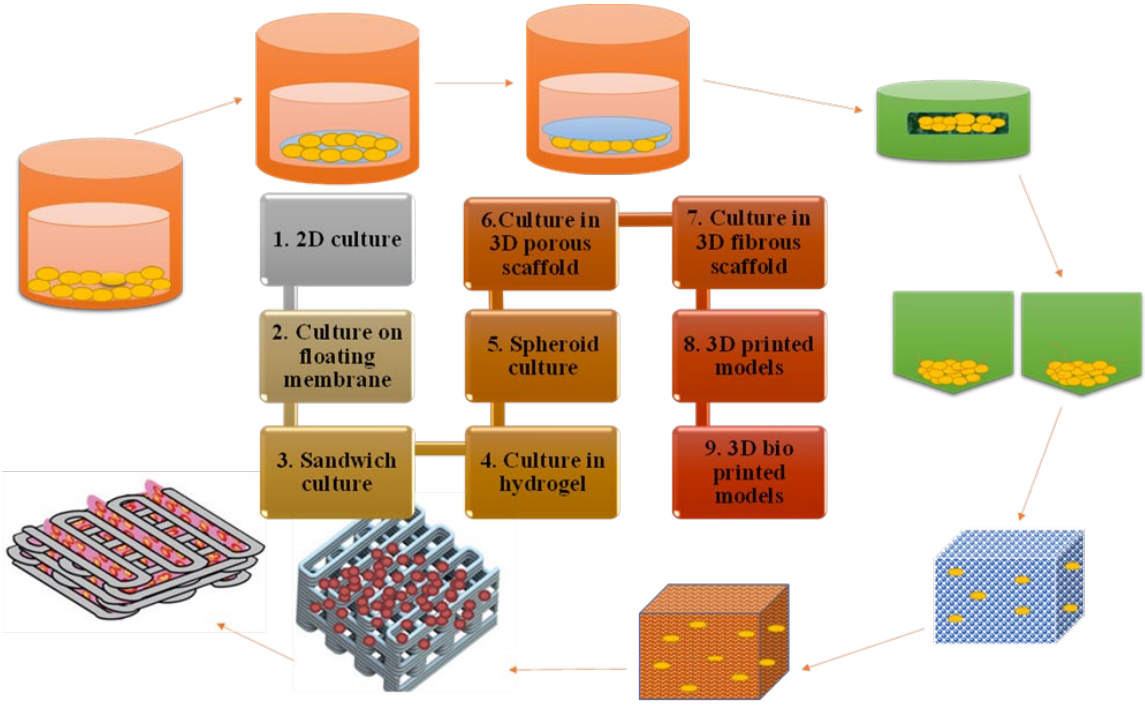

Fig. 1. Conversion of 2D models to 3D bio-printed models

Table 1: Timeline for the development and Invention of 3D bioprinters

Year	Scientist Name	Invention/Development	Ref.
1984	Charles Hull	Invented the first bioprinters and also patented the stereolithographic methods	13
1987		The first commercial 3D printer (SLA-250) has appeared on the global market known as "Stereolithography Apparatus."	14 13
In the 90s	Emmanuel Sachs	He patented and implemented the term "3D Printer." These 3D printers were used to print plastic, ceramic, and metal elements.	15
1996	-Kay C Dee -Rena Bizios	Biomaterials were used in tissue culturing and engineering	16
2001	-Linda Griffith - Gail Naughton	The bladder scaffold was directly printed. Donor cells were directly seeded	17
2002-2003	Thomas Boland	He unproved the inkjet bioprinting technique. Scientists established a bioprinting technique in cells were used due to their high availability.	18
2008	Francois Auger	New 3D tissue constructs with no scaffold	19 20
2009-2016		1. Development of brand-new 3D bioprinter. 2. New bioprinting techniques and products were introduced. 3. Vascular 3D constructs with no scaffold (2009) 4. Injection of hepatocytes into collagen and skin printing (2010). 5. Artificial liver (2012) 6. Artificial cartilage (2012) 7. Tissue integration with a circulatory system (2014). 8. Heart valves (2016)	

Presently, 3D bioprinting techniques are mostly used for fabricating blood vessels, heart valves (Valve interstitial cells), bones, liver cells (for detection of toxin, drugs, and other chemical compounds), skin (healing of wounds and for research purposes) [20]. Currently there are three main types of bioprinters: laser-assisted, inkjet printers, extrusion printers [21]

These techniques have specific requirements for biomaterials used for these purposes such as hydrogels and all types of cells are not compatible. The biomaterials that are used for bioprinting should have specific properties. Inkjet and extrusion-based techniques are the best and most widely used methods for the development of 3D constructs [20].

3. 3D BIOPTINTING TECHNOLOGY

3D Bioprinting Technology is a technique that prints the complex tissues or organs with the use of viable cells and build on biological structures, living cells, and biochemicals that are layer-by-layer in error-free position [22]. It has obtained attention because of its capacity to control some engineering provocation that runs in the area of tissue engineering. Bioprinting is a technology that has a lot of applications, like building up functional tissue or organs to replace the diseased and injured tissue or organ [23]. Bioprinting is held on additive

manufacturing in which we use biomaterials that provide a microenvironment for living cells and the biomaterials that we use, referred to as bio-ink [24].

4. TYPES OF BIOPRINTING

There are three main types of Bioprinting; Jetting based Bio-printing, Extrusion based bio-printing and Integrated bio-printing. These types of bioprinting are explained in detail in this review.

4.1. Jetting based bio-printing

This type of bio-printing is a connection-free method in which 2D, and 3D models are produced by bio ink droplets layered on the substrate. Jetting-based bio-printing evolves by the mechanisms that are used to invent bio-ink droplets through a piezoelectric actuator, thermal technique, pneumatic pressure, and the laser-induced forward transfer method [24]

This thermal method employs the use of a generator that produces heat, through temperature increases within the chamber of the bio ink. This heat produces bubbles and eject small droplets. The bioprinting applies piezo crystal pulse actuator through the utilization of piezoelectric actuator which is arbitrated through electric current, and pulse is generated which results in an emission of a small drop. So, these two techniques are commonly utilized for jetting-based bioprinting and commercially this type of delivery method is used for inkjet printers. The LIFTM (Laser-Induced Forward Transfer Method) technique produces evaporation by the application of laser to generate small droplets [25]. Next in pneumatic pressure, the drops are produced under pneumatic pressure by opening and closing a valve. The components of jetting-based printers have been shown in the Fig 2.

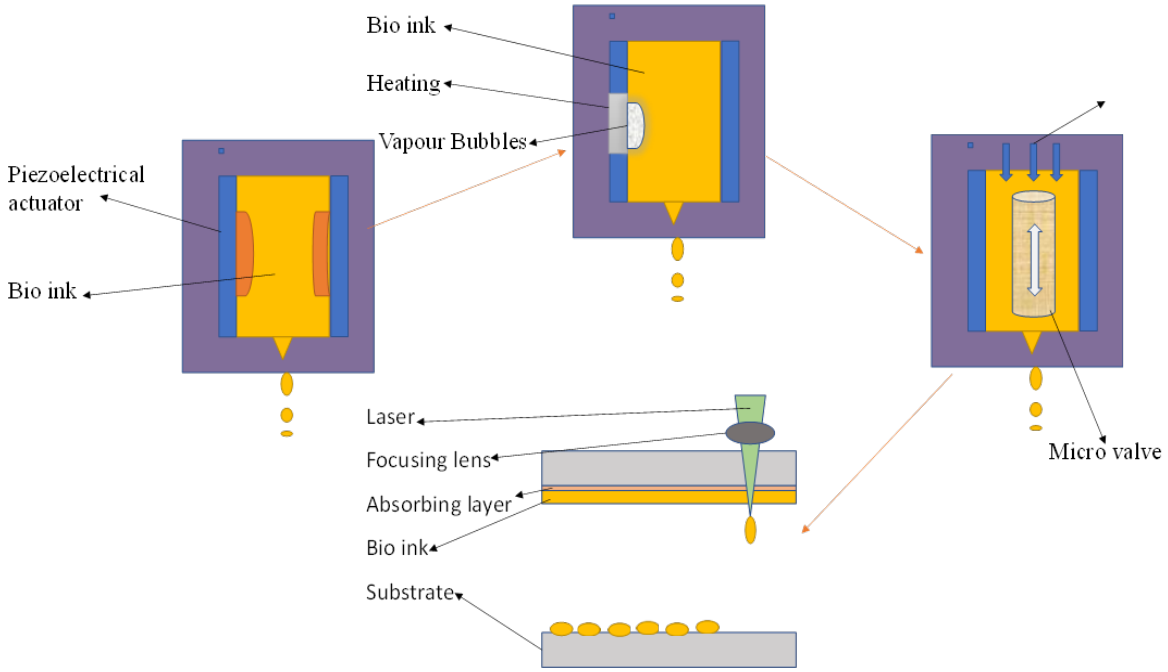

Fig. 2. Jetting Based Printing

4.2 Extrusion based bioprinting:

This type of bioprinting complex distributes ongoing filament of substances that contain cells assorted through micronozzle with a special gel called hydrogel for the manufacturing of 2D or 3D models. The distribution of the gel can be due to syringe pressure and also distribution can be maintained by managing

the amount of pressure [24]. When 2D models print, the hydrogel is solidified through the chemical and physical method, and 3D models can be manufactured by arranging 2D models surface after surface

This type of bioprinting allows much extensive choice of biomaterial. And as a result, manufacture and scaling up 3D models can be attained by the utilization of biomaterial [26].

4.3 Integrated bioprinting:

We know that bioprinting technology is based on cell-laden hydrogel and cell aggregate to manufacture the models. So, this is hard to manufacture 3D models with enough toughness, suitable shape, and size because of stubby mechanical features and insufficient strength of the material of hydrogel. To control and minimize these restrictions, we establish a structure that prints many things like man-made biopolymer and also the hydrogel to generate organs with excessive robustness [27].

And these synthetic biopolymers give physical assist of three-D and the constitutes of the cell-laden hydrogel help in the regeneration of tissues and cells [26].

5. BIOMATERIAL USED IN 3D PRINTING

5.1 Bio inks

Bio inks are used for non-living cells and biological applications. The foremost material which is used for bio-inks are ceramics, thermoplastic polymers, and metals. The main purpose for the manufacturing of organs and tissues through 3D Bioprinting technology is to obtain chemical, mechanical, and morphological properties same to real tissue and organs. Hence, bio-ink has an important role to perform all the aspects of 3D Bioprinting. They save the over artificial organs and tissues against the fabrication procedure such as inappropriate environment and extrusion. Several materials are used as bio-inks to form 3D models including hydrogel, lipids, ceramics, polymers, and elastomers [28].

A perfect bio-ink should have adorable physiochemical properties such as rheological, biological, chemical, and mechanical characteristics. These properties help to build the tissue with sufficient mechanical strength and they are biocompatible and perfect for chemical modification to form tissue [23]. There are 4 types of bioinks; (i) Multimaterial bioinks, (ii) Interpenetrating network bioinks; (iii) Nanocomposites bioinks and (iv) Supramolecular bioinks [29].

5.2 Hydrogels

Hydrogels are one of the essential requirements for 3D bioprinting. Hydrogels are biomaterials that have already been used in 3D bioprinting along with bio inks to make 3d structures [30]. It has some properties similar to the properties of the natural extracellular matrix. Their most important function is to provide a hydrated environment for cell multiplication. Hydrogels may be natural or synthetic. Examples have been given in Table 2.

Table. 2. Examples of different types of polymers used in 3D bioprinting techniques

Examples of polymers:		
Natural hydrogels	Alginates	31 32 33
	Collagen	34
	Gelatin	32 35
	Chitosan	36
	Methyl cellulose	37
Synthetic hydrogels	Poly acrylamide	38
	Poly (vinyl alcohol)	39
	Poly (2- hydroxyethyl methacrylate)	40

They are biocompatible and printable. Even so, they have some limitations as their nature is not strong [41] and products are degradable [42]. Scientists have to make a hydrogel that is capable of bioprinting as well as tissue culturing [43]. There are three types of hydrogels; homopolymeric hydrogels, copolymeric hydrogels and multipolymeric interpenetrating polymer hydrogels [44].

6. IDEAL PROPERTIES OF BIOMATERIAL USED IN 3D PRINTERS

It is essential to find a material that has ideal properties so that it can be used for 3D bioprinting and perform its functions accurately. Biomaterials used for this purpose should be biocompatible, should have some mechanical and functional properties. Biomaterials used in 3D printers should have these properties that make them ideal for use in 3D bioprinting such as biocompatibility, mechanical and structural properties, printability, material biomimicry, degrading of kinetics and by products, porosity and structural support.

6.1 Biocompatibility

The biomaterial used should be non-toxic and less harmful to the tissue cultures and new organs [45]

6.2 Mechanical and Structural Properties

Such material should be used that can provide the required structural, mechanical, rigidity, integrity properties over a period of time [46]

6.3 Printability

The material should have a property to be accurately deposited with desired and temporal control. Biomaterial used should cross-linking mechanisms and shear thinning [47]

6.4 Material Biomimicry

The biomimicry property with ideal physical and chemical properties are based on the composition of specific tissue and localization of ECM (Extra Cellular Matrices) in the desired tissue [48].

For example, Nanoscale property such as ridges, steps, and groove affect, attachment of cell, cytoskeleton assembly, and division of cells [49].

6.5 Degradation of Kinetics and By Products

Embedded cells release protease enzymes. It produces ECM proteins that are required for new tissue culture, which is due to the degradation of scaffold material [50].

Byproducts released due to degradation are also essential as they may not be biocompatible for tissue cultures. The byproducts should be non-toxic, readily metabolized, and easily removable from the body.

6.6 Porosity

3D bioprinters require porous structures for proper transportation of oxygen and nutrients, tissue integration, and vascularization.

6.7 Structural support

3D bioprinting requires good strength to overcome the stress in the host environment

7. APPLICATIONS OF 3D BIOPRINTING IN DEVELOPMENT OF ORGAN SYSTEMS:

Considerable improvement has been achieved in the subject of bioprinting, with different types of printed tissues and organ systems. The human body is composed of several organ systems that work together to sustain homeostasis and normal functioning and natural body operations

An organ system is composed of multiple tissues, organs and anatomical components that work together to carry out a specified role. There are seven organ system in the human physique: skeleton, muscular, nervous, Lymphatic, endocrine, reproductive, integumentary, urinary, respiratory, and digestive system. The bioprinting techniques are being used for artificial development of these organ that have similar functions to original one [2] Different types of cells have been produced by using different types of hydrogels, bioinks, and

growth factors by using different techniques. Examples of cells and tissues that were engineered by using different technique are show in the Table 3.

Table. 3. Examples of tissues and cell of different organ system that were engineered through 3D bioprinting techniques

Organ system	Type of Tissue engineered	Types of cells developed	Bioprinting technique	Biomaterials used	Growth factors used	Ref.
Skeleton system	**Bone tissues**	Bone marrow stomatal cells	Pneumatic extrusion bioprinting method	Alginate hydrogel	Osteogenic marker alkaline phosphatase	[51]
		Human osteogenic sarcoma cells	Extrusion bioprinting method	Alginate/ gelatin hydrocarbon	Agarose and calcium salt of polyphosphate	[52]
		MSC	Inkjet printing	PEG-GEL MA hydrogels	Polydopamine (PDA)BMP 2 peptides	[53]
	Cartilage	Adipose derived stromal stem cells	Inkjet printing	Gelatin methaceyloyl/hyaluronicacid methacryloyl	Lithium acylphosphonite (LAP)	[54]
Muscular system	**Skeleton muscular tissues**	Human skeleton muscle cells (hSkMCs)	Nano fibrous scaffold/ Electrospun Scaffolds	PCL/collagen	BMP-2, FGF-2	[55]
Nervous system	**Brain tissues**	Murine neural stem cells (NSCs)	Inkjet bioprinters	Polyurethane (PU) nanoparticles based bioinks	CNTF, VEGF	[56]
Lymphatic system	**Lymph node tissues**	Artificial lymph node	Extrusion based printing	Self-laden hydrogels	-	[57]
Circulatory system	**Myocardial tissues (blood vessels)**	Cardiomyocyte progenitor cell, Endothelial cell,	Extrusion bioprinting, and inkjet bioprinting	Alginate, fibrin	-	[58] [59] [60]

		Smooth muscle cell, and Mesenchymal stem cell				
Integumentary system	**Skin tissues**	Dermal fibroblast, Epidermal keratinocyte	Inkjet bioprinting	Collagen	-	[61]

7.1 Use of 3D Printers in Skin Engineering

Skin is the largest organ of the body consisting of 15 % of the body total mass of normal human body which mainly constitutes of 3 layers [62]. Some skin illnesses include dermatitis, cellulitis, and even melanoma that can occur in case of any disturbance in normal homeostasis and infection in these layers. The skin consists of a fleshy layer that has several important functions that main normal regulation of homeostasis of the body. The main functions of skin include providing resistance against stressful environmental conditions, thermal regulation, and maintaining excretory mechanisms by sweat glands [63].

7.2 Approaches in tissue engineering of skin

Two major approaches that help or improve skin regeneration are stated below that are used in 3D bioprinting, tissue engineering strategies (Fig 3). The bottom-up approach creates bigger and complex tissues with the help of a process called biological sintering. The elements that can mainly make up building blocks are cell aggregates and Polymer-based microbeads consisting of heterogenous or uniform composition. Smaller blocks discussed are symmetrically arranged in three dimensions which further forms a complex namely biomimetic complex structure. A well-known example is cell sheet technology which makes comparatively thick tissues without using biodegradable symmetry/scaffolds and bioreactor by the help of coating or layering individually a cell sheet that is of few microns in size. Comparatively thick size, one more example can be cell-laden microgels [64], [65]. Other examples can be laser-induced forward transfer [66], [67] as discussed above inkjet printers [68] [69] and techniques including laser techniques that consists of direct writing [70], [71].

The other approach is the top-down approach that converts larger or material in bulk quantity into smaller or more complex structures. this process provides many advantages like stability to the structure and cell migration in the symmetry or structure that can form cell junction or better cell to cell interactions. So, it is concluded that integration of each phenomenon provides a better understanding for skin tissue engineering [72].

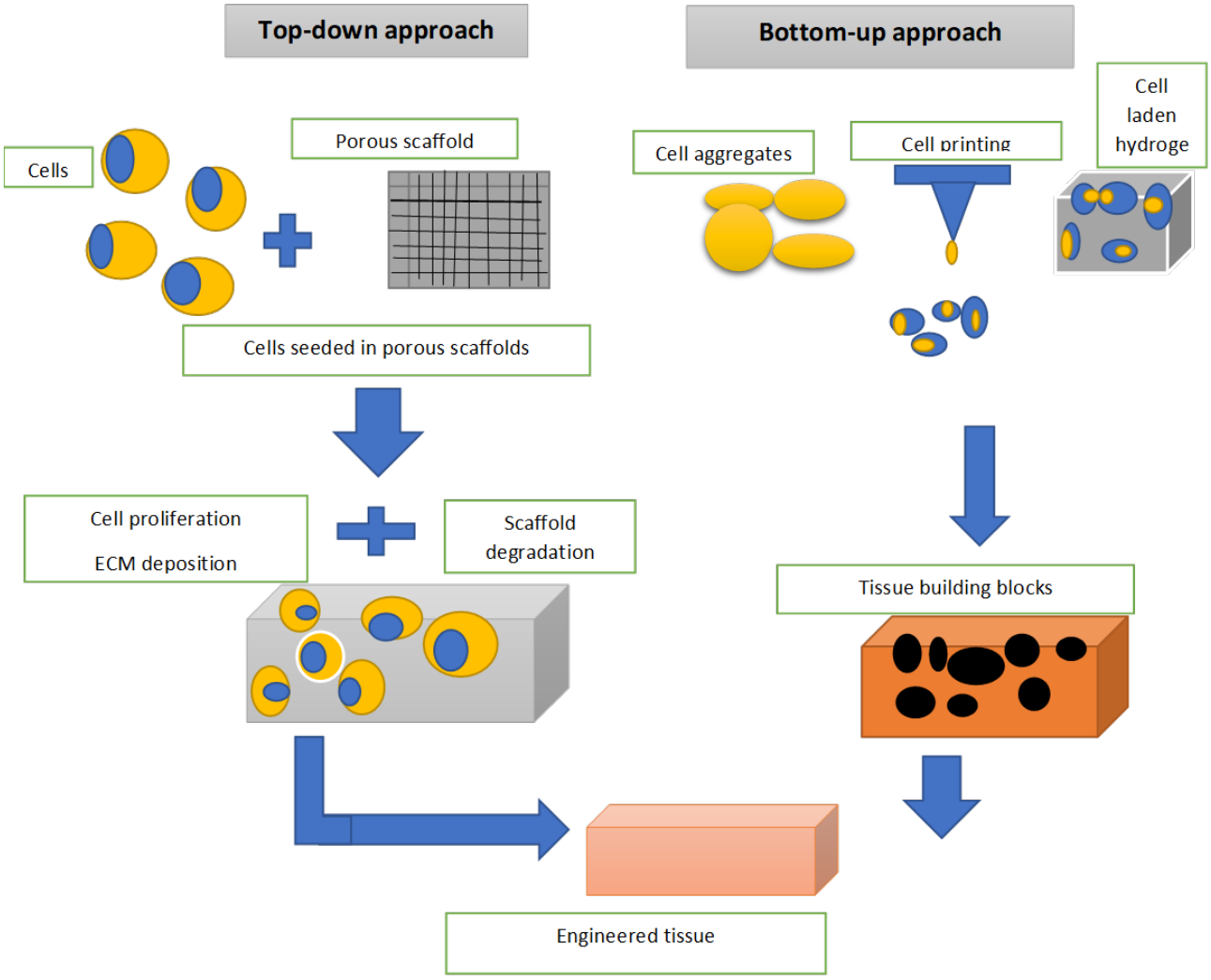

Fig. 3. Approaches of skin engineering using 3D bioprinters

8. LIMITATIONS OF 3D BIOPRINTING

Every technology has to suffer some limitations and has some challenges. Similarly, 3D bioprinting techniques used for organ development and regenerative medicines have some limitations and challenges. Various bioprinting methods have been used to develop different tissue and organs constructs. These methods are responsible for safe delivery of cells, biological molecules, and biomaterials to a specific target. These methods are easily used for constructing simple tissues, and organs. Constructing more complex, solid, and composite organs and tissues always remains a challenge [24].

Presently, it is difficult to construct organs that have a well-integrated circulatory system as the supply of blood, nutrients, and oxygen to the organs may be difficult [2]. Due to some innate properties of biomaterials like hydrogels or bio-inks, these 3D bio-printed constructs have low mechanical strength and integrity. So, they are not fully able to maintain the proper shape and fight against external stress after implantation. The fabrication of the bioprinting constructs has also been a big challenge. The requirement to construct tissues and organs includes different kinds of cells with a resolution in the micrometer unit and complex inner architecture. so, the resolution of cells used in 3D bioprinters is a big problem.

3D bioprinting of skin also has some challenges. Bio printed skin has a long healing time and the procedure is expensive as the skin is heterogeneously made up of different cells. Some easily mimicable skin cells by 3D bioprinters include fibroblasts and keratinocytes [73]. But most complex skin cells like melanocytes, Langerhans cells, and hair follicle cells are difficult to construct. Scientists can use stem cells but there are some ethical issues such as costly procedure, skill requirements and time consuming procedures [74].

8. FUTURE PERSPECTIVE

In a 3D printer, various factors need to be considered on a priority basis to enhance its capability. To enhance the structural integrity, we must consider the biocompatible material that can sustain its integrity [59]. In 3D printing, extrusion-based methods are highly recommended as this method has a maximum resolution that is about 100-300 um [75]. For this purpose, the fabrication resolution of bioprinters should be improved. Additionally, the time of fabrication related to the speed of printing material must be considered [76]. In this regard collaboration of experts from different domains such as biochemistry, engineering, and biomedical will also encourage the various prospects and boost the potential that will be readily available for saving and serving the life of people.

9. CONCLUSIONS

3D bioprinting is an advanced technology that has many uses in every field of arts, medicines, drug screening, etc. It requires specific techniques, types of cells, biomaterials like hydrogels, bio-inks, growth and differentiation factors. 3D bioprinters are used in the development of 11 organ systems. It was first invented by Charles Hull in 1984. There are three basic types of bioprinters: Jetting based bioprinters, extrusion-based bioprinting, and integrated-based bioprinting. Different kinds of bio-inks and hydrogels are being used for 3D bioprinting which may have some ideal properties. Currently, there are two approaches used for the development of 3D bioprinting of skin tissues. This technique also has some limitations as it is very expensive, it consumes a lot of time, and in the transfer of nutrients and gases in bioprinter tissues is difficult but the demand of bioprinters is increasing day by day in different applications.

CONFLICT OF INTEREST

No conflict of interest

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