Review on 3d Printing of Biological Tissues and the Materialization

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Abstract. 3D printing is one of the recently evolved manufacturing techniques which has wide range of applications in engineering, education, art and science. Bioprinting is the recent advancement of additive manufacturing process in the area of medicine. It is a process which involves layering and curing of biological cells or tissues and turning it into a fully functioning organ or hard supports(bones). It has solved the problem of shortage of organs for transplantation. It would be very difficult to find the right organ according to the needs of a particular person but this recent advancement has given a right solution for it. Bioprinters typically uses bioinks which dispenses through the nozzle of the printer for deposition, and a dissolvable hydrogel is used in order to protect and support the tissues of the organic material. This type of printing is done in lab environment only under a controlled system.

1. Introduction
In the end of the ninetieth century researchers were trying out different trial and error methods towards finding apt way and substance that can result into a completely working human body parts [1]. Researchers of the Wake Forest institute had made a seamless work towards the finding of a completely working human bladder and it was developed using a rigid support providing synthetic scaffold in the year 1999. After a triennial period a fully functioning animal kidney which purifies the blood and also secretes urine was developed. Since the innovation of this method has become a lifesaving system for humans, every year the number of patients for organ transplantation gets increased rapidly but the number of donors decreases [2]. However, many times most of the organs donated may not be suitable for transplantation because of various tissue damage and infections which could be transferred to the receiver from the host [3]. Bioprinting has provided students the platform to innovate, design and experiment their ideas in real life [4]. Though Bioprinting is a very helpful manufacturing technique, there are lots of challenges in producing an organic material. In this method the precision is of great importance. Every organ in the body comprises of different kinds and types of tissues, the required cell of kidney, liver, skin and any other organs are obtained so that it will be more useful to create the bio-links which is then made into larger quantity to be fed into the printer to be printed [5].

2. Experimental Techniques
The working of traditional 3D printers is to be known in order to understand the Bioprinting process. This additive manufacturing method can be classified into 3 categories, they are Preprocessing,
Manufacturing and Postprocessing. In the Preprocessing step, the products are designed according to the requirement or blueprints. Once the printing material is selected, the design is converted into machine’s understandable code and then is fed into the printer to print. Bioprinting is very much similar to the tradition of 3D printing apart from the fact that instead delivering materials such as plastic, metal, or ceramic in this case biomaterial. Bioprinting is the method of constructing a living matter by a layer-by-layer arrangement of cells. The advancement in engineering has paved the way for this type of complex processes. The 3D printing process usually follows stacking algorithm in order to deposit the material. The primary material which are living cells in this case are supplied by means of the nozzle to the printing machine. A substance called hydrogel is also supplied to the printer which acts as a binding agent or a “glue”, whose function is to provide structural rigidity to the material. Some common examples for hydrogel are alginate, fibrin and Pluronic F-127 [6]. These are the primary substances which provide protection to the cells. If hydrogel concentration is low then the printing thickness would be limited due to the lack of structural support.

3. Inkjet Based Printers
Basically, this type of printers is capable of spraying ink droplets to the base plate according to the input with great precision and also at very small micron level. Advanced printing technologies will facilitate us to print a living tissue at great precision and accuracy as are capable of ejecting the ink droplets at very high resolution [7]. Makoto et al. has investigated on the biocompatibility of inkjet head and its feasibility of micro seeding with the cell. Living cells can be damaged due to raising and lowering of the temperatures so a method which uses static electrical force to drive away the ink without creating minimal amount of temperature was used. It is claimed that Advancement in the field of tissue engineering is obtained by the process of micro seeding. Similarly, Yuichi et al. has worked to develop the technology where the cells would be printed directly and with high precision using this same technique [8]. For this purpose, Sodium alginate solution and calcium solution were reacted to create a hydrogel and for the construction of the gel structure, sodium alginate solution was ejected from the inkjet nozzle and was mixed with a substrate composed of a calcium chloride. Inkjet bioprinting technology is a promising technique for creating of living tissues, the selection of suitable inkjet system is a challenging task.

4. Extrusion Based Printers
In the field of bio printing Extrusion Based Bioprinting (EBB) is a fastest-growing technology which has made a major difference. It has great ability to print a vast range of biological structures such as tissues, cells, various organic compounds, blood vessels, etc. It also facilitates to print microfluidic components, bio-chips for the purpose of both research and biological usage. Along with this we can also print a wide range of bio-inks which provides a basic structure for the print which includes tissue spheroids, tissue strands, cell pellets, decellularized matrix components, micro-carriers and cell-laden hydrogels. Extrusion based printing technique has number of disadvantages and challenges. This includes the development of new range of bio-inks, print quality which results as limited resolution of the printer, alignment of the cells and maintaining the environment safe and sustainable for the printing process, impediments to organ fabrication and also converting such technology into a easily accessible product [9]. This type of printing technique uses two major operations one is fluid dispersing system other one is automated robotic system [10]. Basically the process oriented with this is the bio inks were deposited layer by layer with great precession and controlled by a computer according to the input feed. Due to continuous and fast deposition of the filaments this method provides great structural integrity. This system is also embedded with easily accessible computer software’s such as CAD which facilitates the user to give the input feed as a CAD file and it prints the required structure automatically [11].
Figure 1. Example of Human-Scale Bio-Printed Tissues [12].

Fig.1. shows the example of printing of tissue like skin in a scale ratio which is as some as that of human scale and it is done by a inkjet printer and these types of system are able to construct tissues either in its own natural environment or an artificially controlled environment. A vascular bypass vessel which facilitates blood flow is produced by micro extruder and fused cellular vascular rods and micro extrusion based bioprinter which facilitated to print aortic valve with two types of cells, aortic root sinus which is a smooth muscle cells and aortic valve leaflet which is a interstitial cells. An air way splint which is used to maintain the opening of the airway from the outside of the trachea is printed using laser bio printing and a basic functional stage of kidneys are printed by using micro extrusion bio printing. All the above given bio printed tissues requires integration of multiple components for the fabrication of fully developed and functionally appropriate sized tissue structures [12].

5. Categories of Bio-Inks and its uses

In the process of bio printing various kinds of bio inks such as cell laden hydrogels, cell-suspensions, cell/tissues spheroids which are spherical in structure, micro carriers and decellularized matrix which is an ECM scaffold of the original tissue [13]. While in case of laser printers and inkjet printers, a fluid kind of bio-inks whereas EBB type uses ink with certain viscosity. Generally, hydrosols are used as the bio-ink in case of the EBB, the choice of the bio-ink differs respectively to the type of regenerative tissue [14]. Bioactive or biological hydrogels such as gelatin, collagen, fibrin and peptide has natural ability to support cell adhesiveness are generally used for cardiovascular bioprinting [15]. KajsaMarkstedt and team have developed human chondrocytes with Nano cellulose which is used for printing of living soft tissues with cells [16]. A bio-ink is the main criteria on which the resolution and the quality of print
depends. The good choice of the bio-ink is essential in order to get a precise and better print quality with excelling overall results.

6. 3D Bioprinting of Myocardium tissue

Heart is a vital organ in a human body which acts as the source for pumping the blood to the entire body through the circulatory system and purifies the blood of the dissolved carbon dioxide and other impurities. Every year there arises about 63,000 heart failure cases in UK [17]. In which about 34,000 are men and 29,000 are female. But considering the number of donors this number is a big sum. In many cases the loss of patient is due to the unavailability of the right heart for the transplantation. Using the bioprinting technique this issue could be overcome. Though bioprinting of human heart and its functional experimentation is in research stages, implementation of this would decrease the number of loss of lives. The Heart is made up of a special form of tissue called as the myocardial tissues this forms a rigid structure of the heart and also helps in the flow of blood through the arteries and veins. In case of damage of a myocardial tissue occurs then the following reflex such as heat, pain, swelling, redness and immobility which are the signs of acute inflammation along with the healing process that is the formation of the granulation tissue through microscopic blood vessels occurs [18]. There are various techniques in order to overcome this tissue damage through traditional methods like the vitro culture method. In tissue culture process there are two methods which is used to deliver oxygen, (a) Is by decreasing the structural size of the developed tissues into fine lumps [19]. and (b) creating a consolidated mesh like structure with orifices’ and passages which will allow the flow even till its middling’s by circulation. The drawback of decreasing the size will result in a number of surgeries to make the implant work in perfect condition and also the development of the iter-vascular tissues by the recipient may take a surplus amount of time[20]. Bio-printing facilitates us to overcome the above disadvantages by printing of accurate 3D element of specific shape and size under controlled condition with uniform distribution of specific cells [21]. Using a different pressure based bio printing technique Gaetani et al created cardiomyocytes obtained from the human cardiac muscle used 3D bioprinting (with pressure based extrusion) to create a construct containing human cardiac-derived Cardiomyocytes [22].

Another avenue of bioprinting was done by FalguniPati and group in this they attempt to Print three-dimensional tissue which are produced by in-vitro method and this system has great ability to produce the structure with great accuracy and functioning, analogues with decellularized Extracellular Matrix (dECM) bio-ink. Decellularization of the natural Extracellular Matrix (ECM) material is done initially to decrease the loss of ECM and also to increase the removal of the cellular material. Decellularization of the adipose gland, cartilage and heart tissue have been successfully done. For translating the dECM to 3D printing system, the dECM was solubilized to get a final concentration of 3%, this resulted in solubilized dECM solution and was acidic in nature and is adjusted to physiological pH prior to encapsulating cells. Using the dECM bio-ink the cell-laden structure was printed [23].

7. 3D Bio-printing of Heart Valves

There are four heart valves within the heart namely Mitral valve, Tricuspid valve, Aortic valve and Pulmonic valve these valve are responsible for the flow of blood in an unidirectional manner and these valves are branched and connected to different sub systems followed by arteries and veins which at last is attached to the fibrous[24]. Mitral incompetence is a state in which the heart’s mitral valve does not closes tightly and facilitates back flow of the pumped blood into the heart. If the problem occurs this will not allow the free flow of blood to all body parts and also oxygen level in the blood decreases resulting in a physical tiredness of the recipient. In this case heart valve replacement surgery would be required. The most common method for heart valve replacement surgery is bioprosthetic and mechanical heart valves [25]. But there are several drawbacks in both the cases that includes, the patient undergoing heart valve replacement surgery would have to take anticoagulants for the lifetime.
Figure 2. Bioprinting process of different heart valves [18].

Figure (1-2) represents flat value where (3) is the microscopic view scaled to 50µm, (4-6) represents axisymmetric valve and (7-13) represents anatomical valve. The figures (1,4,5,11) are the images of the 3D modelled heart valve in a manufacturing software; images (2,6,3) are the completely printed bioprinting valve (9,10) are the valve scan view, thresholded, and segmented into separate STLs for the leaflet and the root and (12) is fluorescent image of first printed two layers of aortic valve conduit.
8. Conditions for an ideal bioprinter
An ideal bioprinter is a dream come true phenomenon, scientists have been working on it for years to get a better precession and accuracy of the printed. The quality of print is one of the major conditions for the development and lasting of the product. Basically a perfect Bio-Printer is one which plays a accurate role of laying extracellular matrix in thin sheets as a basic bonding material and to arrange cells in a different pattern according to the input but the main things plays a major role over here is the size variation of the printing elements. An illustration shows that for the formation of perfect vascular structure and formation of tissue boundaries the epithelial and endothelial has to be arranged with proper calibration in with variations in geometries such as cylindrical and planner. Since the extracellular matrix has to printed with different orientations facilitates the cells to be offered with polarity, Ex: electrospinning process is carried out to give polarity to the cardio-myocytic cells. In addition to the above listed things sizing of the components which does these function also matters [26].

9. Conclusion
Ever since introduced, the 3D Bioprinting has gained the major attention over it. There had been major improvement since 1983. The promise of printing human organs began in 1983, when Charles Hull invented stereo lithography. Especially, the work of 3D bioprinting is concentrated in the field of cardiovascular tissues such as myocardium, cardio valves and arteries etc. According to the works of the authors mentioned, there are even more scope of improvement and many new techniques that have been introduced in order to make the printing with good resolution and fabrication. However, some techniques and methods are still being imaginary and many challenges remain for generating tissue or organ analogs.
with fully functional capabilities. Therefore, more research works and time can be dedicated to work on more high-performance bioinks and high-resolution bioprinter. There is much promise future for combining 3D bioprinting techniques with other Tissue Engineering. Bio fabrication and biological techniques will enable significant improvement for cardiovascular tissue engineering applications. The successful and complete innovation of a 3D Bioprinter with an efficient bioink would result in major savings of money and lives.

10. References
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