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Limitations and Principles of Safety in the 3D Bioprinting Process

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----------ABSTRACT----------
The importance of biological printing is in tissue structures that imitate the micro and real environment of human tissues and organs. This is very important in medicinal tests and clinical tests. In biological printing, cells or molecules are printed directly on a substrate in a particular pattern so that the cells can be kept together and formed the required 3D structure. This technology is widely used in medical science, including tissue regeneration, diagnosis of drugs, tissue transplantation, cosmetic surgery, and more. In this study, we have tried to point out existing damage and safety tips in this technology. Studies show the challenges facing this technology, such as high clarity in cell construction, lack of mechanical resistance and adequate integration of bio -printing structures made with soft hydrogel, material printing speed, recurreability and cell preservation. Also, work safety instructions with bio -printers including personal hygiene, printer cleaning, work room cleaning, ventilation system, periodic medical tests for operators are provided. Bioprinting technology is developing and requires further research to remove restrictions and reduce risk management.
KEYWORDS: *bioprinting, three- dimensional, tissue, safety, limiteds*

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I. INTRODUCTION

Biofabrication is a growing research technology that consists of a formal and tissue -based structure. Conventional biofabrications include washing materials, drying with freezing, and so on. Although the above methods are capable of a 3D set To rely on bio, they create materials but face obstacles to diversity and diversity in the production process [1]. Recently, three -dimensional bio -printing has been created as a new method and has good control over tissue architecture with repeatability [1]. 3D printing is a method in which materials are layered on top of each other, which leads to the production of the desired product [6]. This method was used for the first time in the medical field to produce bladder tissue at Harvard Medical School with joint use. From polymer and collagen, it was layered with patients' cells by hand. In the early 2000s, 3D printing started in medical science [6]. The most important goal in 3D bioprinting is to create a suitable replacement for laboratory methods on animals and to replace tissue implants with living cells. The importance of this process is in the mechanism of growth and reproduction of living cells. The sensitivity of living cells complicates bioprinting. Further research in bioprinting is focused on the path that will lead to the development of living structures relying on the function of tissues and organs [4].

II. MATERIALS AND METHODS

In recent years, 3D bioprinting has caused fundamental changes in regenerative medicine and tissue engineering. Private companies are commercializing this technology based on the research done, but the development of bioprinting faces limitations that are discussed in this study [10].

II-1-History of bioprinting

The first method for three-dimensional printing of non-living materials was based on stereolithography, which was implemented by different groups. In 1981, Kodama made the first 3D printing based on rapid prototyping, in which light was used to create bonds between resins [16]. After that, in 1984, by Hull, the production of models of layered resins was carried out in bioprinting. Forgacs in 1996, by studying the behavior of the cell, determined that a new space was provided for the structure of the cells. The use of biomaterials in bioprinting began in 2000. In 2003, the first bioprinting machine was designed and built by Boland. In 2009, the first commercial bioprinters were released to the market [26].

II-2-Familiarity with 3D printing

3D printing is the process of making a hardware product from a software file. The production of this product is done by layering raw materials on top of each other until the final object is created, which is called volumetric 3D printing. At present, researches about 3D printing technology are not finished [11].

3D printing is implemented with various technologies. Some production methods are the main and common ones, and another group uses sub-methods or technologies under the set of main methods to make the product. The most important differences in bioprinting are related to the type of raw materials, product durability, surface finish, production speed and product manufacturing cost. The different methods of 3D printing are: Stereolithography (SLA), Selective Laser Sintering (SLS), Fused Deposition Modeling (FDM), Digital, Light Process (DLP), Multi Jet Fusion (MJF), PolyJet, Direct Metal Laser Sintering (DMLS), Electron Beam Melting (EBM)[15].

II-3-Familiarity with 3D bioprinting

With the beginning of the 21st century, 3D printing entered medical science. The application of this technique was developed in surgery, orthopedics, tissue engineering researches, etc. In this technology, the use of organic solvents and the production of non-conventional pore structures were eliminated, and biocompatible materials such as hydrogels and composite materials, which form completely like natural tissue, were replaced [22].

II-3-1-Bioinks

Bio-inks are vital materials taken from living organisms and include living cells and carrier molecules that help the growth process. Carrier materials are actually polymer gels that act as scaffolds during bioprinting. And the cells are placed on them and grow. Retention of water in the structure of these compounds creates good stability. Selection of desirable biological inks causes them to have acceptable physical, chemical and rheological properties [4]. The important properties of biological ink include density, gelation, and cross-linking ability. These indicators affect the quality of printing, cell viability, and biological product stability. The existence of a hydrogel scaffold capable of maintaining living cells provides favorable conditions. It is very important to create. If the hydrogels are hard, they may put pressure on the cells and disrupt their proliferation and movement. Finally, hydrogels must maintain a state of balance in the printing process [8].

Unlike the materials used in 3D printing, the bio-ink must be non-toxic and the printing temperature must be in a range that does not harm living cells and carrier materials. Biological essences are classified into four main functional, fugitive, support and structural groups according to their role [5].

II-3-2-structure of bioinks

Carrier materials used in biological inks include natural and synthetic polymers. Synthetic polymers are involve polycaprolactone (PCL), pluronic, polyvinylpyrrolidone (PVP), polyethylene glycol (PEG) as well as natural polymers such as gelatin, hyaluronic acid, collagen, and matrigel. Other groups of biological substances used in biological ink include elastomers, hydrogels and ceramics [5]. According to the conducted researches, it seems that the most common materials used in the structure of biological inks are hydrogels. Examples of these materials are peptide-polymer designed hybrids and spider silk protein-based hydrogels. In addition to that, other carrier agents, which are cellular materials and nanoparticles, play a role in drug release and improving mechanical properties. Some compounds such as microgels can also be added to the structure of biological essences [2]. Figure1shows the application of 3D bioprinting for the manufacture of therapeutic materials.

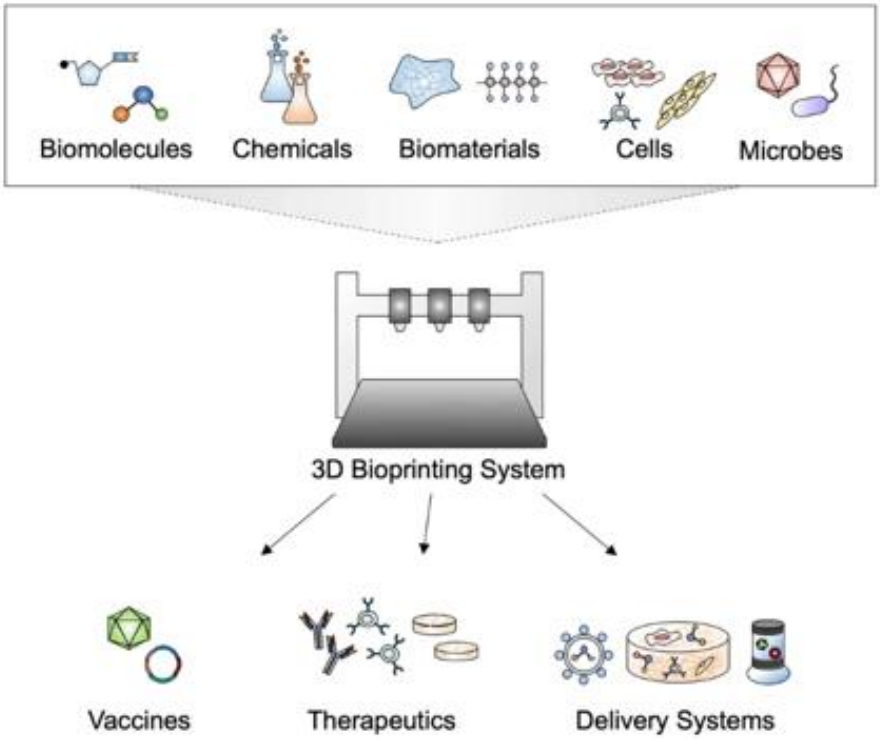

Figure1 - Applications of 3D bioprinting for manufacturing of therapeutics [26]

II-4-The mechanism of action in the 3D bioprinting process

II-4-1-Biomimicry

In fact, biomimicry is the reproduction of the internal and external components of the cell in the tissue and the function of the organ in nature and the living environment. In the process of bioprinting, the proliferation of cells and carrier materials on the scaffolds must be controlled because they affect the adhesive properties, size and morphology of the cells. For better performance of bioprinting, cell arrangement and extracellular matrix along with the gradient of soluble and insoluble factors are required [4].

II-4-2-Autonomus self- assembly

The mechanism of tissue reproduction and growth takes place with the help of living embryonic cells. Cell reproduction with the help of cell signals leads to tissue organization in the desired way. The primary stimulus in this process will guide the cell in its growth path. For a better performance of tissue creation, there must be complete information about the process of growth and reproduction of embryonic cells [4].

II-4-3-Mini tissues building blocks

In this method, small units are created by the process of self-assembly of tissue structure and become macro-tissue based on biological organization, and tissue cell reproduction continues [4].

II-5-Basic steps of 3D bioprinting

Despite the variety of different methods of bioprinters, the steps of the bioprinting process are almost the same. These steps include pre-bioprinting, bioprinting and post-bioprinting [7]. Figure 2 shows the overview of bioprinting steps.

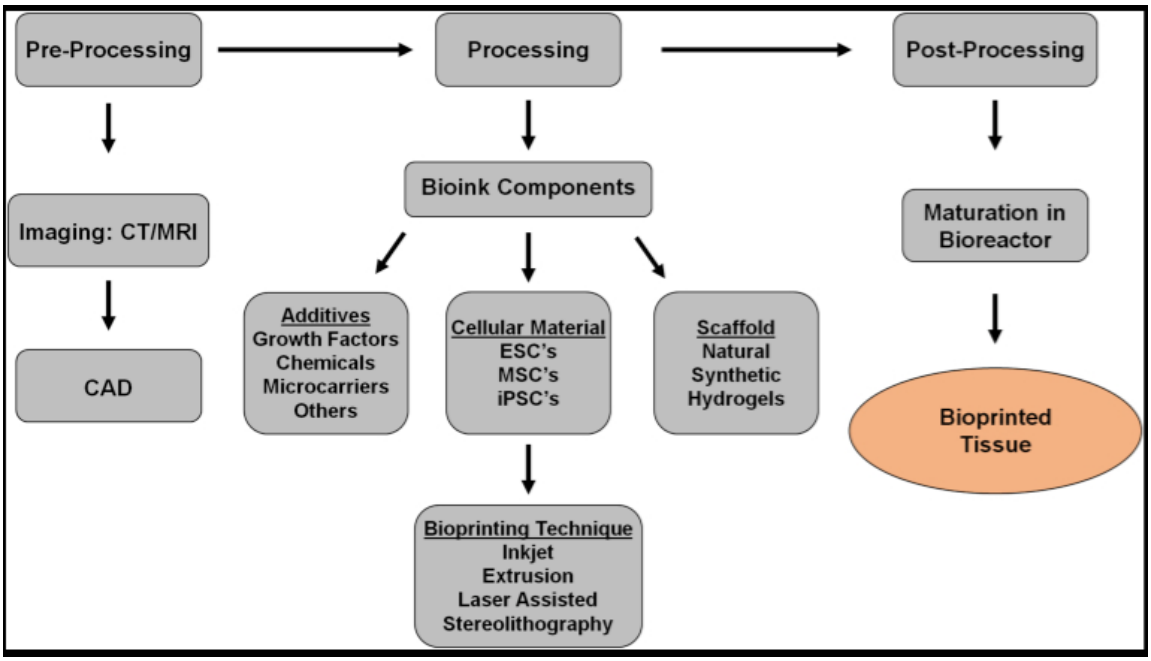

Figure 2 - Bioprinting overview schematic [9]

II-5-1-Pre-bioprinting

In this step, the shape of the model to be made is prepared first. Then, biological materials are selected to perform the work, and after that, a biological model of the desired tissue is presented. Applied techniques at this stage include CT or MRI. The obtained images are reconstructed and oxygenation is done to keep the cell mass alive [4]

II-5-2-Bioprinting

In this step, the biological ink is placed in the printer to create a three-dimensional structure, then a mixture of living cells and carrier materials are placed in the printer cartridge, and the operation of forming the structure on the scaffold is performed by layering in the direction of the three-dimensional texture [4].

II-5-3-Post-bioprinting

At this stage, the printing stability operation takes place, which is to maintain the structure and mechanical stability of the product. At this stage, physical and chemical stimulation should be done to protect the growth of tissues [4].Figure 3 shows common 3D bioprinting techniques.

II-6-3D bioprinting production technologies

Bioprinting is done with two main methods with scaffolding and without scaffolding. In the scaffold type, the cellular material sits on the weft of the scaffold. This material can be layered on the surface of hydrogel or nanofibers in the form of biological ink. In the non-scaffold type, cellular material is deposited directly in spherical, cylindrical or honeycomb form [29]. There are various technologies for 3D bioprinting, which include: Extrusion based, Inkjet-based, Pressure-assisted, Laser-assisted, Stereolithography, Embedded, Fused deposition modeling (FDM), Vat polymerization, magnetic bioprinting based, Bioplotting.

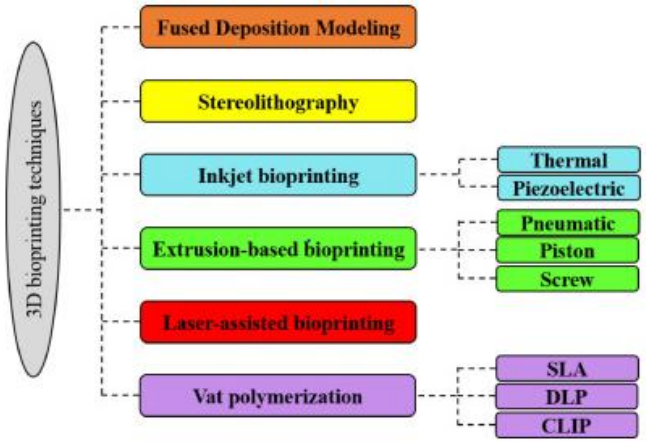

Figure 3 - common 3D bioprinting techniues [5]

II-6-1- Extrusion-based bioprinting

This type of bioprinting is very common due to its high flexibility and cheapness [3]. The biological inks used in this method are made of materials such as alginate, gelatin, collagen, fibrin, chitosan, silk, hyaluronic acid, and agarose. Extrusion-based bioprinting is used to produce a wide range of cellular tissue [17].

II-6-2- Inkjet-based bioprinting

In this method, cell proteins stick on the substrate. With a graphite printer, the two-dimensional texture is placed on a flexible substrate. Inkjet printers were replaced by the presence of biological materials, creating 3D bioprinting. In this method, with the help of heat or sound, cells are placed on the surface. Quantitative changes in biological materials affect print quality [20].

II-6-3- Pressure-assisted bioprinting

In this method, biological materials are formulated as a paste or solution. With the help of pneumatic pressure, biological materials are placed in a continuous string from the nozzle neck on a fixed bed in a layered manner, and finally a three-dimensional structure is created [19].

II-6-4- Laser-assisted bioprinting

In this method, the printer is without a nozzle, which gives a very good result. The use of the automation system has caused the biological material to be printed directly on the substrate, which has high resolution and good accuracy. The obtained product has good stability and survival [18].

II-6-5- Embedded bioprinting

This method is used to make robots, complex organs and sensors. Biological essences are placed in an elastomeric tank. The biological materials used are biocompatible and have acceptable mechanical resistance. The biological ink used is made of hydrogel/microgel, which minimizes the force of gravity during the printing process [21].

II-6-6- Stereolithography

This method is a watt polymerization technique that uses ultraviolet rays for the laser-assisted printing process. The resins in the printer's tank perform the layering operation on the substrate in two directions, bottom-up and top-down. In this method, the surface of the product is smoother. Also, in this method, hydrogel is used together with a laser with a specific wavelength with the aim of protecting living cells. Biological materials are limited in this method because the assurance of biocompatible materials will disrupt the drug delivery activity [22].

II-6-7- Fused deposition modeling

Biological materials in this method are thermoplastic polymers. The deposition of these materials is placed on the substrate as a layer of molten materials and then becomes a solid material. The advantages of this method include high production accuracy, low cost and coarse cellular materials [27].

II-6-8-Magnetic bioprinting based

In this technique, multidimensional bioprinting is provided in the laboratory, where two methods are used to transfer cells onto the substrate. The first method is reverse magnetic penetration printing, and the second method uses nanoparticles such as magnetic iron oxide in the printing process. As a result of the weak magnetic force, cell structures are placed on the substrate and create the texture of the product [24].

II-6-9-Droplet-based bioprinting

In this method, cellular materials are placed in the form of continuous drops from the printer on the substrate. The dependence of the density and size of biological materials is effective in the shape and form of the droplet. The most important advantage of this method is the compatibility of 90% of biological ink with the product [12].

II-6-10-Acoustic bioprinting

In this technique, liquid droplets inside the cell are deposited from a tank in a mild acoustic environment on the substrate and the product is produced. The three-dimensional cellular structure produced is completely controllable. The effect of the sound current causes different orientations in the bioprinting process. In this method, because there is no nozzle, clogging usually does not occur [24].

II-7-The equipment used in the 3D bioprinting process

Bioprinters are a set of equipment that implements the process of placing biological cells on a scaffold. This equipment includes print modules, biological materials placement chamber, height controllers, monitoring system, device skeleton and some side equipment.

II-8-Pathology of bioprinting

3D bioprinting is rapidly developing, but there are obstacles that need to be overcome.

A) Currently, there are limited biological inks that, in addition to printing, can show the function of tissue architecture [28].

B) During the bioprinting process, the shear stress applied to the cell is harmful to the cell and may have an adverse effect on its genetic structure [28].

C) The conducted studies mainly rely on 2D bioprinting, but it is still not completely clear in the practice of 3D cell culture [28].
D) Vascularization after the bioprinting process to deliver nutrients to the cell and merge host vessels with printed vessels is one of the limitations of this method [28].
E) For cell survival, factors must be taken into account, including cell storage in the printer, thermal damage during the printing process, and mechanical forces applied to the cell. Also, cell viability has an inverse relationship with pressure [30].
F) The morphology of printed cells depends on the size and shape of the device's nozzle holes. Usually, large square holes are more suitable for cell safety [30].
G) Toxic macromolecules may be created during the sticking phase of the biological ink on the substrate [30].
H) The stiffness of the scaffold is effective on the quality of bioprinting. The mechanical properties of the cell can be seen on the rigid surface compared to the flexible substrate [30].
I)Despite many clinical trials and the wide application of bioprinting in many cardiovascular, liver, bone and cancer diseases, the number of approved cell therapies is low [31].
J)The large variety of cell therapy needed to fight diseases is different [31].
K)compared to the number of cells produced in the bioprinting process [31].
L)Regarding the premature aging of cells in the production of bioprinting products, the research is still not completely clear [31].
M)The development of cell therapy requires a significant budget, and its commercialization requires further research and comparison with other current methods [31].
N) The substances in bio-ink are still in the research stage and there are safety concerns about them [31].
O) Bioprinting supplies and equipment must be sterile and this may be dangerous for cells. Another important point is to ensure the biocompatibility of the non-living biological materials in the biological substance. Also, the durability of cultured cells is not clear [31].
P) Some living cells are of animal origin and may cause severe immune reactions after transplantation [31].
Q) The standards of bioprinting are limited and until today only the extrusion method of bioprinting and bioinks based on biomaterials have been able to get a standard certificate [31].
R) Bio-based inks should have characteristics such as mechanical integrity, biocompatibility, stability, and optimal printing ability [32].
S) Due to the nature of bioprinting technology and its very important application in the medical industry and the dependence of this technology on software systems, cyber security is very important for it. The market of medical activities is estimated to be several billion dollars per year [23]. The manipulation of information related to bioprinting processes and cyber attacks on their software cause disruptions in the functioning of their processes and irreparable damages [24]. Therefore, their protection in Against the cyber activities of criminals and wrongdoers, it is very necessary [33].
T) Another important point in the bioprinting industry is the damage to moral issues in society. By spending a lot of money, rich people can regenerate and use the cells needed for their damaged tissues, and even with the advancement and development of this technology, the use of new living cells in the body of these people may cause them to live longer. but other groups of the society may not be able to pay the costs and the damage caused by this issue may damage the moral structure of the society [35].

II-9-Safety principles in the process 3D bioprinting

The potential risks of 3D bioprinting include the following:

- The operator of the bioprinter is exposed to aerosols contaminated with pathogens and gets sick through breathing [34].
- The lack of a standardized standard for the permissible threshold limit (OEL) when exposed to possible pollutants during the bioprinting process. It is necessary to create a specialized safety office in research centers related to bioprinting [34].
- Printer devices must be sterilized after work and placed in a closed cabinet under a suitable cover [34].
- It is necessary for the operator to use special personal safety equipment during work, including overalls, gloves, glasses, filter masks, etc [34].
- Conducting medical tests for the personnel who work with the bioprinting device should be done in periods of 3 to 6 months and undergo periodical medical examinations [35].
- Mucous membrane exposure to bloodborne pathogens while prepping human-derived bioink or cleaning the printer [37].

- Inhalation of bloodborne pathogens through aerosolized human-derived or other potentially infectious [37].
- materials during the printing process or deposition of bioink [37].
- Inhalation of respirable particulate or volatile organic compounds (VOCs) while printing scaffold [37].
- Before the start of the work, read the instruction of the bio -printer manufacturer companies and its safety items must be observed [38].
- When the bio -printer is working, the operator does not close it At the top of the printer, install the appropriate ventilation system and, at the start of the work, be directed out of the transmission of contaminated air [38].
- Avoid eating and drinking in bio -printer work rooms. The bio -printer work room should be permanently cleaned [38].
- Cleaning of the enclosure and surfaces should be moist so that dust is not spread in the air After the work, the operator must take care of personal hygiene and his hand and face washing [38].

III-Results

The comparison between 3D bioprinting techniques shows that in the methods based on projection and scanning, the high resolution and speed of product manufacturing depends on the optics of the device. In extrusion and drip systems, the printing speed is slower. The emergence of techniques that examine the entire print volume at once, such as tomography and holography, can solve the problem of printing speed. In the case of vat polymerization printing, they need various processes that are time-consuming. The use of microfluidic systems solves this problem [13].It was found that extrusion-based bioprinting has a simple and cost-effective function and is suitable for most biomaterials [14].Bio-based inks should be resistant, stable, bio-compatible and safe. With the advancement of biotechnology and polymer nano-materials, bio-printing will develop faster [25]. Table 1 summarizes the comparison of the most important 3D bioprinting methods.

Table 1- Comparison of the most important 3D bioprinting techniques [36]

Parameters	Inkjet	Laser	Extrusion	Stereolithography	Microfluidic
Printing process	Serial (drop by drop)	Serial (dot by dot)	Serial (line by line)	Parallel and continuous (projection based)	Organ-on-a-chip
Cost	Low	High	Medium	Low	Low
Cell viability	>85%	>95%	40-80%	>85%	>80%
Print speed	Fast	Medium	Slow	Fast	Fast
Supported viscosities	3.5-12 mpa/s	1-300 mpa/s	$30 – 6 \times 10^7$ mpa/s	No limitation	0-30 pa/s
Resolution	High	High	Medium	High	High
Cell density	Low	Medium	High	Medium	High
Representative materials	Alginate, collagen	Alginate, collagen	Alginate, collagen,Gelma	Gelma	Alginate, collagen,Gelatin

Bioinks should be able to perform bioprinting and tissue architecture to restore proper function of the produced organ, and should not alter gene expression profiles [23]. Despite the research done on 3D bioprinting, most of the materials used in bioink are taken from animal cells, which is necessary to direct biological materials to human cells [30].

IV-Discussion

Bioprinting is a relatively new technology, and the negative points about it have not been fully studied. Some of the limitations in this method are related to technical conditions such as biological materials, implementation methods, equipment and devices, and observing the principles of product preservation. Other ambiguities in the bioprinting process are the existence of some social and moral conflicts about it. Basically, many technologies become successful when people like them and use them. Many researchers believe that for a bright future for bioprinting, the following steps should be done: 1- Drug tests 2- Bioprinting of simple organs 3- Bioprinting of complex organs [6].

An important point in 3D bioprinting is its short study time and long-term success has not yet been established for it. In addition, there are legal problems in the use of biological materials in biological ink for printing, which must be solved. For example, animal cells that are used may lead to protests by environmentalists. It is also possible that the results of tissue production and placement in the patient's body may lead to problems in the user in the long term, in this case the level of responsibility and accountability of the doctor or the medical

team implementing this technology is not clear. Due to its high importance, the equipment used in the medical industry must be patented and pass the (ethical test) course [35].

V-conclusion

3D bioprinting technology has a great future ahead. The development of this technique in medical science is very surprising, but it seems that it still has a long way to go. Researchers in the scientific centers of the world are trying to improve the strengths of this method and reduce its weaknesses. The development perspective for this technology is in clarifying the factors that are currently defined as limitations. These factors include ethical issues, social problems, attention to safety factors for system managers, quality of biological essences, biological materials, quality of bioprint implementation methods, etc. Considering that, the most important principle in medical science techniques and treatment methods is patient satisfaction. Efforts should be made to assure patients that 3D bioprinting technology helps to regenerate body tissues and organs.

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