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Review Article

3D BIOPRINTING - CHANGING THE SHAPE OF MEDICAL PRACTICE

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ABSTRACT

3D Bioprinting is regeneration of tissues and organs suitable for transplantation. The innovative idea needs knowledge from various sectors of sciences like Biotechnology, Bio material sciences, physics, engineering, medicine and pharmacy. This paper discusses methods of 3D bioprinting, 3D bioprinters, approaches, Materials and scaffolds, Printability, degradation kinetics, cell sources, challenges. Researchers continue to improve 3D printing technology as commercial and industrial inters growing in this revolutionary area where regeneration of human tissues possible which are unable to self regenerate like bone, cartilage and nervous system.

INTRODUCTION

Three-dimensional (3D) printing is driving major innovations in many years, such as engineering, manufacturing, art, education and medicine. Recent advances have enabled 3D printing of biocompatible materials, cells and supporting components into complex 3D functional living tissue. 3D Bioprinting is being applied to regenerative medicine to address the need for tissues and organs suitable for transplantation. Compared with non-biological printing, 3D Bioprinting involves additional complexities, such as the choice of materials, cell types, growth and differentiation factors, and technical challenges related to the sensitivities of living cells and the construction of tissues. Addressing these complexities requires the integration of technologies from the fields of engineering, biomaterials science, cell biology, physics and medicine.

3D Bioprinting has already been used for the generation and transplantation of several tissues, including multilayered skin, bone, vascular grafts, tracheal splints, heart tissue and cartilaginous structures. Other applications include developing high-throughput 3D-Bioprinted tissue models for research discovery and toxicology. Printing has a revolutionary effect on society, affecting education, politics, religion and language across the globe. This technology, indeed, will be able to build *ex-novo* organs using biocompatible materials and human cells; replace the allograph transplants, eliminating waiting lists that often make the difference between life and death; and provide more predictive, less expensive experimental models, replacing animal tests. Production of 3D complex structures has been applied by the industry to produce customized objects, such as pieces of bicycles and jewels.

Definition

3D bioprinting is the process of creating cell patterns in a confined space using 3D printing technologies, where cell function and viability are preserved within the printed construct (Chua C.K. *et al.*, 2015). Generally, 3D bioprinting utilizes the layer-by-layer method to create tissue-like structures that are later used in medical and engineering fields. Bioprinting covers a broad range of materials. Currently, bioprinting can be used to print tissues and organs to help research drugs and pills (www.explainingthefuture.com 2016*)*. In addition, 3D bioprinting has begun to printing of scaffolds. These scaffolds can be used to regenerate joints and ligaments.

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History

The subsequent development of the industrial-scale-printing press in 15th century, printing has been defined as the great contribution to civilization; today, the new frontier of the printing technology is 3D-printing. The inventor of 3D printer was Charles Hull, who first came up with the idea in 1983; Hull coined the term "steriolithography" in his U.S patent 4,575,330-entitled "Apparatus for production of three-dimensional Objects by steriolithography"-issued in 1986. He later founded the company 3D systems, which develops the first 3Dprinter, called a "steriolithography apparatus" in 1988. By this new technology an object can be imagined, designed and then produced by using the same

Machine, the 3D printer. The first organ to be created with a bioprinter, using stem cells as 'ink', will be a thyroid gland. 3D printing has become a useful and potentially transformative tool in a number of different fields, including medicine.

3D Bioprinting

3D bioprinting generally follows three steps, pre-bioprinting, bioprinting and post Bioprinting. Bioprinter is displayed in figure 1 (www.explainingthefuture.com)

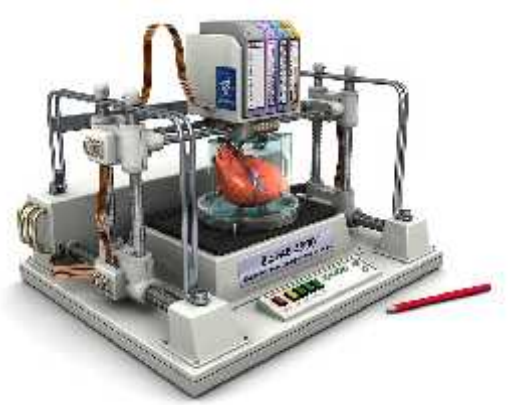


Fig 1 3D Bioprinter

Pre-bioprining

Pre-bioprinting is the process of creating a model that the printer will later create and choosing the materials that will be used. One of the first step is to obtain a biopsy of the organ. The common technologies used for bioprinting are computed tomography (CT) and magnetic resonance imaging (MRI). In order to print with a layer-by-layer approach, tomographic reconstruction is done on the images. Now 2D images are then sent to the printer to be made. Once the image is created, certain cells are isolated and multiplied (Shafiee *et al.,* 2016). These cells are then mixed with a special liquefied material that provides oxygen and other nutrients to keep them alive.

Bioprinting

In the second step, the liquid mixture of cells and nutrients are placed in a printer catridges and structured using the patient's medical scans (Cooper-White M *et al.,* 2016). When a bioprinted pre-tissue is transferred to an incubator, this cell-based pre-tissue matures into a tissue. Artificial organs such as livers and kidneys made by 3D bioprinting have been shown to lack crucial elements that affect the body such as working blood vessels, tubules for collecting urine, and the growth of billions of cells required for these organs. Without these components the body has no way to get the essential nutrients and oxygen deep within their interiors (Harmon K. *et al*., 2013).

Every tissue in the body s compartmentalized of different cell types, many technologies for printing these cells vary in their ability to ensure stability and viability of the cells during the manufacturing process.

Post-bioprinting

The post-bioprinting process is necessary to create a stable structure from the biological material. If this process is not Well-maintained, the mechanical integrity and function of the 3D printed object is at risk (Shafiee *et al*., 2016). In order to maintain the object, both mechanical and chemical stimulations are needed. These stimulations send signal to the cells to control the remodeling the growth of the tissues. In addition, in recent development, bioreactor technologies allowed the rapid maturation of tissues, vascularisation of tissues and the ability to survive transplants (Ozbolate *et al*., 2015). Bioreactors working in either providing convective nutrient transport, creating microgravity environment, changing the pressure causing solution to flow through the cells or add compression for dynamic loading. Each type of bioreactor is ideal for different types of tissue, for example compression biorectors are ideal for cartilage tissue.

Bioprinters

Bioprinting began from 2D ink-based printers modified to become cell-printers. The ink in the catridges was replaced with a biological material and the paper was replaced with an electronically controlled elevator stage to provide controll of the Z axis (Third dimension in addition to the X & Y axis). Medical imaging technology is an indispensable tool used by tissue engineers to provide informatioin on a 3D structure and function at the cellular, tissue, organ and organism levels. In tissue engineering, 3D-bioprinting can be essentially of two types: with or without incorporating living cells on to the solid surface. The most important factors in 3D bioprinting namely surface resolution, cell viability and the biological materials used for printing. Three concepts of 3D bioprinters are nowadays available:

- Inkjet bioprinters
- Microextrusion bioprinters
- Laser- assisted bioprinters (LAB)

The parameters for comparision of various Bioprinters are given in Table 1.

Inkjet Bioprinters

Inkjet printers are the most commonly used type of printer for both non-biological and biological applications. The first inkjet printers used for bioprinting applications were modified versions of commercially available 2D ink-based printers (Xu T *et al.,* 2008). Inkjet bioprinter has been displayed in fig 2 (www.labromancy.com)

Inkjet based bioprinters are custom-designed to handle and print biological materials at increasing resolution, precision and speed. Inkjet printers use thermal forces to eject drops of liquid onto a substrate, which can support or form part of final construct.

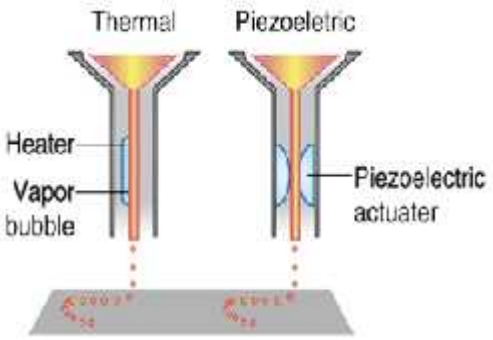


Fig 2 Inkjet bioprinter

Thermal inkjet printers function by electrically heating the print head to produce pulses of pressure that force droplets from the nozzle. Several studies have demonstrated that this localized heating, which can range from 200 °C to 300 °C, does not have a substantial impact either on the stability of biological molecules, such as DNA (Okamoto T *et al*., 2000). The advantages of thermal inkjet printers include high print speed, low cost and wide availability.

However, the risk of exposing cells and materials to thermal and mechanical stress, low droplet directionality, non uniform droplet size, frequent clogging of the nozzle and unreliable cell encapsulation pose considerable disadvantages for the use of these printers in 3D bioprinting. Many inkjet printers contain a piezoelectric crystal that creates an acoustic wave inside the print head to break the liquid into droplets at regular intervals. Applying a voltage to a piezoelectric material induces a rapid change in shape, which in turn generates the pressure needed to eject droplets from the nozzle (Tekin E *et al.*, 2008). Ultrasound parameters, such as pulse, duration and amplitude, can be adjusted to control the size of droplets and the rate of ejection. Commercially available inkjet bioprinters are also relatively cost-effective owing to their simple components and readily available design and control software. Droplet size and deposition rate can be controlled electronically, and can range from <1 pl to >300 pl in volume (Sekitani T *et* al., 2008). with rates of 1–10,000 droplets per second.

Advantages

- Inkjet printers include the capability to generate and control a uniform droplet size and ejection directionality as well as to avoid exposure of cells to heat and pressure stressors (Saunders R *et al*., 2004).
- Additionally, the sheer stress imposed on cells at the nozzle tip wall can be avoided by using an open-pool nozzle-less ejection system.
- This reduces the potential loss of cell viability and function, and avoids the problem of nozzle clogging.
- Inkjet printing is the potential to introduce concentration gradients of cells, materials or growth factors throughout the 3D structure by altering drop densities or sizes (Philippi *et al*., 2008).
- Low cost, high resolution, high speed and compatibility with many biological materials.

Disadvantage

Common drawback of inkjet bioprinting is that the biological material has to be in a liquid form to enable droplet formation; as a result, the printed liquid must then form a solid 3D structure with structural organization and functionality.

Uses

Inkjet bioprinting approach includes the regeneration of functional skin (Skardal A *et al*., 2012) and cartilage. The high printing speed of the approach enables direct deposition of cells and materials directly into skin or cartilage lesions.

Microextrusion Bioprinters

The most common and affordable nonbiological 3D printers use micro extrusion. Micro Extrusion bioprinters usually consist of a temperature-controlled material-handling and dispensing system and stage, with one or both capable of movement along the X, Y and Z axes, a fiberoptic light source to illuminate the deposition area. A few systems use multiple print heads to facilitate the serial dispensing of several materials without retooling (Mironov V *et al*., 2009). Micro extrusion bioprinter has been displayed in fig 3 (http://bio-impression.weebly.com/le-tpe.html)

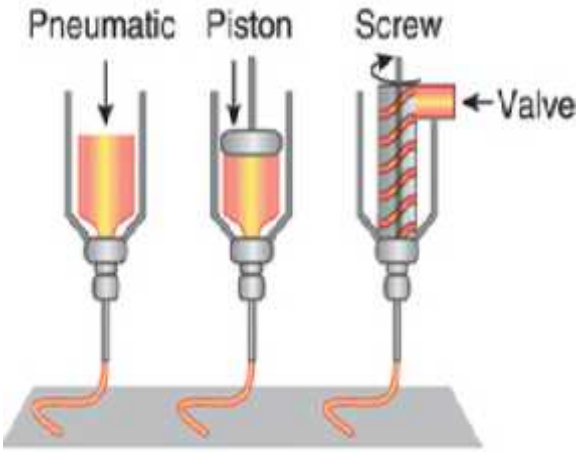


Fig 3 Micro extrusion bioprinter

Nearly 30,000 3D printers are sold worldwide every year, and academic institutions are increasingly purchasing and applying micro extrusion technology in tissue and organ engineering research. Microextrusion printers function by robotically controlled extrusion of a material, which is deposited onto a substrate by a micro extrusion head. Microextrusion yields continuous beads of material rather than liquid droplets. Small beads of material are deposited in two dimensions, as directed by the CAD-CAM software, the stage or micro extrusion head is moved along the z axis, and the deposited layer serves as a foundation for the next layer. Microextrusion methods have a very wide range of fluid properties that are compatible with the process, with a broad array of biocompatible materials described in the literature. Materials with viscosities ranging from 30 mPa/s to >6 × 107 mPa/s (Jones *et al*., 2012). Have been shown to be compatible with microextrusion bioprinters, with higher-viscosity materials often providing structural support for the printed construct and lower-viscosity materials providing a suitable environment for maintaining cell viability and function. Several biocompatible materials can flow at room temperature, which allows their extrusion together with other biological components, but crosslink into a stable material at body temperature (Schuurman W *et al*., 2013).

Alternatively, materials that flow at physiologically suitable temperatures (35–40 °C), but crosslink at room temperature may also be useful for bioprinting applications (Smith *et al*., 2007). Materials with shear-thinning properties are commonly used for microextrusion applications. The high shear rates that are present at the nozzle during biofabrication allow these materials to flow through the nozzle, and upon deposition, the shear rate decreases, causing a sharp increase in viscosity. High resolution of microextrusion systems permits the bioprinter to accurately fabricate complex structures designed using CAD software and facilitate the patterning of multiple cell types.

Advantages

- In 3D bioprinted organs the most common technology used for scaffold-less tissue spheroid bioprinting is mechanical microextrusion.
- Microextrusion bioprinting technology is the ability to deposit very high cell densities. Achieving physiological cell densities in tissue-engineered organs is a goal for the bioprinting field.

Disadvantages

- Cell viability after microextrusion bioprinting is lower than that with inkjet-based bioprinting; cell survival rates are in the range of 40-86%, the rate decreasing with increasing extrusion pressure and increasing nozzle gauge (Smith *et al*., 2004).
- Although cell viability can be maintained using low pressures and large nozzle sizes, increasing print resolution and speed is a challenge for many users of microextrusion bioprinting technology.
- It is more expensive.

Uses

Microextrusion bioprinters have been used to fabricate multiple tissue types including aortic valves (Duan *et al*., 2013). Branches of vascular trees and *in vitro* pharmacokinetic as well as tumor models.

Laser-Assisted Bioprinters

Laser-assisted bioprinting (LAB) is based on the principles of laser-induced forward transfer initially developed to transfer metals, laser-induced forward transfer technology has been successfully applied to biological material, such as peptides, DNA and cells (Chrisey *et al*., 2000). Laser assisted bioprinter has been displayed in fig 4 (www.labromancy.com).

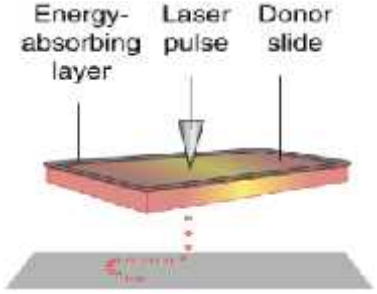


Fig 4 Laser-assisted bioprinter

The resolution of LAB is influenced by many factors, including the laser fluence (energy delivered per unit area), the surface tension, the wettability of the substrate, the air gap between the ribbon and the substrate, and the thickness and viscosity of the biological layer (Guillemot *et al*., 2010). Although less common than inkjet or microextrusion bioprinting, LAB is increasingly being used for tissue- and organ-engineering applications.

LAB is based on a pulsed laser beam that acts on a laser energy-absorbing layer (Gold or Titanium) and a layer of biological material (cells and/or hydrogel) prepared in a liquid solution. LAB is compatible with a range of viscosities and can print cells with negligible effect on cell viability and function. LAB can deposit cells at a high density with microscale resolution of a single cell per drop. The application of LAB to fabricate a cellularized skin construct demonstrated the potential to print clinically relevant cell densities in a layered tissue construct, but it is unclear whether this system can be scaled up for larger tissue sizes (S.V Murphy *et al*., 2014).

Advantages

- LAB is nozzle-free the problem of clogging with cells or materials that plague other bioprinting technologies is avoided.
- LAB is compatible with a range of viscosities (1–300 mPa/s) and can print mammalian cells with negligible effect on cell viability and function.
- High resolution of LAB requires rapid gelation kinetics to achieve high shape fidelity, which results in a relatively low overall flow rate (Guillotin *et al*., 2011).

Disadvantages

Preparation of each individual ribbon, which is often required for each printed cell or hydrogel type, is time-consuming and may become onerous if multiple cell types and/or materials have to be co-deposited.

Uses

- LAB is used to fabricate a cellularized skin construct demonstrated the potential to print clinically relevant cell densities in a layered tissue construct but it is unclear whether this system can be scaled up for large tissue sizes (Michael *et al*., 2013).
- *In vivo* LAB has been used to deposit nano-hydroxyapatite in a mouse calvaria 3D defect model.

Approaches

Researchers in the field have developed approaches to produce living organs that are constructed with the appropriate biological and mechanical properties. 3D bioprinting is based on three main approaches.

- Biomimicry
- Autonomous self-assembly
- Mini-tissue building blocks

Table1 Comparison of Bioprinters types

	Bioprinter type			
Parameters	**Inkjet**	**Microextrusion**	**Laser-assisted**	**References**
Material viscosities	3.5-12mPa/s	30 mPa/s to $> 6\times10^7$ mPa/s	1-300 mPa/s	(Guillemot *et al*., 2010) (Kim *et al*., 2010) (Chang *et al*., 2011) (Guillotin *et al*.,2011)
Gelation method	Chemical, Photo-crosslinks	Chemical, Photo cross linking, temperature Sheer thinning.	Chemical, photo-crosslink	(Murphy *et al*., 2013) (Smith *et al*., 2007) (Koch *et al*., 2010) (Michael *et al*., 2013)
Preparation time	Low	Low to medium	Medium to high	(Peltola *et al*., 2008) (Norotte *et al*., 2009)
Print speed	Fast (110,000 droplets per second)	Slow (10-50 µm/s)	Medium to fast (200-1600 mm)	(Guillotin *et al*., 2010) (Demirci *et al*., 2007) (Smith *et al*., 2004) (Nair *et al*., 2009)
Resolution or droplet size	< 1 pl to >300pl droplets per second)	5µm to millimeters wide	Microscale resolution	(Campbell *et al*., 2005) (Philippi *et al*., 2008) (Xu *et al*., 2005)
Cell viability	> 85%	40-80%	> 95%	(Xu *et al*., 2006) (Chang *et al*., 2008) (Hopp *et al*., 2005)
Cell densities	Low,$<10^6$ cells/ml	High, cell spheroids	Medium, 10^8cells/ml	(Mironov *et al*., 2011) (Marga *et al*., 2012)
Printer cost	Low	Medium	High	(Jones *et al*., 2012)

Biomimicry

Biologically inspired engineering has been applied to many technological problems, including materials research, cell culture methods and nanotechnology. 3D bioprinting involves the manufacture of identical reproductions of the cellular and extracellular components of a tissue or organs (Ingber *et al*., 2006). Mimicking the branching patterns of the vascular tree or manufacturing physiologically accurate biomaterial types and gradients. For this approach to Succeed, the replication of biological tissues on the microscale is necessary. Understanding of the microenvironment, including the specific arrangement of functional and supporting cell types, gradients of soluble or insoluble factors, composition of the ECM as well as the nature of the biological forces in the microenvironment is needed. The development of this knowledge base will be important to the success of this approach and can be drawn from basic research in fields of engineering, imaging, biomaterials, cell biology, biophysics and medicine.

Autonomous self-assembly

Another approach to replicating biological tissues is to use embryonic organ development as a guide. The early cellular components of a developing tissue produce their own ECM components, appropriate cell signaling and autonomous organization and patterning to yield the desired biological micro-architecture and function (Marga *et al*., 2007). A 'scaffold-free' version of this approach uses self-assembling cellular spheroids that undergo fusion and cellular organization to mimic developing tissues. Autonomous self-assembly relies on the cell as the primary driver of histogenesis directing the composition, localization, functional and structural properties of the tissue. It requires an intimate knowledge of the developmental mechanisms of embryonic tissue genesis and organogenesis as well as the ability to manipulate the environment to drive embryonic mechanisms in bioprinted tissues.

Mini-tissue building blocks

The concept of mini-tissues is relevant to both of the above strategies for 3D bioprinting. Organs and tissues comprise smaller, functional building blocks (Mironov *et al*., 2009) or mini-tissues. These can be defined as the smallest structural and functional component of a tissue, such as a kidney nephron. Mini-tissues can be fabricated and assembled into the larger construct by rational design, self-assembly or a combination of both. There are two strategies: first, self-assembling cell spheres are assembled into a functional macro-tissue using biologically inspired design and organization, second accurate high- resolution reproductions of a tissue unit are designed and then allowed to self-assemble into a functional macrotissue. The main steps in the bioprinting process are imaging and design, choice of materials and cells, and printing of the tissue construct is displayed in Fig 5 (www.nature.com) the printed construct is then transplanted, in some cases after a period of *in vitro* maturation, or is reserved for *in vitro* analysis.

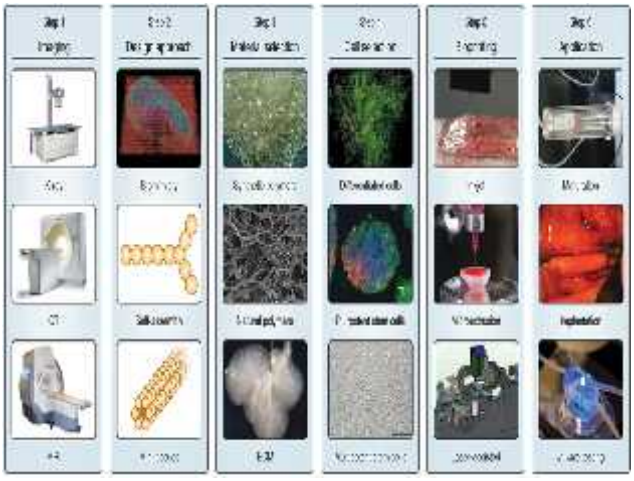
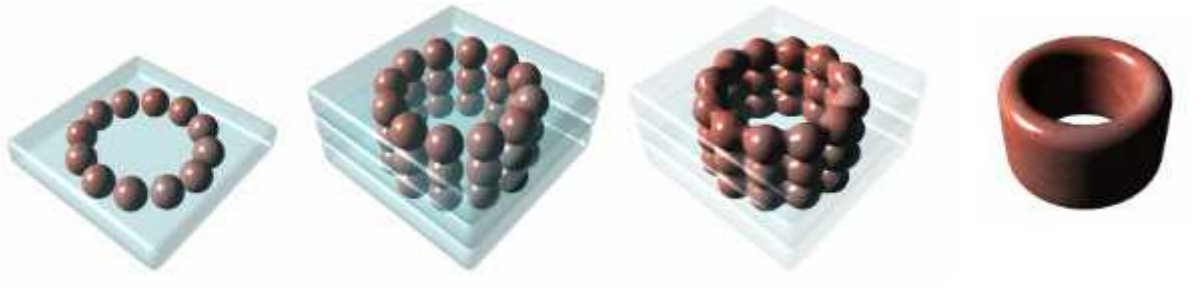


Fig 5 Steps involved in 3D Bioprinting.

MATERIALS AND SCAFFOLDS

3D printing technologies were designed for nonbiological applications, such as the deposition of metals, ceramics and thermoplastic polymers, and generally involved the use of organic solvents, high temperatures or crosslinking agents that are not compatible with living cells and biological materials. The process involved in printing a tissue has been displayed in fig 6 (www.techli.com).

For example, inkjet printing requires materials with a rapid crosslinking time to facilitate the layering of a complex 3D structure. Microextrusion, however, can incorporate highly viscous materials to maintain a 3D shape after deposition, with final crosslinking occurring after fabrication. Thermal inkjet printing and LAB both involve the localized heating of the material to deposit cells.



[A] Bioink spheroids Printed into layer of Biopaper gel

[B] Additional layers printed to build object

[C] Bioink spheroids fuse together and biopaper dissolves

[D] Final living tissue

Fig 6 How to print a tissue

But 3D bioprinting field has been to find materials that are not only compatible with biological materials and the printing process but can also provide the desired mechanical and functional properties for tissue constructs. Materials currently used in the field of regenerative medicine for repair and regeneration are predominantly based on either naturally derived polymers (alginate, gelatin *etc*) or synthetic molecules (polyethylene glycol). The advantages of natural polymers for 3D bioprinting and other tissue engineering applications is their similarity to human ECM, inherent bioactivity. The advantage of synthetic polymers is that they can be tailored with specific physical properties to suit particular applications.

Challenges in the use of synthetic polymers include poor biocompatibility, toxic degradation products and loss of mechanical properties during degradation. Even so, synthetic hydrogels, which are both hydrophilic and absorbent, are attractive for 3D bioprinting regenerative-medicine applications owing to the ease of controlling their physical properties during synthesis. Materials must have suitable crosslinking mechanisms to facilitate bioprinter deposition, must be biocompatible for transplantation over the long-term, and must have suitable swelling characteristics and short-term stability.

Printability

Some types of bioprinting technology, such as inkjet, have limitations on material viscosity, where as others, such as microextrusion, may require the material to have specific crosslinking mechanisms or shear-thinning properties. Processing parameters, such as nozzle gauge, determine the shear stress to which cells are exposed (Nair K *et al*., 2009). As well as the time required for the material to be deposited to forms 3D structure (Murphy *et al*., 2013). Bioprinted organs have been displayed in fig 7 (www.telegraph.co.uk)

Fig 7 Bioprinted organs

Materials with either low thermal conductivity or the ability to cushion the cells during delivery may increase cell viability and function after printing (Hopp *et al*., 2012). Although postprinting cell viability can range markedly based on printer specifications, material properties, resolution and cell types, inkjet bioprinting studies usually quote cell viabilities in excess of 85%, microextrusion printing studies report viability ranges of 40–80% and LAB studies report viability in excess of 90% (Chang *et al*., 2008).

Degradation Kiinetics and Byproducts

As a material scaffold degrades, the embedded cells secrete proteases and subsequently produce ECM proteins that define the new tissue. The degradation kinetics of the materials must be understood and controlled. The first is the ability to control degradation rates, ideally matching the rate of degradation with the ability of cells to replace the materials with their own ECM proteins. This is challenging because materials with suitable functional and mechanical characteristics for a given tissue may not match the ability of the cellular components to replace

the material upon degradation. Degradation byproducts are also important because they often define the biocompatibility of any degradable material. The degradation products should be nontoxic, readily metabolized and rapidly cleared from the body. Toxic products can include small proteins and molecules but also non physiological pH, temperature or other factors that can be detrimental to cell viability and function. For example, some large-molecular-weight polymers that are initially inert can be broken down into oligomers or monomers that can be recognized by cells and cause inflammation and other detrimental effects.

Structural and Mechanical Properties

A material is essential for the maintenance of a 3D structure, in resisting or producing specific forces or as an anchoring point for mechanical leverage, then maintenance of these properties is essential for continued function of the construct. Materials must be carefully selected based on the required mechanical properties of the construct, and different Structural requirements will be needed for diverse tissue types ranging from skin and liver to bone. In this care must be taken to design a material with specific structural and degradation properties while avoiding potential foreign body responses or toxic degradation byproducts in the construct.

Cell Sources

The choice of cells for tissue or organ printing is crucial for correct functioning of the fabricated construct. Tissues and organs comprise multiple cell types with Current options for printing cells involve either the deposition of multiple primary cell types into patterns that faithfully represent the native tissue or printing stem cells that can proliferate and
Differentiate into required cell types.

Cells chosen for printing should closely mimic the physiological state of cells *in vivo* and are expected to maintain their *in vivo* functions under optimized conditions specific and essential biological functions that must be recapitulated in the transplanted tissue. Precise control of cell proliferation *in vitro* and *in vivo* is important for bioprinting.

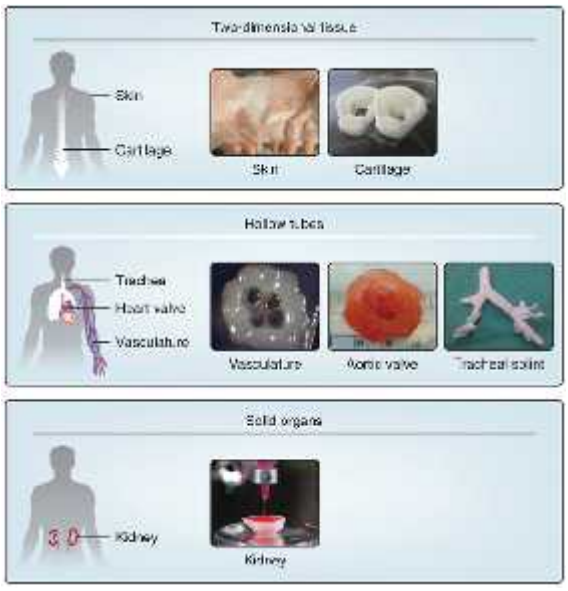


Fig 8 Examples of human-scale bioprinted tissues

Too little proliferation may result in the loss of viability of the transplanted construct; too much proliferation may result in Apoptosis.

Although the field is at an early stage, it has already succeeded in creating several tissues at human scale that are approaching the functionality required for transplantation. Technological challenges include the need for increased resolution, speed and compatibility with biologically relevant materials. Examples of human-scale bioprinted tissues have been displayed in fig 8 (www.nature.com)

Currently, the materials being used for printing are selected either because of their compatibility with cell growth and function or because of their crosslinking or extrusion characteristics. For this reason, many published studies use a limited range of materials, including collagen, hyaluronic acid, alginate, modified copolymers and photo cured acrylates

Challenges

- The final step of this process is making printed organ cells behave like native cells.
- There's the issue of getting blood to all of the cells in a printed organ.
- Scientists have printed larger blood vessels, as the technology improves, the next will be fully functional replacements of organs.
- Currently bioprinting doesn't offer sufficient resolutions to create tiny, single cell thickness capillaries.

CONCLUSION

3D printing has become a useful and potentially transformative tool in a number of different fields, including medicine. This technology is revolutionary mainly in the field of regeneration of those human tissues unable to self regenerate, such as bone, cartilage, nervous system. Today it is possible to build artificial bone from calcium phosphate, which is a component of both human bones and teeth; the printer's product should be able to integrate directly into a patient's body, where it will fuse with the existing bone. Due to clinical, commercial, industrial interest of this area of research several industries and start-ups based on bioprinting are rising worldwide. Researchers continue to improve existing medical applications that use 3D printing technology and to explore new ones. Bioprinting "fever" is gradually affecting the globe and certainly we will witness a strong scientific and industrial development in the next few years however this technology leap-frogging to breakthrough innovation.

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