REVIEW ARTICLE

3D Bioprinting: The Roller Coaster Ride to Commercialization

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Abstract: Three-dimensional (3D) bioprinting as a technology is being researched and applied since 2003. It is actually several technologies (inkjet, extrusion, laser, magnetic bioprinting, etc.) under an umbrella term "3D bioprinting." The versatility of this technology allows widespread applications in several; however, after almost 20 years of research, there is still a limited number of cases of commercialized applications. This article discusses the potential for 3D bioprinting in regenerative medicine, drug discovery, and food industry, as well as the existing cases of companies that create commercialized products and services in the aforementioned areas and even in fashion, including their go-to-market route and financing received. We also address the main barriers to creating practical applications of 3D bioprinting within each sphere the technology that is being studied for.

Keywords: 3D bioprinting, Commercialization, Regenerative medicine, Drug discovery, Food

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1 From 3D printing to 3D bioprinting

Three-dimensional (3D) printing is the technology of fast prototyping and additive manufacturing used to create the complex architecture of high accuracy through stage process of product construction according to the specified digital mode[1]. Hull has received the patent for photopolymerization-based stereolithography (SLA) technology in 1986. This work was the first in the area of 3D printing techniques. Nowadays, several technologies are united by the term "3D printing:" Fused deposition modeling; SLA, digital light processing; ColorJet printing; multiple jet modeling; selective laser sintering; selective laser melting; and direct metal laser sintering. Boland has suggested the bioprinting method based on traditional two-dimensional (2D) inkjet technology in 2003[2]. In the same year, Mironov *et al*. have proposed the method of extrusion 3D bioprinting with the use of tissue spheroids as "building blocks"[3].

The implementation of an automated additive process eases the fabrication of 3D products on the basis of high-precision control of their architecture, external shape, inner geometry of pores, and the correlation between high reproducibility and repeatability[4-6]. Due to these features, 3D bioprinting technology appears

to be an extremely promising approach in the fabrication of cell material-containing biomimetic scaffolds (substrates) that serves as the basis for the creation of living and functional 3D constructs for the benefit of regenerative medicine.

Thus, 3D bioprinting is the technology of layer-by-layer fabrication of 3D tissue and organ constructs according to the assumed digital model using living cells as printing material.

For now, however, the lack of cell material is one of the limiting factors for bioprinting technology development. With advances in cell technology, this situation is going to change, but today, the bioprinting technology depends on development; it has niche implementation. It is like having Google or Baidu web search engines without the development of the internet. Therefore, the technology comes into use in new areas, such as food arrangement, fashion industry, and space science. We have also noticed the development of bioprinting technology itself; the new technologies for cell materials positioning in 3D space are emerging in addition to the "golden triad" (inkjet, extrusion, and laser bioprinting). Some of them will be discussed in more detail later on.

2 *In situ* bioprinting

One of the new approaches developed in 3D bioprinting is *in situ* bioprinting that is the replacement of tissues and organ defects using bioprinters directly during surgery. This method is considered advantageous in view of the possible "physiological" solution to the vascularization problem due to progenitor cell migration in the printed tissue-engineered construct and vascularization process that starts in surrounding recipient tissues. The idea of *in situ* bioprinting was first proposed by Weiss *et al.* in 2007[7]. However, there were only few experiments on *in situ* bioprinting since then due to the difficulties with forming of the construct directly in the wound (on non-horizontal surfaces). As a consequence, it is necessary to have interactive software for analyzing the shape and depth of the tissue defect with the immediate consideration of this information for bioprinting. Moreover, there are special requirements for extrusion biomaterials that particularly have to be polymerized instantly in the wound without any influence from additional factors such as ultraviolet radiation or chemical cross-linking agents. Nevertheless, *in situ* bioprinting has several significant advantages over other bioprinting techniques. Thus, applying direct bioprinting in tissue defect excludes the need to prepare the substrate that minimizes the risks of *in vitro* contamination. Furthermore, *in situ* bioprinting can exclude the need of stem and progenitor cell differentiation *in vitro* for critical or large defects, and reducing fabrication time and costs. The stem cells are immediately placed in the natural, growth-factor-rich environment that ensures organotypic differentiation when printed by stem or progenitor cells *in situ*. More importantly, *in situ* bioprinting can achieve the needed hierarchy of different cells' placement and orientation in the defect, while in technologies of prepared scaffolds transplantation the substrate can change its shape due to swelling compression or any other deformations.

There are few experiments on *in situ* bioprinting but they confirm its advantages as stated above. Skardal *et al.* have demonstrated the possibility of inkjet *in situ* bioprinting using fibroblasts and keratinocytes for burns restoration[8]. Kerikel *et al.* have published the results of successful experiments on bone defect restoration using laser *in situ* bioprinting[9]. This technology seems particularly advantageous in terms of using it in hospitals to restore lost functions.

At present, we can find the presence of bioprinters in hospitals. For example, a bioprinting center has opened in Brisbane, Australia (Institute of Health and Biomedical Innovation 2017). These developments lead to the appearance of a business model which allows the printing of constructs in specialized labs, and the direct application of *in situ* bioprinting at the patient's hospital bed.

Poietis, a French-based company, has entered into a clinical research collaboration with the Assistance Publique – Hôpitaux de Marseille (AP-HM) to pursue a clinical trial for bioprinted skin tissue. Through the partnership, Poietis and AP-HM aim to carry out a Phase I clinical trial for an Innovative Advanced Therapeutic Medicinal Product for skin healing issues. The timeline for this phase is 2 years[10].

Such technical solutions could be used for chronic wounds such as diabetic, venous, and pressure ulcers and burn wounds that affect over 7 million patients in the United States with an annual treatment expenditure of US$ 25 billion[11]. Globally, this statistic increases to 11 million injuries per year. Chronic, large or non-healing wounds are especially costly because they often require multiple treatments; for example, a single diabetic foot ulcer can cost approximately US$ 50,000 to treat. Full-thickness skin injuries are a major source of mortality and morbidity for civilians, with an estimated 500,000 civilians who were treated for these injuries in the United States each year[12-15].

Bioprinting technology could also be used for military purposes. For example, for military personnel, burn injuries account for 10 – 30% of combat casualties in conventional warfare. US company nScrypt partnered with the U.S. Military in the Uniformed Services University 4D Bio3 Program to develop a special bioprinter for point-of-care bioprinting in unexposed conditions[16]. It is possible that this program will bioprint meniscus from live cells and hydrogel-based scaffolds.

Economic impact on potential markets of *in situ* bioprinting could be considered as following:

The global wound care market is estimated to reach US$ 25 billion by 2024 (Wound Care Market by Product, Research and Markets, 2019).

We are targeting the following segments:

- Skin grafts for patients with post-operative defects after removal of skin tumors or diabetic ulcers
- Plastic surgery applications
- Orthopedic surgeries
- Military and civilian field surgery for burn victims.

We estimate that the skin bioprinting market will reach around US$ 1 billion by 2024 (3D Bioprinting Market Report, Roots Analysis, 2014).

A further US$ 130 million is the estimated size of the cartilage bioprinting market by 2024.

Since *in situ,* bioprinting has a lot of advantages as automatization of the application process that allows to create multi-layered constructs (of complex geometrical shapes) made of configurable hydrogel solution with autologous patient cells. The bioprinting process is carried out with high precision and can be conducted from various angles. Moreover, the computer vision system brings adaptability to the system, allowing us to use it on various wounds and defects without additional reconfiguration of the software, while IR proximity sensors ensure trajectory adjustment for breathing and other physiological processes. *In situ* bioprinting will allow to print structures with small (300 μm) pores, which help maintain optimal temperature and humidity inside the healing wound, thereby speeding up the healing process and lowering the incidence of complications.

However, it also has some disadvantages (not only technological ones) such as bioprinters, cell material and biomaterials of substrates (scaffolds) registration for their use in clinical practice, clinical and pre-clinical trials, and intellectual property registration. Nevertheless, we see positive changes in approaching the idea of printing tissues and organs, not just scientific publications.

3 Space bioprinting

As we divined in our previous review, commercial companies started to provide B2S (business-to-science) services of conducting experiments for research institutions on the international space station (ISS)[17]. Commercial companies, such as 3D Bioprinting Solutions, Techshot, and nScrypt, have set series of experiments in space. Interestingly, for these experiments, bioprinting technology was used not only for regenerative medicine purposes but also for other research purposes, such as:

- Bacteria behavior in space study
  Conventionally, genetic antibiotic resistance research is carried out on 2D cultures on earth. However, such experiment design ignores the fact that bacteria in living organisms tend to form 3D biofilms, which have the unique phenotypic antibiotic resistance, due to the fact that antibiotic molecules do not diffuse into the full volume of a biofilm.
- Protein structure modeling in space
  There is a lot of interest in structure prediction as a screening process for proteins that are

not tenable for experimental determination. Structure prediction depends on protein crystallography, which allows us to create a mathematical model of the protein in question.

- Biomaterial research
  The calcium phosphate particles can be used for bone defect regeneration. Microgravity allows obtaining biocompatible octocalcium phosphate phase rapidly in the final product. Thus, magnetic levitation of calcium phosphate particles is a promising approach for rapid 3D fabrication in the field of bone tissue engineering.

More companies plan to participate in space experiments. Cellink, a Swedish 3D bioprinter manufacturer, has announced a strategic collaboration with Made In Space, a microgravity manufacturing specialist, to identify bioprinting opportunities for the ISS (https://cellink.com/cellink-partners-with-made-in-space-for-microgravity-bioprinting/). US companies such as Allevi and Made In Space are also developing 3D bioprinter for space[18].

In space, companies try to use two main approaches: (i) Using classical extrusion bioprinting technology (main challenge to overcome microgravity, and especially using hydrogels for scaffold material printing) and magnetic/acoustic approach, and (ii) using novel technology that applies microgravity as an additional trigger for biofabrication (main challenge to design 3D model of the printed construct).

These technologies are compared in **Figure 1**.

Here, we would like to discuss in detail the acoustic and magnetic bioprinting technologies as the new directions of bioprinting.

The use of magnetic forces in tissue engineering has begun with a series of studies by Ito *et al.*[19]. The developed approach was defined as "magnetic force-driven tissue engineering." Magnets and magnetic fields were used to place cells with magnetic nanoparticles on various scaffolds in initial series of experiments. The next step in the development of this approach was the use of magnetic forces to control the movement of tissue spheroids containing magnetic nanoparticles in 2D space[20,21]. Recent works have shown that superparamagnetic nanoparticles of iron oxide in moderate concentrations are not toxic and are recovered by binding iron ions in the body[22].

Demirci *et al.* were the first who have used the method of magnetic levitation of cells without its saturation with magnetic nanoparticle[23]. Diamagnetic objects ranging in size from several millimeters to centimeters were used in these experiments. Their final equilibrium configuration depended on the balance of magnetic and gravitational forces (in special paramagnetic environment, in the gradient magnetic field created by special magnets, and in the absence of direct contacts between its components). Such approach allows to manage building blocks in paramagnetic environments to fabricate 3D construct[24]. Gadolinium salts were added as the additional agent to enhance the medium paramagnetic properties in their experiments[25,26]. Gadolinium salts can be included in some contrast mediums used in magnetic resonance imaging (e.g., Omniscan), so they are allowed for clinical use. Nevertheless, gadolinium salts in high concentrations can cause toxic effect on cells and tissue spheroids. This approach also creates certain risk of osmotic pressure imbalance due to excessive ion concentration in the paramagnetic medium.

Another approach in the development of "scaffold-free" technology is the management of cell material (including tissue spheroids) using ultrasonic waves or so-called acoustic bioprinting[27]. One of the approaches in acoustic bioprinting is to control cells using so-called "acoustic tweezers."

The mode of action of "acoustic tweezers" is as follows: Piezoelectric substrate and two transversely-spaced pairs of interdigital transducers generate standing acoustic-surface waves that capture and move cells. The change of the cell position occurs due to the change in acoustic amplitude and transducers pair phase. Since phase and amplitude can be set and changed easily, the accuracy of cell movement will be limited only by the equipment resolution. Whereby, the cell movement speed can reach 5 μm/s[28]. Some studies have illustrated that such manipulation with cell material does not affect its viability, functionality, and genes expression[29,30]. Moreover, it has variety of advantages in comparison with approaches

	Additive printing	Formative printing
Scaffold	Required	Not required
Printing speed	Medium	High
Printing area	Limited by axes of the bioprinter	Limited by cuvette volume
Viability of cells	Might decrease, due to the extrusion process	Might decrease, due to the concentration of magnetic/paramagnetic supplements
Biosafety	Sealing of the whole bioprinting system is required	Biosafety is inherent, cuvette is a hermetic medium

Figure 1. Comparison of additive and formative bioprinting.

described above, such as: (i) Ability to control cells in closed systems that significantly reduces the risk of possible microbial and fungal contamination; (ii) allows not to use any cell material labeling for manipulation; and (iii) allows to avoid any physical impact on the cells. At the same time, "acoustic tweezers" have the capacity that is 106-fold lower than optical tweezers[31]. Thus, "acoustic tweezers" work in the frequency range similar to the one that is used in medical ultrasound equipment (like ultrasound diagnostic apparatus for the imaging of the fetus in the womb)[32]. The platform consisting of "acoustic tweezers" can be built in the unified software and hardware complex without the use of nozzles and other expensive elements of classic bioprinters necessary for biomaterial management (nozzle-free approach).

The simultaneous use of magnetic and acoustic fields for cell material control using an inhomogeneous magnetic-acoustic field is possible. The principle of this method involves fast levitation fabrication of construct in inhomogeneous magnetic field from cells and/or tissue spheroids chaotically distributed in the active volume of liquid medium. The construct is fabricated in the area where there is the "magnetic-acoustic trap" (area of gravitational, magnetic, and acoustic fields crossing). The gravitational forces are compensated, and tissue spheroids experience forces pulling them together. The final construct can have spherical, annular, ellipsoidal, or other shapes defined by the specific configuration of the magnetic-acoustic field[33]. The described approach involves the development of complex acoustic and magnetic waves design and requires special skills and competencies in experimental physics as well as the availability of specialized equipment. That is why this bioprinting method is not widely applied as it is still on the engineering development stage. Using this method, we create not only the construct model but also field (or several fields) configuration that will determine the object's shape.

Thus, whichever method of bioprinting in microgravity could be used, the main purposes for tissue engineering in space are:

3.1 Investigation of gravity-free effect on human tissues

Tissue engineering constructions are used to study the gravity-free effect on human tissues on earth and in space. First tissue construction (cartilage)[34] was created in zero-gravity in space on the Russian space station "MIR" by the team of the Massachusetts Institute of Technology (MIT) under the supervision of Professor Robert Langer using rotation bioreactor Synthecon developed by NASA. Cell suspension forms tissue aggregates (tissue spheroids) in this rotation-type bioreactor.

3.2 Drug discovery and disease modeling (including possible diseases during long space flights)

During the great voyages of discovery through world oceans, seamen suffered from an awful

disease – scurvy that was caused by chronic vitamin C deficiency as a result of the lack of fresh fruit and vegetables. This condition happened when the seafaring people were at sea continuously for probably more than 3 months, at some stage in the voyaging and the price was more than 2 million lives[35]. This case shows that humans have to prepare for investigating not only new deep space but also possible risks and dangers during these flights.

3.3 Investigation of space radiation effect on human tissues

Another separate, essential branch of space biological science is space radiation studies. Recently, the possibility of creating permanent bases on the Moon, sending manned spacecraft to Mars, and establishing planetary settlements on the planet has been in discussion more frequently according to the vast experience of habitability of space. Space radiation is known to have a negative effect on the human body, especially in space flights outside the earth's protective magnetosphere. Bioprinting technology allows to create radiation-sensitive organs, so-called sentinel organs, as models for further studies of radiation effects.

4 Drug discovery

The pharmaceutical industry could strongly benefit from having means of early detection of negative side effects of potential drugs. This allows pharmacologists to save time and money on formulations as well as cases when a side effect was only discovered when the drug is already in the market but needs to be recalled, with obvious legal and financial ramifications.

2D-based assays are currently the main technology being used for pre-clinical studies. The problem with this technology is that in two dimensions, the cells have a limited amount of contacts between themselves. Most of their contacts are with the culture medium or the surface they are on. This in turn leads to limited modeling of real human tissue interactions (signaling, biomarkers, etc.) This is the reason why the drugs tested in 2D fail when tested *in vivo*. Finally, a new alternative has become available – tests 3D tissue and organ constructs. They can also be used for testing cosmetic formulations as an alternative to animal tests which are getting banned in different jurisdictions (like the EU since 2013).

The increased effectiveness of 3D cultures comparing to 2D analogs is widely accepted. However, the question on how to obtain standardized fabrication methods that allow reproducibility of constructs, rapidness, and cost-effectiveness required for drug testing remains. 3D bioprinting with its automation capabilities and possibility to form complex structures seems the most likely candidate for providing the answer. Other advantages of 3D bioprinting in comparison to other fabrication methods include the ability to create channels for vascularization inside the constructs as well as allowing coculture to form heterotypic constructs under the conditions similar to a typical tissue environment. This increases interest in applying 3D bioprinting to various stages drug discovery (**Figure 2**)[36-38].

Biofabrication strategies that are being used include rotating flask methods, liquid overlay, hanging drop, and magnetic levitation. Manual cell seeding or fabrication of a mold are typically used for these methods[39]. Bioprinting offers higher precision, resolution, and accuracy in comparison to the methods listed above[40]. Other advantages of bioprinting include easier fabrication of spatially-patterned coculture models, low risk of cross-contamination while handling different cell types (in a limited physical space), precise control over delivery of growth factors and genes, and controlled architecture with high-throughput. Bioprinting also enables fabrication of constructs with desired pore sizes associated with a specific type of tissue as well as a controlled architecture. A crucial advantage of bioprinting is that it can be utilized under physiologically-amenable conditions (e.g., pH, humidity, and temperature), while adding genes and proteins that help modulate the behavior of cells. Moreover, using magnetic levitation for bioprinting of tissue spheroids improves the throughput and resolution of bioprinted constructs[41].

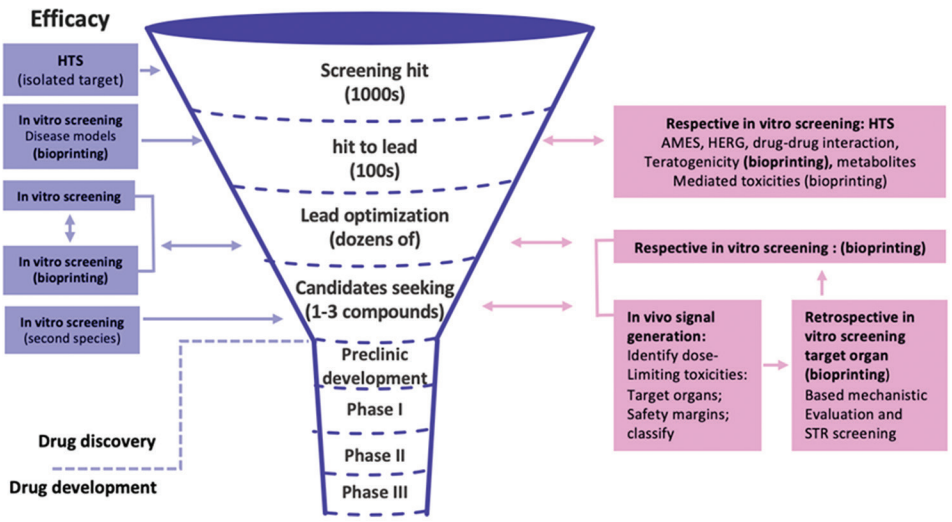

Figure 2. The role of bioprinting in different stages of drug discovery.

In comparison to other fabrication methods, 3D bioprinting offers increased ability to position multiple cell types precisely mimicking the native tissue design. Another opportunity provided by bioprinting is creating a 3D construct considering coculture and vascularization possibilities by depositing multiple cell types (**Figure 3**). The ability to process several materials, complex spatial positioning and make bioprinting a very adaptive technology and increased cell-matrix interactions improves the viability of cells for longer periods. Recent studies have demonstrated that bioprinting allows high-throughput fabrication in generating[42,43].

Drug testing requires perfusion, which is relatively easy for bioprinted constructs. There is increased demand for patient-specific diseased models for personalized drug discovery and therapeutic planning, for which bioprinting has already demonstrated undoubted potential, especially in the application of induced pluripotent stem cells (iPSCs)[44,45]. It is necessary to note, however, that there have not been cases of completely functional tissue made of bioprinted iPSCs[45]. Considering all of the advantages detailed above, there are multiple *in vitro* models for drug testing that has already been created (detailed in **Table 1**)[46].

3D bioprinting technology has provided major advantages in the fabrication of 3D tissue and

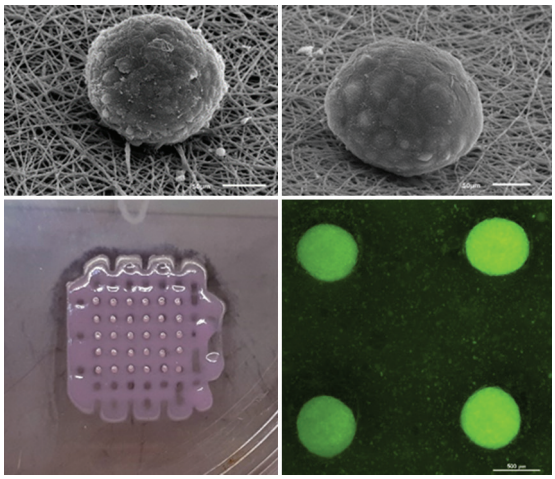

Figure 3. Type-specific 3D spheroids robotically inserted into the collagen grid represent *in vitro* model of human tissue.

organ models in comparison to other fabrication methods. Its unique properties make bioprinting a prime candidate for high-throughput industrial applications, even though vascularization remains a challenge. An interesting approach with a lot of promise for fabrication of *in vitro* tissue and organ models is combining bioreactors with on-chip perfusion. Naturally, bioprinters will need to increase their user-friendliness and lower the costs to become commercially viable on a large scale. In summary, bioprinted 3D models will likely soon be adopted by pharmaceutical companies for drug discovery.

Table 1. *In vitro* organ models for drug testing manufactured through bioprinting.

Organ/Tissue model	Bioprinting modality	Bioink	Cell types	Drugs tested	References
Liver	Sacrificial bioprinting	GelMA	-HepG2/C3A cells, HUVECs	-Acetaminophen, Trovafloxacin,	[47,48]
	DBB	Alginate	-HepG2 and human epithelial cells	-Levofloxacin -Amifostine	[49,50]
Vascular network	Indirect bioprinting	Gelatin/Pluronic	MSCs	Rho-kinase inhibitors	[51-53]
Alveolar model	Valve-based printing	Matrigel™	Type-II alveolar epithelial cells and endothelial cells		[54]
Cornea	EBB	Collagen/ alginate	keratinocytes	-	[55]
Intestine	EBB	Scaffold-free	Epithelial cells of human intestinal origin and myofibroblasts	Indomethacin	[56]
Kidney	EBB	Gelatin/fibrin as ECM and Pluronic as sacrificial ink	Proximal tubule epithelial cells	Cyclosporine A, cisplatin, resazurin	[57]
Muscle	EBB	Alginate/Pluronic	C2C12 cells	Cardiotoxin	[58,59]
Heart	EBB	Fibrin	Rat heart origin primary cardiomyocytes	Epinephrine and carbachol	[60]
Glioma	EBB	Alginate/gelatin/ fibrinogen	Breast cancer cells	Temozolomide	[61]
	DBB	Matrigel	OVCAR-5 cells and MRC-5 fibroblasts	Prolactin, estrodine	[62]
3D neoplastic tissues	EBB	Alginate/gelatin/ fibrinogen	HeLa cells	Paclitaxel	[63]
Skin	LBB	Collagen/ Matrigel™	HaCaT keratinocytes/ NIH3T3 fibroblasts	All-trans retinoic acid, dexamethasone, doxorubicin, S'-fluorouracil, and forskolin	[64,65]

The legal aspect of 3D bioprinting is becoming increasingly important with the growing usage of this technology. There are concerns that while bioprinting is regulated by existing laws that govern medicine and medical research, this current framework does not allow us to mitigate risks to patients, as well as address the requirements of health-care providers and manufacturers. At the very least, that is the situation in the US and EU[66]. There is no specific regulatory framework or even strategy toward 3D bioprinting developed in countries that lead the way in biofabrication research and industry applications. This is further complicated by the fact that 3D bioprinting is a truly unique technology in the sense that it combines 3D printing techniques, materials science and cell biology, and meaning this technology combines the challenges of all possible applications (organ transplantation, medical devices, and cell therapy)[67,68]. Separate regulation does not account for the combined use of the technologies and applications listed above. There is also a question of multiple actors involved in the production chain.

There is also the issue of informed consent, which is not clearly regulated when it comes to novel medical technologies. Ideally, a consent form should inform about all potential risks and adverse effects as well as list a detailed composition of a bioprinted product and fully describe the implantation process. Donors should be better informed of how their cells, tissues, or organs can be utilized now or in the future and this information should be made available with strict access guidelines through specialized databases.

This brings on another issue of data protection on a global scale which requires specialized infrastructure for storing encrypted files with data about cells, tissues, and organs received by patients. This information should also be in a unified format, accessible by commercially used bioprinters, with measures to protect intellectual property also in place. Clearer guidance would also assist innovators, who had to be able to better understand how their products are to be classified once released into the market. One suggested approach to licensing in bioprinting is placing responsibility on companies to share benefits and at the same time emphasizing the role of public research.

A draft version of guidelines was released in May 2016 by the US Food and Drug Administration (FDA) for manufacturers of medical devices that work with additive manufacturing[69]. While it was meant to provide manufacturers with the agency's initial outlook on manufacturing 3D printed devices, it does not address the use or incorporation of biological, cellular, or tissue-based products in additive manufacturing. Products that contain living human cells/tissues (including specific medical devices) and are intended for transplantation in human patients are qualified by the FDA as human cells, tissues, and cellular and tissue-based products (or combination products). Similar classification criteria exist in the EU, but without a general definition or specific regulation for combination products. They are currently regulated as medicinal products or medical devices[70,71].

In summary, international cooperation is required to create clear legal guidelines regulating 3D bioprinting while ensuring that intellectual property, safety, and bioethics are addressed on a global scale. Hopefully, together with educating medical professionals and general population, this will enable future innovations and active medical applications of 3D bioprinting.

5 Financing

3D bioprinting industry is not currently being widely used in healthcare, and its large commercial success is likely to be at least 15 – 20 years away, when bioprinted human organs will become available for transplantation at the costs comparable to the current market. However, a few companies have already launched products into the market and have raised investments through various available means.

There is an important distinction between investments in bioprinting companies focused on regenerative medicine and companies that are working toward creating a cultured meat product. While the former has produced a couple of notable initial public offering (IPOs) (Organovo, Cellink), the latter have also recently begun to attract investor interest, which led to some major investment rounds. In this chapter, we are covering regenerative medicine companies, and you can read about cultured meat investments in chapter 9.

The first bioprinting company that raised significant amounts of capital is Organovo which had set to create tissue models for drug discovery. Organovo went public in 2012 using a reverse IPO and over the next few years raised about US$ 128 million in several installments[72]. This was a crucial breakthrough for the whole bioprinting industry not to mention the company itself. In December 2019, Organovo and Tarveda Therapeutics announced a merger agreement under which Tarveda would execute a merger with a subsidiary of Organovo; the joint company would use the name Tarveda Therapeutics, Inc. and trade on Nasdaq.

CELLINK decided to pursue their IPO just several months after the company was created. However, that was not without reason, as at the time, their bioprinter (priced at US$ 10,000) was sold in 25 countries, mostly to research institutions. CELLINK listed on Nasdaq First North, and notably their IPO was oversubscribed by 1070%. Cellink's current market cap is at US$ 400 million[73].

Cyfuse Biomedical K.K., a manufacturer of 3D bioprinters from Japan, closed its Series B private placement funding in 2015. Cyfuse raised about US$ 12.5 million, bringing the total amount of investments to about US$ 17.8 million[74].

Poietis, a bioprinting company that was one of the first to create a commercially available

bioprinted tissue has raised €5 million in their Series A round in 2018. Poietis has established partnerships with companies such as Badische Anilin und Soda Fabrik and. The contributors to this round included Nouvelle Aquitaine Co-investment Fund. This has provided the company with financial resources that will enable them to accelerate compatibility with regulations and good manufacturing practices requirements. The company has announced that it could produce the first bioprinted tissues for implantation into patients in 2021[75].

In 2019, Aspect Biosystems, a Canadian biotech company, have raised US$ 20 million during their Series A from Radical Ventures, a VC firm focused on companies that aim to solve global problems as well us Pangaea Ventures, Pallasite Ventures, and Rhino Ventures. The company specializes in microfluidic 3D bioprinting. Aspect Biosystems have created a bioprinting platform for production of human tissues[76].

Allevi, formerly known as Biobots, creates desktop 3D bioprinters. The company's clients include companies such as AbbVie, GSK, and Johnson & Johnson. The company was founded in 2014 and has US$ 3.6 million in funding raised up to date[77].

We may expect the industry to start consolidating in the next few years through mergers of bioprinting companies with each other and with strategic partners through acquisitions, reflecting the general trend of the increased interest of industry leaders in regenerative medicine and drug discovery through testing on 3D models.

6 Food industry

Another emerging industry where bioprinting is widely used is alternative protein food technology. There are two major fields of alternative protein: Plant-based meat products and cultured meat products. Plant-based products use specific plant proteins and other supplements to imitate taste and texture of meat, while cell-based products aim to grow various cell types, which constitute natural meat tissues *in vitro* and then assemble these cells into a final product. Both fields use additive manufacturing approaches as means to confer complex specific geometric shapes, organoleptic qualities, or nutritional characteristics to the final product.

While none of the cell-based meat startups have not rolled out their products on any market yet, plant-based alternatives have been growing by leaps and bounds over the recent years, culminating in the successful IPO of Beyond Meat, an American company, that produces a range of meat alternatives using a mix of various plant proteins, and achieved more than 200% growth[78]. Spain-based Novameat[79] and Israeli Redefine Meat[80] now use 3D printing to create more sophisticated plant-based products; while the former focuses on exact material formulation and simulation of proper meat texture, the latter develops multi-layered plant-based steak structure.

Cell-based meat (also known as *in vitro* meat or IVM) is another approach to production of alternative protein. Despite not being present on the market, cultured meat products still attract attention from potential consumers (**Figure 4**), some studies show that as many as 66% of US adults would try meat grown from animal cells[81].

Cultured meat startups vary widely in their approach to product development (**Table 2**). Since one of the major barriers, serum-free medium formulation, was overcome in November 2019 by Mosa Meats that was the first to introduce a cell-based burger patty[82], several companies now focus on using bioprinting to create unique products.

Since the cost of final product is still expected to be prohibitive during early stages, there are various possible applications, for example, production of cultured meat in space[83]. Space launches incur high costs and constant resupply is not a viable solution for long-term manned missions. Therefore, creation of sustainable food production systems is a high priority for multiple space agencies.

Keeping crewmembers fed during such expeditions will face several challenges, and cultured meat products can potentially solve most of them. Using water recovery system in combination with serum-free medium produced

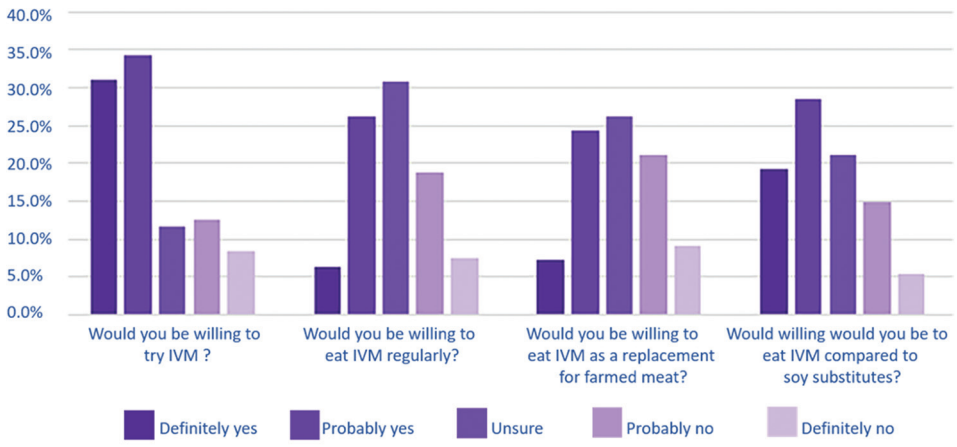

Figure 4. Consumer survey on *in vitro* meat.

Table 2. Cell-based meat companies.

Cell source species	Bioprinting	Non-bioprinting companies	
Mammals	Meal Source Technologies	Wild Earth	Bio. Tech. Foods
	Aleph Farms	LabFarm	New Age Meats
	Fork and Goode	Mosa Meat	Meatable
	Meatech	Biftek. Co.	MiraiFoods
		Higher Steaks	Biofood
		Cell Farm	
Seafood	BlueNalu	Avant	Wild Type
		Finless Foods	Seafuture
		Future Fields	Shiok Meats
		Clean research	
Poultry		JUST	Gourmet
		Bond Pet Foods	SuperMeat
		Clear Meat	Peace of Meat
		Integriculture	Vital Meat
Multi-species	Future Meat Technologies	Memphis meats	Cellular Agriculture
		VOW Foods	Mission Barns

by algae will create self-sustaining systems, which would in turn eliminate any need for resupply runs. Moreover, the self-sustaining systems allow astronauts to directly control and adjust their diet to combat specific medical conditions such as muscle atrophy and osteopenia, which arise during prolonged space travel. In addition to meeting specific medical and nutrition requirements, these systems also allow crewmembers to select palatability preferences as bioprinting can be used to create distinct texture and palatability characteristics in the food that can help crewmembers to endure all the various psychological hardships of long-duration space travel.

Early prototypes of this technology were produced in joint experiments by 3D Bioprinting Solutions[84] and Aleph Farms[85,86]. In these experiments aboard the ISS, magnetic bioprinting was used to assemble spherical constructs out of spheroids, made from animal and fish cells. Both 3D Bioprinting Solutions and Aleph Farms carried out extensive research in tissue engineering, developed originally for regenerative medicine purposes, to create food products from multiple tissue types (e.g., myoblasts, fibroblasts, adipocytes, and blood vessels). Multiple companies across the world embrace this approach, transferring bioprinting advances from tissue engineering to food tech.

BlueNalu, which employs several former members of Organovo, plans to print thin slices of layered fish tissues that consist of muscle cells, connective tissue, and fat cells, and combine it with industrial-scale bioreactor system[87]. Meatech and Future Meat Technologies, two startups from Israel, also plan to use bioprinting as a method to combine multiple tissue types to create a final product which would resemble natural meat cut texture. However, beyond bioprinting, they use different technologies; Meatech plans to use umbilical cord samples as the prime source of cells, while future meat technologies, according to their patents, plans to focus on bioreactor system development[88]. Fork and Goode, an American company led by Gabor Forgacs which is one of the bioprinting pioneers, has not unveiled any product plans, but they have multiple patents relevant to the printing of food products which were filed by Organovo several years ago[89].

However, the application of bioprinting in consumer goods is not limited to the food industry only. Cellular agriculture in textile production also uses its techniques. BioLogic, based in MIT, uses bioprinting to create responsive biomaterial for sportswear[90,91], while Modern Meadows employs bioprinting to create artificial leather textiles. This novel approach has the potential to solve the issue of animal abuse in fashion industry, but there is more – technologies used by this two companies, BioLogic's approach in particular, allow us to create smart fabrics that could potentially revolutionize a wide range of industries. Sensors and actuators on nanoscale will transform everything, from fashion to military applications, since they can adjust the properties of a wide range of materials in real time[92].

7 Conclusion

The practical applications described in this article demonstrate the evident potential of 3D bioprinting for a number of industries. There is undoubtedly increasing interest from both private investors and governments as bioprinting can be applied to solving crucial and fundamental problems such as the lack of donor organs or the environmental impact of meat industry. This should propel both the science and the opportunities for commercialization forward. Once the limiting factors such as the lack of adequate cell material are successfully addressed, we shall no doubt see more commercial products and services in regenerative medicine, space science, drug discovery, food industry, and perhaps even beyond that.

Conflict of interest

The authors declare no conflict of interest.

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