Perspective

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3D bioprinting: challenges in commercialization and clinical translation

Sanjairaj Vijayavenkataraman*[1,2]

[1]The Vijay Lab, Division of Engineering, New York University Abu Dhabi, Abu Dhabi, UAE

[2]Department of Mechanical & Aerospace Engineering, Tandon School of Engineering, New York University, Brooklyn, NY 11201, USA

*Author for correspondence: vs89@nyu.edu

3D Bioprinting has become a revolutionary tool in the field of tissue engineering and regenerative medicine. Bioprinting industry has seen a tremendous growth in the past decade, with a number of bioink companies and bioprinter companies on the rise. While the growth of bioprinting has been tremendous in terms of research and reach, permeating into life sciences research where two-dimensional cell culture has been the norm, we are yet to witness a commercial success in terms of clinical translation. This perspective article aims to highlight some of the lesser-discussed challenges in the field that are to be overcome to fully translate the use of bioprinting into the clinics and make it a standard of testing in the pharmaceuticals industry.

Tweetable abstract: This article highlights some of the lesser-discussed challenges in the field that are to be overcome to fully translate the use of bioprinting into the clinics and make it a standard of testing in the pharmaceuticals or skin care industry.

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The market landscape of 3D bioprinting

3D Bioprinting is a pioneering technology within the field of Tissue Engineering and Regenerative Medicine (TERM). With its ability and demonstrated potential to fabricate tissues such as skin [1,2], muscle [3], cartilage [4], bone [5] and neural tissues [6], the market demand and potential of this technology is increasing at a rapid pace. The rate at which bioprinting technology and its ancillary products and services were commercialized over the last decade is mind-blowing. Estimates suggest that the market size of 3D Bioprinting in 2022 is US$2.13 billion and it is expected to grow up to $8.3 billion in 2030 at a compound annual growth rate (CAGR) of 18.51% [7]. The number of bioprinting companies increase year on year with both new players such as Bico (CELLINK) and Poietis and established players such as GE Healthcare foraging into Bioprinting. A sample list of companies mentioned in a market survey [7] is shown in Box 1. This is just a sample list of companies included in one of the many market surveys but there are many other companies other than what was mentioned above.

The key drivers of such a market demand are twofold: (i) increasing organ demand for transplantation; and (ii) need for *in vitro* tissue/organ models for drug/cosmetic testing to replace/mitigate the use of animal models. While the demand for organ transplants continues to increase, the supply has been stagnant for decades, with just 10% of the global demand being satisfied [8]. Given the potential of bioprinting to fabricate functional tissues and maybe organs in the future, bioprinting research grows at a rapid pace. The *in vitro* tissue/organ model for drug or cosmetic testing is a huge market that can be captured by Bioprinting well ahead of the regenerative medicine market. With the ability to pattern biomaterials and cells in complex and biomimetic architectures, bioprinting has the potential to not only fabricate biomimetic normal tissues but also various diseased tissues that could be used as *in vitro* disease models for studying the pathophysiology of the disease, in addition to being used as drug testing platforms.

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Box 1. A sample list of companies involved in Bioprinting.

List of companies involved in Bioprinting

- 3D Bioprinting Solutions
- Advanced Solutions Life Sciences, LLC
- Allevi Inc.
- Aspect Biosystems Ltd.
- Bico group ab,
- Bio3D Technologies Pte. Ltd.
- Cellink Global
- Collplant Biotechnologies Ltd.
- Cyfuse Biomedical K.K.
- Electro Optical Systems
- EnvisionTEC, Inc.
- Foldink Life Science Technologies
- Formlabs, Inc.
- GE Healthcare
- Inventia Life Science PTY LTD
- Optomec Inc.
- Organovo Holdings Inc.
- Pandorum Technologies Pvt. Ltd
- Poietis
- Precise Bio
- Regenovo Biotechnology Co. Ltd
- Renishaw PLC
- Revotek
- Rokit Healthcare
- Stratasys
- Vivax Bio, LLC

Though the market demand for 3D Bioprinting is huge and there has been considerable success in the market growth and commercialization, the full potential of the technology is not realized yet, in the sense, the technology is not yet fully translated into the clinics nor has become a standard of testing in the pharmaceuticals or skin care industry. There are still many challenges that are to be overcome to achieve this feat which is briefly dealt with in this perspective article.

Challenges in commercialization & clinical translation

The challenges in commercialization or clinical translation are many and multifold in the field of Bioprinting. A recent review [9] captured the current challenges in the clinical translation of 3D bioprinted human tissues. This perspective discusses the other significant but lesser-discussed challenges.

Bioprinters

With so many commercial bioprinters in the market, bioprinters are becoming more and more sophisticated and affordable. While multi-nozzle printers are still sold at a higher price, with heavy competition and more players, the price is expected to come down. I personally feel the technical aspects of bioprinters are being addressed by the companies and universities very well and the current technical challenges such as scalability, multi-axis movement, multiple printing modalities, etc., are being focused upon with improvised versions visible in the near future. One challenge related to scalability (printing human scale tissues and organs) will be to maintain the cells without damage during the long printing process. Bioprinting is a slow process, as is known in the field. With scalability comes the challenge of cell survival during the long printing process. While new bioprinting processes such as tomographic volumetric bioprinting [10] might significantly reduce the printing time and other processes such as the freeform reversible embedding of suspended hydrogels (FRESH) [11] might enable the possibility of printing inside a extracellular matrix (ECM) bath with culture media and growth factors that would preserve the printed cells throughout the printing process, conscious research in this direction is a need in the field. Another associated challenge will be *in situ* monitoring of the printing process and printed tissues/organs. There have been considerable perspectives on *in situ* process monitoring in bioprinting [12] as it is a widely researched area in the

general additive manufacturing (AM) field and the concepts specific to bioprinting have to be customized. However, *in situ* monitoring of the printed cells might be a challenge as the cell viability and other biological characterization are usually done post-printing. It becomes important when we aim at human scale tissue/organ fabrication as that would save a considerable amount of time, cost and effort. The earlier you can detect that the process parameters are not favorable to the cells, the sooner you can stop the process or make amends. To give an example within the metal AM, infrared cameras can capture the heat distribution or heat map of the structures as they are being printed using laser-based powder bed AM processes (e.g., selective laser sintering or selective laser melting). If any anomaly is detected, either toll path correction is made, or the printer can be stopped. A similar system to monitor the cells during printing will be a very useful tool. It could be as simple as fluorescing cells detected with optical systems as they are printed or other complicated optical/electrical/electronic systems.

A futuristic goal could be to make bioprinters accessible to the clinicians at the clinic. The bioprinters are not simple enough to be operated by a clinician. The setup and preparation require special skills and time. A clinician will not be able to do the end-to-end bioprinting process and must be assisted by a technical team. If we are talking about a bioprinted tissue for transplantation involving autologous cells, the clinician can do a biopsy but the subsequent isolation and culture of cells, preparation of cell-laden bioinks, design of the tissue constructs, optimizing the bioprinting parameters and post-printing procedures require a team of individuals with different skillsets. Future bioprinters could be made clinician-friendly with fewer printing options, easy to use and maintain, and customized for specific types of tissues that are in high demand rather than a completely open system.

Bioinks

Bioinks is the backbone of those bioprinting processes which requires cells to be suspended in a viscous solution, usually hydrogels, to achieve desired printability and structural stability. Extrusion bioprinting is the best example, where bioinks determine the success of the bioprinting process. Bioprinting processes like inkjet printing where cells are suspended in a very low viscous fluids such as culture media, the bioink properties might not be as critical as that with extrusion bioprinting. Given the critical importance of bioinks, the various aspects of the bioinks, including the source, batch to batch variation, and tissue-specificity are the important challenges to be overcome to aid in successful commercialization and clinical translation.

Source of bioinks & batch to batch variation

Bioinks are predominantly hydrogel-based and hydrogels are "*crosslinked polymeric substances capable of absorbing and retaining large quantities of water*" [13]. There are two main types of hydrogels that are used, namely natural and synthetic polymer hydrogels. While synthetic polymer hydrogels have the advantage of tunable properties and very limited batch-to-batch variation, they find limited application in bioprinting due to lack of bioactive molecules or cell adhesive sites [14]. The commonly used synthetic polymer hydrogels in bioprinting are polyethylene glycol (PEG) and Pluronic®. Needless to say, natural polymer hydrogels such as collagen, gelatin, chitosan, alginate, and fibrin, possess inherent advantages as bioinks and hence most commonly used. However, the source of these hydrogels are mostly animal sources with a few plant-based hydrogels. For example, hydrogels such as collagen, gelatin, and fibrin are derived from vertebrates whereas alginate and agarose are obtained from algae or sea weeds [15].

The sources of these bioinks pose two problems. The first problem is the source itself. For applications in regenerative medicine, natural polymer hydrogels might cause potential harmful immunological reactions. Williams [16] had the view that decellularized tissues might elicit adverse innate or adaptive immune responses and cited the example of a ECM product CorMatrix®, which although approved for use by several regulatory bodies in different jurisdictions showed significant chronic inflammation and fibrosis in the long-term [17]. There are several methods discussed in the literature to remove the endotoxins responsible for immunotoxicity and general biocompatibility in natural polymers such as collagen [18] and process-induced immunotoxicity of synthetic polymers such as polycaprolactone [19]. Specific studies on bioinks with respect to quantifying the endotoxins or other immunotoxic compounds and methods to decrease their levels are required. For other applications such as cellular agriculture or cultivated meat, the use animal source of biomaterials and bioinks is oxymoron as the whole point of cultivated meat is to reduce animal sources to mitigate global warming.

The second problem is the batch-to-batch variation. Almost all the commercially available natural polymers have an inherent issue of batch-to-batch variation. While this is a very common problem faced by the bioprinting community, it is less discussed in the scientific field, to the best of my knowledge. It is very hard to overcome this challenge as each animal is different and the ECM materials taken from the animals will be different in their

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properties as well. One example is how the ECM properties, including the mechanical, structural and biological properties, are different depending on the age of the animal [20]. Several other factors including the environmental factors will play a role in the ECM make-up and properties. In addition to the source variations, process-induced variations that affect the chemical composition/structure, molecular weight, mechanical/rheological properties, and degree of substitution/functionalization (e.g., degree of methacrylation of methacrylate-based bioinks) also contribute to batch-to-batch variation.

Tissue-specific bioinks

Almost all commercial bioprinting companies that sell bioinks have a catalogue of tissue-specific or cell-specific bioinks. For example, bioinks for skin, bioinks for neural tissue, etc. It is very logical to have tissue-specific bioinks because each tissue is different in terms of its ECM composition and properties. As a case and a point, in our recent works on tunicate-derived bioinks, we formulated a tunicate dECM/alginate bioink, successfully incorporated and printed human mesenchymal stem cells (hMSCs) and differentiated the hMSCs to chondrocytes post-printing [4]. We attempted to use the similar tunicate dECM/alginate bioink to print human neural stem cells (hNSCs) [6] but the post-printing cell viability of hNSCs were very poor. We had to include Matrigel as a component in the bioink to print hNSCs successfully and differentiated them into peripheral neurons [6]. A perfect recipe for tissue-specific bioinks is not available yet and it takes a lot of time and efforts to have such a recipe. While there have been attempts to develop a universal bioink [21], nothing solid has materialized yet commercially.

Most of the commercial bioinks pass through only basic cell viability tests such as biocompatibility and post-bioprinting cell viability. However, there are many other cellular functions that might be influenced by the bioink. For example, the bioink might support post-printing cell viability but not cell differentiation. Hence, evaluation of bioinks based on specific applications or a rigorous complete evaluation of a bioink of various cellular functions might potentially be a good step to commercialization and clinical translation.

While hydrogel based bioinks suffer from poor mechanical properties, I do not see it as a challenge for commercialization/ clinical translation as there are many ways to overcome them – by additives or by using hybrid bioprinting techniques or combining bioprinting with scaffold-based approaches. Long-term studies (28 days or longer) of bioinks are necessary as many studies only report short-term studies (24 h, 48 h or up to 7 days). Long-term studies will not only help evaluate the long-term biocompatibility or cellular functions but also the change of properties of bioink with time (degradability, breakdown of crosslinks resulting in structural failures, interaction of new ECM produced by the cells with the bioinks and its effects). Studies in large animal models are necessary as studies with bioprinted tissues on large animal models are far few in the literature.

Bioprinting parameters & standardization

The other important challenge in translation of this technology is the standardization of process parameters. The outcome of bioprinting is very sensitive to a range of process parameters [22] and any change in the process parameters including the external environment might result in a completely different outcome. Depending on the type of bioprinting process, the process parameters that will influence the properties of the bioprinted constructs will vary. For example, extrusion pressure and speed might be the important process parameters when it comes to extrusion bioprinting while the impact of heat energy or vibrating frequencies and voltage becomes important with inkjet bioprinting [23]. The impacts of various process parameters on the cellular activity in different bioprinting processes are summarized in a recent review [23]. However, cellular viability is not the only concern. There are other important properties of the bioprinted constructs such as its mechanical strength, structural stability, and long-term culture capability that are to be ensured. While there has been attempts to standardize the bioprinting process parameters before [22,24], they are limited either with respect to one process or one specific bioink or one specific tissue or just one specific aspect (such as mechanical property or cellular activity). A more thorough open database of bioink optimization will help the field progress to a greater extent.

Also, the variations involved in the process, such as the nozzles and nozzle dimensions in case of extrusion bioprinting, the type of cell culture media used for formulating the bioink, and other external factors such as temperature and humidity of the bioprinting chamber (if not controlled) will play a huge role in determining the properties of the bioprinted tissue. These parameters, sometimes, are not measured or reported in the literature. With these gaps and misses, the repeatability of the process and the reproducibility of the bioprinted construct becomes challenging and hence a barrier to clinical translation.

Hurdles in clinical translation

There are many hurdles when it comes to clinical translation of bioprinting tissues and organs. The first and well-known challenge is the source of cells. If the cells are patient-derived, which is the best-case scenario in terms of immune response, the challenge of harvesting the cells from the patient, the feasibility and time taken to expand the cells in the laboratory, achieving a critical mass of cells (sometimes billions of cells) for bioprinting a tissue of required scale, bioprinting of the tissue and maturation of the bioprinted tissue before transplantation is a huge process riddled with challenges and uncertainties. One alternate is to go with induced pluripotent stem cells (iPSCs) [25]. The use of iPSCs is very useful when it comes to difficult to access tissues such as spinal cord or brain cells to take a biopsy or sensitive cells such as lung cells which are difficult to maintain in culture, as easily accessible and easily maintained cells such as skin cells (fibroblasts) can be isolated and made into iPSCs which can then be programmed to differentiate into different types of cells. However, this process still does not solve the long time duration issue. One of the possible solutions is to develop an immune neutral stem cell type that is compatible with most human beings, it could be synthetic or genetically-modified iPSCs [26–28]. If we manage to achieve a biological breakthrough by establishing such a cell type, off-the-shelf bioprinted tissues and organs might become very much achievable and that would solve the problems associated with patient-derived cells and immune rejection.

While scaling up of bioprinting technologies to print human-scale tissues and organs has been discussed extensively, the development of associated technologies like bioreactor for tissue maturation has not been discussed in detail. However, with the increasing attention given to cultivated meat and lab-grown meat, the bioreactor technologies at industrial scale and other challenges in the tissue engineering field such as serum-free media are being addressed. I hope the field of TERM will take advantage of these technologies to accelerate the scalability aspects of engineered tissues.

Another challenge in clinical translation of bioprinted tissues is the compatibility for long-term storage and transportation. Freshly printed tissues or organs may not be a feasible option, given the long lead time for printing and tissue maturation. While procedures similar to organ transport can be used (when the donor and the recipient is not at the same physical location), the time has always been critical. Hence, technologies related to long-term storage and revival of these tissues has to be focused upon.

Last but not least, the ethical, legal, and social aspects of bioprinted tissues and organs [29] need to be considered carefully and addressed suitably. The regulatory aspects of bioprinting and bioprinted tissues cannot be undervalued when it comes to commercialization. Regulatory aspects are multi-faceted, starting from intellectual property (IP) of bioinks, bioprinters, and bioprinted products to specific regulatory guidelines for creation, use, and approval of bioprinted products. No regulatory body had come up with a precise and specific regulatory document regarding bioprinting yet. The US FDA released a draft policy on "Technical Considerations for Additively Manufactured Devices" in 2016 but it does not include cell- or tissue-based products as they "may necessitate additional regulatory and manufacturing process considerations and/or different regulatory pathways" [30]. The real challenge lies in the complexity of the bioprinting process itself and its potential in fabricating complex combination products, posing challenges in 'product classification'. An example of a complex combination product could be a drug-eluting tissue construct fabricated using 3D printing and 3D bioprinting; this product could include a 3D-printed scaffold made of synthetic biopolymer, the synthetic biopolymer being functionalized with an anti-inflammatory or pro-regenerative drug capable of eluting the drug at a controlled rate, and bioprinted human-derived cells suspended in a natural human decellularized ECM bioink onto the synthetic polymeric scaffold. Given such a highly complex combination product, product classification and regulatory consideration for approval of such a product becomes highly challenging [31]. This is significant for clinical translation of bioprinted products as the safety evaluation of individual components of such a combinatorial product has to be different from the combination product itself. One area of concern with clinical trials of complex bioprinted products is the potential irreversibility of treatment and the related consequences [30]. The reversibility of treatments with bioprinted tissues/organs is very difficult. Removal of tissue after implantation might result in irreversible damages to the normal functioning of the body and many times complete removal of the tissue after implantation might not be possible (such as residual cells, vascular networks, etc.) resulting in other complications. These, along with other ethical, regulatory, and social aspects, need to be addressed for successful clinical translation of bioprinting.

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Conclusion & future perspective

In summary, the challenges to be overcome and therefore the future perspectives for the field of Bioprinting are:

- Printing human scale tissues and related technologies such as bioreactors for maintaining the cell viability and functionality both during and after bioprinting.
- *In situ* monitoring of the printed cells becomes important when we aim at human scale tissue/organ fabrication as that would save a considerable amount of time, cost, and efforts. The earlier you can detect that the process parameters are not favorable to the cells, the sooner you can stop the process or make amends.
- Future bioprinters could be made clinician-friendly with fewer printing options, easy to use and maintain, and customized for specific types of tissues that are in high demand rather than a completely open system.
- Steps to overcome the challenges associated with the immunogenicity aspects and batch-to-batch variation given the animal origin of bioinks; synthetic bioinks are one promising option but the challenges of making them more bioactive and cell-friendly have to be overcome.
- A perfect recipe for tissue-specific bioinks is not available yet. While there have been attempts to develop a universal bioink that will suit any and all cells, any and all functionality (cell adhesion, migration, differentiation, etc.), this is a humongous task to attempt.
- Long-term studies in large animal models with bioprinted constructs is a must for further clinical translation.
- Develop an immune neutral stem cell type (synthetic or genetically-modified iPSCs) that is compatible with most human beings to make off-the-shelf bioprinted tissues and organs possible, solving the problems associated with patient-derived cells and immune rejection.
- Ethical, legal, and social aspects of bioprinted tissues and organs need to be considered carefully and addressed suitably.

Executive summary

Market landscape of 3D bioprinting

- Given the potential of bioprinting to fabricate functional tissues and maybe organs in the future, commercial bioprinting landscape grows at a rapid pace, with both new players and established players foraging into the bioprinting space.

Challenges in commercialization & clinical translation

- There are several challenges that can be grouped into four namely challenges related to the bioprinters (scalability, maintenance of cells during the printing process when printing human scale constructs); challenges related to bioinks (potential problems with the animal origins of bioinks and batch-to-batch variation of bioinks and its implications), challenges related to tissue-specific bioinks and its significance, and clinical translation hurdles including the ethical and legal aspects of bioprinting.

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