Creating Tumor Models With 3D Bioprinting For Cancer Research

Trinabh Chakravarty

Student, R N Podar School

Abstract

Cancer, across the globe, remains the second most common cause of deaths second only after Ischemic heart disease. Typically, a tumor microenvironment (TME) is a heterogenous collection of stromal cells, cancer cells, immune cells, endothelial cells and extracellular matter. Conventionally, most research works on cancer are based on 2D modelling which suffers an inherent limitation of not fully imitating the complexity of human tissues. The 3D bioprinting has gained the world's attention for its ability to mimic the pathophysiology of TME. This pioneering technology has the ability to imitate the TME and hence, simulate cancer tissues to be tested for possible treatments. This way the researchers can test customisable, replicable and reproducible constructs under in vitro environment and understand their in vivo behaviour. This way, the drugs can be tested and evaluated for their impact, side effects and results just like they would have been on in vivo human cells.

At the same time, the technology has not been able to reach its true potential due to the challenges involved. Researchers maintain that accurate mimicking of the cancer tumours requires collaborative understanding and application of more than technology and manufacturing process. Despite recent developments in the field of bioprinting in breast cancer tissues, findings of several studies have failed to give out reproducible results. A collaborative multidisciplinary research and development effort is needed to make any significant development in both the 3D bioprinting technology and the printable media technology.

Nearly all the researchers have agreed in the tremendous potential that the 3D bioprinting technology has in the field of medicine, medical treatment, research and development. The technology offers great promises for regenerative medicine wherein complex anatomical structures like skin, bone tissues, cartilages and so on can be customised and manufactured on demand. It is argued that the conventional methods are far less accurate compared to bioprinted TMEs however, more needs to be researched and developed in this area to increase its commercial viability. As our understanding of and expertise in this area improves, the effectiveness and accuracy of this technology shall also improve. The increased application of this technology shall increase the scale of production and hence, gradually bring down its cost.

Keywords: 3D bioprinting, Tumours, Cancer, Drug Development

1.   Introduction

Cancer, across the globe, remains the second most common cause of deaths second only after Ischemic heart disease. According to the data released by the WHO (2019), cancer was responsible for over 10 million death in 2022 alone which was nearly one-sixths of the total deaths across the globe. A report by the Global Burden of Disease (GBD) (2015) estimated that, on an average, 19.3 million new cancer cases are reported each year with an overall five-years survival rate of 64%. Luo et al. (2019) identify lung cancer (25%), colorectal cancer (11%), and pancreatic cancer (7%) as the top-three causes of cancer-related mortalities across the globe. Clearly, considering its mortality rate and increasing incidence, experts across the globe have started showing interest in research on cancer, its causes and treatment.

According to Nagai and Kim (2017), cancer tumors are caused when cells in the body start multiplying uncontrollably with a tendency to penetrate and destroy healthy human tissues. The cancer cells have the tendency to spread throughout the body and hence, creating interference with others systems of the body as well. Tumors, due to the rapid and uncontrolled multiplying ability of the cancer cells, have complex pathophysiology which is still not fully understood. Typically, a tumor microenvironment (TME) is a heterogenous collection of stromal cells, cancer cells, immune cells, endothelial cells and extracellular matter (Sharma et al., 2023). This combination while aids tumor progression, also interferes with the metastasis and hence, one's response to the treatment.

Gao et al. (2021) further argue that multiple factors including the TME collectively influence the treatment outcome and the patient's response and wellbeing. For any treatment to be effective, the treating doctor needs to fully understand these factors and how they interact with the proposed treatment. Mostly, the treatment is given based on the doctor's understanding of patient's response which is gradually adapted and modified. Conventionally, most research works on cancer are based on 2D modelling which suffers an inherent limitation of not fully imitating the

complexity of human tissues. The 3D bioprinting has gained the world's attention for its ability to mimic the pathophysiology of TME (Kačarević et al. (2018).

Datta et al. (2020) define 3D bioprinting as the advance technology using which one can create complex three-dimensional biostructure mimicking human tissues and/or organs using bio-ink or a combination of biomaterial, growth factors and living cells. The technology is able to create precise replication of human tissue or organ through layer-by-layer deposition of biomaterial (bio-ink) just the way it should have been in a TME. According to Shukla et al. (2022), the 3D printing as a revolutionary step forward in the field of cancer research with multiple possibilities and clinical applications. This pioneering technology has the ability to imitate the TME and hence, simulate cancer tissues to be tested for possible treatments. Sharifi et al. (2021) maintain that creating TME using bioprinting can produce more accurate, predictable and replicable results.

Knowlton et al. (2015) studied the benefit of 3D bioprinting technology in context of cancer research and argued that it offers great potential. It was pointed out that the technology can be used to produce a patient specific TME for the medical practitioner using which a more accurate and effective treatment can be designed, tried and tested before being actually applied on to the patient. It was argued that majority of the commonly given treatments like chemotherapy, radiation therapy and so on have their own side-effects which can be minimised if the treating physician has the knowledge on TME behaviour to the proposed treatment. The potential use of this technology can allow generating personalised treatment approach which so far remained a distant goal in this area.

2. Rationale Behind Research

While cancer remains one of the most dreaded diseases in the world, our understanding on cancerous growth and the reasons behind them remains limited. Zhang et al. (2016) maintains that cancerous growth interferes with the other cells, organs and the functions of the human body causing further complications. In order to effectively treat cancer, we must make efforts to better understand its operation and associated complexities.

According to Almela et al. (2021), majority of our present understanding on cancer is primary based on conventional methods like tissue culture, animal tissues and 2D modelling and testing on animal tissues and organs. While experimenting on human cells and organs is not always a possibility, the use of 3D bioprinting allows researchers to simulate human cells and organs to do their experiments without endangering human lives. While possibilities for the 3D bioprinting are limitless, its actual application in this area has remained limited. This research explores how the 3D bioprinting is being used in this area and its outcomes.

Staros et al. (2022) discuss the serious side effects of the cancer treatment and argues that our better understanding of how cancerous growth works and interferes with the functions of the human body can help improve the quality of treatment, better outcomes and better patient wellbeing. The 3D bioprinting technology has the ability to create human tissues and organs that mimic the human tissues and organs. Using this, the researchers shall be able to better understand what creates these side effects and how can they be minimised or eliminated.

Despite its potential in cancer research, the use of 3D bioprinting has only started and remains relatively limited (Chameettachal et al. (2019). This research explores the present and the potential use of 3D bioprinting technology in cancer research and treatment using findings of the previous researchers and scholars. This research is being conducted to determine how the application of 3D bioprinting technology can used to improve our understanding of the disease, its treatment and the side effect caused. Being based on the findings of the previous researchers, this research shall consolidate our collective understanding in this area while also determining the way forward in this direction.

Gao et al. (2021) further argue that developing drugs for cancer treatment is a long, time-consuming and expensive process because much of research and development work done in this area is based on 2D cell modelling and testing on animal tissues. Moreover, the findings are not as accurate and reliable because of the lack of adequate preclinical models. The use of 3D bioprinting can revolutionise the research and development efforts in this area.

It is argued that direct testing on synthetically manufactured human tissues and organs presents a more effective and reliable testing platform for drug developers (Li et al., 2023). The use of this technology is not only expected to reduce the time consumed in developing a drug but also the costs involved. Findings of this research shall explore the beneficial use of 3D modelling can be used to improve drug development and testing.

3. Use of 3D Bioprinting in Cancer Research

3.1. 3D Bioprinting

3D printing has been the recent-most buzzword in the area of medical research, especially cancer research, drug development and testing and treatment strategies. Mazzocchi et al. (2019) define 3D bioprinting as an additive manufacturing process wherein, 3D printing technology has been extended to construction of living tissues, organs and so on. The process involves layer-by-layer construction of tissues based on information available on their construction as found in real-life situations. 3D bioprinting uses biomaterials which is a combination of synthetic and natural substances mixable with the living material to produce a mimicked structure.

Ma et al. (2018) describes 3D printing as the latest addition to the toolkit used in cancer research and understanding. 3D bioprinting has long been amidst us although recently, it has been discussed in context of cancer research and development. The first mention of 3D bioprinting can be found in 1984 when Charles Hull developed a technology to artificially produce a mimicked structure of human tissues using a mix of natural and synthetic material (collectively called bio-ink) and filed an application to get it patented. In 1988, Robert J. Klebe, another researcher in this area developed a technology that uses inkjet printer to print 3D bio-printed models of human tissues (Leberfinger et al., 2019). Eversince then, the technology has gained everyone's attention for the plethora of possibilities that it offered for researchers and medical practitioners.

The 3D printing modelling uses a silicon-based customisable mould in and around which the material containing living cells, extracellular matter and growth material is used to create an imitation of human tissue. The method uses a layer-by-layer construction to make a grid of vascular channels to house the living and non-living material imitating the human tissue or organ (Tripathi et al., 2023). While living cell materials like mesenchymal stem cells, endothelial cells, immune cells etc. are arranged to imitate the tissue structure, the extracellular material provides stability to the 3D printed tissue.

According to Bartolo et al. (2022), the development of 3D printing technology has opened several doors for researchers across the globe working in a wide range of areas like 3D printed skin and bone grafts, implants and even full 3D printed organs and so on including cancer research. It is argued that the application of 3D printing is specifically relevance for cancer research because of the nature of the disease. Cancer is caused due to uncontrolled and malignant growth of body tissues which, prior to 3D printing technology, was impossible to replicate to experiment upon. The 3D printing technology allowed researchers to create identical replica of cancerous human tissues and hence, improve the accuracy of the findings. According to Murphy et al. (2020), the 3D printed human tissues can maintain their structure, shape and function for over six weeks and hence, allow researchers to observe the outcome of their tests in greater details.

3.2. 3D Printing and Cancer Research and Drug Development

3D bioprinting is a novel approach that has opened several new possibilities in several new areas of study. Researchers now have real-life models to work upon and note the outcomes for more accurate and reliable results. Augustine et al. (2021) describe 3D bioprinting modelling as a technology that has suddenly rendered most conventional modelling methods like animal tissue sampling, 2D bioprinting, cell culture and so on obsolete. It is argued that the 3D bioprinting allow researchers to create customised bio-samples which simulates the subject of their respective research. According to Sharma et al. (2023), the 3D bioprinting technology allows creators to manufacture tissues and/or organs, one layer at a time, in a predetermined manner to create complex structures like vascular network, cell layering, extracellular material and so on in a

specific order and proportional mix to allow imitating the real-life tissue. By creating nearly exact and sustainable replica of human tissues and/or organ, results using 3D bioprinting are far more accurate, reliable and replicable.

Vijayavenkataraman et al. (2018) further note that the ability of 3D bioprinting to replicate human tissues has opened new horizons in regenerative medicinal research. Research works and experiments are ongoing in areas such as bone repair, skin grafting, cartilage healing, vascular recreation, organ recreation and so on. 3D bioprinting can use patient specific cell to create tissues and organs which drastically reduces the chances of rejection.

Cui et al. (2017) argue that one of the most typical challenge being faced by cancer researchers is the anatomy of the cancer tumour which is a complex combination of both cancerous and non-cancerous cells. The application of 3D bioprinting in context of cancer research and development and describes how 3D bioprinting can be used to create tissues with specific combination of body cells (both cancerous and non-cancerous) and extracellular substances that closely replicates cancerous tissues called tumour microenvironment (TME) which can sustain in a controlled environment for a considerably long period of time.

According to Sztankovics et al. (2023), the 3D bioprinting technology can produce several similar cell/tissue samples allowing them to conduct controlled experiment for better results. This allows researchers to not only observe how tumor behaves under different conditions but also its reaction to different treatments. Moreover, the findings using 3D bioprinted samples are more accurate and reliable because they arrive from an exact replica of cancer tumour. Wang et al. (2018) studied 3D bioprinted cancer models for their accuracy and effectiveness in cancer research. The findings showed that the results using 3D bioprinted models were atleast four times more accurate and reliable compared to those using animal tissue and cell culture.

Ma et al. (2020) further maintained that the application and use of 3D bioprinting modelling has various advantages in cancer research. Citing the example of *in vitro* tumor models, the researchers observed that the use of 3D bioprinting enables the researchers to work on models that closely replicates cancer tumors in real-life situations. The detailing ability of 3D bioprinting modelling allow researchers to replicate the complex high-resolution microstructures including cellular structure, vascular structure, cell layering and extracellular material. This has hugely beneficial implications in research and development of cancer research. The 3D bioprinting provide researchers with preclinical models to test their research upon with assurance that the findings are likely to be both accurate and reliable.

Nie et al. (2020) discuss 3D bioprinting in context of cancer drug research and development citing another context. According to them the technology uses 'additive manufacturing' using a mix of customisable combination of cellular and extracellular material. This way the technology can

create customisable, replicable and reproducible constructs replicating the in vivo conditions under *in vitro* environment. This way, the drugs can be tested and evaluated for their impact, side effects and results just like they would have been on *in vivo* human cells. It is also argued that identical samples allow researchers to compare the effects of different salts on the same TME and hence, understand how and why aspect of treatment outcomes.

The drugs developed using 3D bioprinting technology are likely to be far cheaper due to the saving in time and effort needed to get the test results. Nagai and Kim (2017), for instance, observed that the conventional methods of cancer drug screening methods often fail to demonstrate the complex interaction taking place within a TME often resulting in high rejection rates. The use of 3D bioprinted model, being close of in vivo tumors, allows researchers to predict the outcomes using less time and resources. This saves time, efforts and money needed in drug development. For instance, Wang et al. (2018) studied the impact of chemotherapeutic drugs like Doxorubicin and Paclitaxel on 3D bioprinted breast tumor-stroma models to determine the comparative effect of the two drugs. The results were compared after 72 hours of treatment of two identical TME of breast cancer. The findings indicated that Paclitaxel caused lesser damage compared to Doxorubicin.

Augustine et al. (2021) maintain that the 3D bioprinting has application in the field of research and development of cancer drugs and treatment. The technology can be used to produce accurate TME on larger scales allowing researchers to test drugs and find out cell-cell and cell-matrix interactions without risking human body or organ. Moreover, since the 3D bioprinting-generated TME better replicates cancer tumor compared to other methods like 2D modelling or animal cell testing, the researchers in this area can get a better and closer insight into tumor biology, drug response and possible side effects.

Staros et al. (2022) further note that the use of 3D bioprinting allow researchers to observe how cancerous cells metastasize allowing designing of more effective treatment strategies for better patient care and wellbeing. It is argued that, going further, the ability of the bio-printed 3D TME to allow researcher to observe the interaction between cancer cells, immune cells and stromal cells and extracellular matter shall help in better understanding on how a given cancerous growth affects the body and hence, can be prevented from metastasizing.

Wang et al. (2018) further maintain that the 3D bioprinting technology has helped us in better understanding how cancer operates and spreads over time allowing us to design strategies to control its metastasis. It is argued that because most tumour are formed under skin and are surrounded by other tissues, understanding how they operate, spread and cause damage to body becomes difficult. Through TME created using 3D bioprinting technology allows researchers to better study cancerous growth from structural, functional and operational perspective.

To summarise, 3D bioprinting has been one of the latest additions in the toolkit for researchers working on cancer research and drug development. Cancer tumour microenvironment, unlike other diseases, are a complex combination of normal cells, cancerous cells and extracellular material which makes it difficult for researchers to accurately predict its behaviour, metastasis and/or the drug testing results. 3D bioprinting allow researchers to customise TME in a replicable and reproducible manner opening several opportunities for them. They can study the behaviour of TME in an *in vitro* environment, as it would have behaved under *in vivo* environment.

3.3. Challenges In the Use of 3D Bioprinting

Over years, 3D bioprinting has emerged as a pioneering new technology having application in many different areas of medicine including research, treatment, drug development and testing and so on (Knowlton et al., 2015). At the same time, the technology has not been able to reach its true potential due to the challenges involved (Tripathi et al., 2023). Simulating tumours, in itself, present tremendous hope in fields like regenerative medicine, drug screening, pathology, research and development and so on. 3D bioprinting can potentially offer in vitro simulation of in vivo presence and function of various types of cancer tumours, however, the outcome of such simulation largely depends upon the degree to which such simulations imitate the in vivo tumours.

Bojin et al. (2021) argues that the technology is still in its evolving stage with many new developments still to be achieved and categorises the challenges being faced by the 3D bioprinting technology into three broad categories namely material, technological and application related challenges. It is imperative that the industry addresses these challenges to be able to realise its true potential. Kronemberger et al. (2021) note the challenges being faced by the 3D bioprinting and maintains that the technology itself, despite its potential, is fairly new. While much has been talked about the technology in theory, much of it still remains to be seen in practical terms. In context of cancer research and treatment, it is observed that the technology needs to evolve further.

Dong et al. (2024) maintain that accurate mimicking of the cancer tumours requires collaborative understanding and application of more than technology and manufacturing process. This complicates the very process of bioprinting. Shukla et al. (2022) studied the inkjet bioprinting technology with Laser Assisted Bioprinting (LAB) for their comparative benefits and limitations and observed that while both the technologies have successfully achieved singular cells per droplet, effectiveness of their outcomes may vary. The inkjet manufacturing process may have limitation in printing sustainable printing 3D structure, the LAB technology is too expensive to be applied in all research works. It is also argued that different technologies are available in different regions preventing researchers from adopting a global approach to cancer research.

Many identical sample tumours may be needed to conduct a series of controlled tests however the reproducibility of this technology has been questioned more than a few times. According to Ma et al. (2020) despite recent developments in the field of bioprinting in breast cancer tissues, findings of several studies have failed to give out reproducible results. It was observed that such variations in replicating the breast cancer tissues was primarily due to variations in the materials used in scaffolds and the printing methods. This, according to the researchers, indicates that the technology has not reached the level of accuracy needed to give predictable results. A need is felt for more research in the area of printable ink for better outcomes.

Santoni et al. (2022) further note that a collaborative multidisciplinary research and development effort is needed to make any significant development in both the 3D bioprinting technology and the printable media technology. For instance, to bio-print a cancer tumour with reasonable accuracy, the printing team needs to understand not only the anatomy of the tissue being reproduced but also the printable media and the technology available. Considering the complexity of the disciplines involved, it becomes difficult for the researchers to generate matching samples and getting accurate outcomes.

It is also argued that the sustainability and longevity of the tissue samples printed using 3D bioprinting technology is questionable limiting its possible applications in various areas. According to Arslan-Yildiz et al. (2016), the average life of a 3D bioprinted TMEs is six days in a controlled *in vitro* environment. This short-life limits the several possible uses of TME manufactured tissues in regenerative medical purposes.

Human anatomy is highly complex. The technological advancements in their present state are not capable enough to accurately and satisfactorily replicate human tissues and/or organs. In order to effectively do this, a multidisciplinary and collaborative approach is needed wherein experts in printing technology, printing media technology, human anatomy, pathology tissue engineering, biology, materials engineering, chemistry, physics, and nano-medicine experts and so on are required (Padhy et al., 2024).

The challenges remain imminently visible limiting the optimal use of this incredible technology. The findings suggest great potential of this technology however, much needs to be worked upon in this area to realise it. Presently, the technology is being uses selectively considering its relative effectiveness compared with the conventional research tools like the use of animal tissues, cell culture, 2D printing and so on.

3.4.   3D bioprinting- The Way Forward

Nearly all the researchers have agreed in the tremendous potential that the 3D bioprinting technology has in the field of medicine, medical treatment, research and development. According

to Staros et al. (2022), the technology has the potential to bring us one step closer to creative power in manufacturing the human anatomy. The technology offers great promises for regenerative medicine wherein complex anatomical structures like skin, bone tissues, cartilages and so on can be customised and manufactured on demand. In context of the use of regenerative medicine in cancer treatment, experts in this area have conceptualised its application in the fields such as lung cancer, bone cancer, breast cancer and so on wherein damaged tissues can be replaced using artificially manufactured healthy tissues causing less damage to the patient (Vijayavenkataraman et al., 2018).

Researchers like Kačarević et al. (2018) and Mahfouzi et al. (2021) also foresee its future application in research and development wherein customised TME can be manufactured and/or replicated to be used in drug screening, testing and experimentation. It is argued that the conventional methods are far less accurate compared to bioprinted TMEs however, more needs to be researched and developed in this area to increase its commercial viability.

Tan et al. (2021) further observed that the synthetic manufacturing of human tissues outside the human body also opens doors towards better understanding of human anatomy and pathology. Prior to this technology, the best in vitro alternative remained tissue culture and 2D printing however, they had limitations. As the technology advances, the human race shall gain better understanding of how our body works. In context of cancer research, Kronemberger et al. (2021) maintain that, as the technology advances, it shall find its applications in understanding how cancer tumour forms, interacts with and affects the healthy tissues. This shall provide us a better understanding on how to prevent and/or control the metastasis of cancer cells.

The possibilities are immense however, the limitations regarding our own understanding of the 3D bioprinting technology and its implementation is preventing us from exploring new areas. As our understanding of and expertise in this area improves, the effectiveness and accuracy of this technology shall also improve. According to Dong et al. (2024), the increased application of this technology shall increase the scale of production and hence, gradually bring down its cost.

4.   Conclusion

Despite best efforts to provide medical care, cancer remains the world's second largest cause of deaths. Scholars have agreed that new ways need to be found which aid better understanding of cancer pathology and remedial treatment. 3D bioprinting is pioneering new technology that has opened plethora of opportunities in several areas including cancer research, pathology, drug research and development and so on.

3D bioprinting allow researchers to synthetically manufacture human tissues and organs which can be used in cancer research and regenerative medicine. Researchers have shown that,

compared to conventional research methods, findings of the research using 3D bioprinted samples are more reliable and accurate saving both time and efforts for researchers.

At the same time, scholars in this area have also agreed that this technology is fairly new and is evolving with time. Despite its potential, more needs to be researched and developed in this area before realising it. Work needs to be done on printing technology, bio-ink media, human anatomy, pathology and so on to get better outcomes.

The future of this technology seems very promising. Several new applications of this technology are being anticipated including regenerative medicine, organ transplant, bio-healing, skin and bone grafting and so on. Researchers have expressed hope towards its potential contribution in cancer research and treatment.

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