Mandala 2:00
Team 9

APPLICATIONS OF BIOPRINTING IN CANCER RESEARCH

Daniel Tometich (djt55@pitt.edu), Jake Atkinson (jla118@pitt.edu), Jerry Wiernusz (gjw26@pitt.edu)

***Abstract*---*Three dimensional bioprinting is an emerging development in the medical industry with huge potential benefits in the cancer research field. Bioprinting holds many advantages over the traditional methods of fabricating cells and tissue. With the use of 3D bioprinting, scientists and engineers can create three dimensional models made from hydrogels, bioinks, and living cells to model the expansion, progression, and other characteristics of different types of cancer. Stereolithography is a common method of bioprinting. This technique uses a laser to harden heat-sensitive plastic or hydrogel into pre-programmed shapes, which are created using any 3D modeling software. Stereolithography has many important applications, including the creation of three-dimensional models of tissue for use in cancer research. In order to print an accurate model of human tissue, real tissue is scanned by an X-ray, MRI, or other medical device. This scan can be modeled and edited on a computer. Once this base structure is created, researchers can seed it with different types of cells to simulate different cellular environments, like different types or stages of cancer, and run various trials in a setting more realistic than older methods of cancer modeling. Although treatments for cancer have yet to be optimized, the evolution of bioprinting is leading towards a better solution. By providing better, more accurate models of cellular environments, bioprinting allows scientists to test and develop new ways of preventing, slowing, and eliminating cancer cells. This research can revolutionize the field of medicine and improve the quality of life, ease of treatment, and survival rate of cancer patients around the world.*

Key words---Biomaterials, Bioprinting, Cancer treatment, Cell environments, Stereolithography

CANCER: THE PRESSING ISSUE

With cancer evolving into the prevalent disease it is today, a cure, or even just a way to stop the expansion, is becoming more necessary. In 2014, over 14 million people around the world had been diagnosed with cancer [1]; in 2018, that number grew to a staggering 18 million [2]. Not only are people being diagnosed at a record pace, but the mortality rate is also at an all-time high. In 2018, over 9 million people died from cancer [2]. Between 2012 and 2018, the number of new cases of cancer rose 28.2%, while the deaths rose by 16.5% during that same time frame [2]. While the number of instances grew over 4 million, the number of deaths increased by only 800 thousand. The slight growth of the death toll, compared to the large increase in victims, could potentially be linked to the decrease in the popularity of smoking, the leading cause of cancer and cancer-related deaths [2]. Unsure if the minimal increase of deaths (compared to the number of new diagnoses) is the direct impact of improved knowledge, engineers are developing new technologies, such as 3-dimensional bioprinting, to research the deadly combatant.

Under current research techniques, cancer treatment options have the lowest success rate among the world's largest diseases [3]. A new method of research, called bioprinting, is on the upswing. Three-dimensional (3D) bioprinting is a process of constructing functioning models of organs, tissues or cell environments for the uses of research and potentially eventual transplantation [4]. 3D bioprinting allows for scientists to examine live, accurate models of cancer cells. Through their examinations, they can test and develop new ways of slowing down the progression or even eliminating the disease. With this advanced technology entering the cancer research market, medical experts are optimistic that the estimate of new diagnoses in 2030 will be lower than they were in 2018.

The Issue of Sustainability

All medical technologies and innovations, to be practical and useful, should strive to be sustainable. Sustainability in the medical field has several distinctions from sustainability for other fields, such as energy production. The National Institute of Health, in their mission, state that their goal is to use medical knowledge to "enhance health, lengthen life, and reduce the burdens of illness and disability" [5]. This definition of sustainability does not simply mean that

the technology is supportable by the environment, but it also must have a meaningful impact on the overall quality of life as specified by those three criteria. Bioprinting has a great potential to fit this definition, because it can lead to new discoveries and more accurate ways of modeling diseases. Also, bioprinting can be used in the field of tissue engineering, which involves the creation of living tissue and organ parts for use in transplants or surgeries. While bioprinting can improve quality of life in this way, there are other aspects of sustainability, such as environmental and economic, that do need to be considered.

While the main purpose of medical innovations is to improve this quality of life, they still need to be sustainable in other ways. One of these ways is that the technology must be environmentally aware and mindful of plant and animal species. Bioprinting can fit this requirement because it reduces the need for drug testing and medical research using live organisms as test subjects. In her article, "Animal Testing and Medicine", Dr. Hajar describes how in the past, animals have been used for testing to better study cell environments than possible with 2-dimensional methods or research. Not only can these tests be useless, because the results might not be true for humans, but also the animals are captured and eventually die because of the diseases and medications that are tested on them. Not only is this unjust, but in recent years, laws have been passed in other countries to prevent testing on animals [6]. Should these laws be implemented in the United States, the demand for bioprinting for research could skyrocket; also, as bioprinting becomes used more and more, the need for animal testing will decrease.

Another aspect of sustainability that needs to be considered is economic viability. Simply put, this means that, like other technologies, bioprinting uses financial and physical resources, and so it is not free or unlimited. Therefore, new products or innovations should use materials that are readily available and be as cost-efficient and time-efficient as possible. As will be discussed, bioprinting struggles in these areas; however, its benefits to the medical field help compensate for these drawbacks, and the technology is constantly evolving and improving its efficiency.

HISTORY OF BIOPRINTING

Three-dimensional printing, also referred to as additive manufacturing, was first introduced in the late 1980's [7]. It has since erupted into its own industry and is changing the ways people can interact in the world around us [8]. Initially, most progress made in the technology was to generate structures faster and run more efficiently [7]. However, recent advances, such as new printable materials, have allowed for the printing to be used in other fields for a variety of different uses [7]. It has since captivated the automobile, aviation, and cake-decorating industries as well as many others including the medical field. It has become such a large part of the medical field that the term bioprinting was introduced. Robert Langer and Joseph Vacanti introduced the idea and technology of bioprinting when they combined cells with materials in the mid 1990's. The initial reasoning behind their idea was to manufacture and eventually implant tissue engineered organs into people [9]. Advances in the new process were rapid and by the end of the 1990's the first bladder was printed [10]. This quickly advanced into creating and modeling live organs to better understand how different diseases affect them and how problems, such as failure, arise [10]. Although implanting fully bioprinted organs in patients has yet to be implemented, there have been a multitude of purposes in the pharmaceutical industry, including the creation of artificial cellular environments for the study of biological processes and diseases, such as cancer [10].

MECHANICS OF BIOPRINTING

The process of bioprinting involves many preliminary steps, the printing itself, and some post-processing before the tissue/organ sample is ready to be used in research. Before a model of a biological system can be produced, the 3D printer must have an actual model with which to build the sample. To generate this model, the tissue or organ is imaged using any traditional medical device, like computed tomography (CT) or magnetic resonance imaging (MRI). These images are then processed by a computer to form three-dimensional models of the sample. Researchers can use computer-aided design (CAD) software to adjust the models as needed. Once this preliminary processing is complete, the actual materials for printing are prepared. Primary cells are taken from a patient and cultured in a lab to produce enough cells to make the tissue. The researchers then create or choose bioinks with desired properties that are like the tissue or organ being created. These bioinks are seeded with cells, then loaded into the actual bioprinter, which creates the tissue or organ based on the provided 3D model. Once the assembly is complete, the organ or tissue often undergoes maturation or other subtle processes, until it is ready to be used as a model for drug testing and disease research.

Imaging

When first scanning a patient to gain information about the structure of a certain tissue or organ, researchers have a wide array of imaging technologies at their disposal. Most of these technologies work by creating layers of two-dimensional images of the tissue sample or organ; however, the method by which they form these images varies from device to device. Computed tomography, for example, uses ionizing radiation (like X-rays) to form high-resolution images that are good at displaying harder tissues. Magnetic resonance imaging (MRI) pulses radio waves into the tissue.

These pulses activate a "response signal" by exciting hydrogen atoms. The pattern of these signals is then used to construct the medical image [4]. MRI is best used at displaying soft tissue. A third major imaging technique is ultrasound, which uses high-frequency sound waves that reflect off the tissue in different patterns, which are interpreted by a computer to form the image. Ultrasound is safer than MRI or CT, because it doesn't make use of radiation, and it also can be used to measure how tissues vibrate in response to the sound; however, it has a much lower resolution compared to MRI or CT.

There are other methods to image tissues, such as microscopy or positron emission tomography (which uses antimatter), but MRI, CT, and ultrasound are the most common techniques, and the three of them can account for most tissue types within the body. Imaging techniques can also be combined, if the tissue/organ being scanned has different kinds of tissue. Whatever the case, once the tissue or organ is imaged, it then needs to be modelled into a format that bioprinters can read.

Materials

Most recent bioprinters read 3D models in stereolithography (STL) format. This type of computer file processes three dimensional images by breaking them into an array of small geometric segments. Once this file is created, researchers use some CAD software to design the tissue's internal structure: nutrient channels, extra supports, or other vital components not captured by the 3D model. Some bioprinters have their own compatible software for use in 3D design. Once the model is complete, it is uploaded to the bioprinter so the actual tissue/organ can be printed.

To print the sample, the bioprinter also needs materials. Most bioprinted constructs are formed from a hydrogel seeded with living cells and other nutrients as needed. These hydrogel "bioinks" have a high-water content that helps shield the cells from harm [11], because the printing process can cause shear forces that can damage the cells in the bioink. Creating the proper hydrogel is difficult, because it needs to have the structural integrity to hold itself together, as well as be fluid enough that cells can migrate throughout the structure. The desired properties can also vary depending on the object being printed; as a result, there is no set formula for a hydrogel, as it can vary by researcher and purpose.

Creating a proper, viable bioink is one of bioprinting's biggest challenges. Because the materials need to be sturdy without losing biocompatibility, researchers must carefully select materials. Many polymers and compounds that are traditionally used in 3D printing and tissue engineering are possible candidates; however, some can be unideal because they are too biologically active, which can cause premature/undesired effects in the bioink. To prevent this, scientists and engineers are looking at hydrogels and new biopolymers, which can better model the nanoscale features of the extracellular matrix [12]. However, these hydrogels, in becoming more biocompatible, tend to sacrifice structural integrity. A consequence of these effects is that the range of useful biomaterials is not large enough to sustain rapid growth in the bioprinting industry. However, efforts are being made to combine different types of materials to produce desired bioinks. For example, researchers led by Hyun-Wook Kang combined tricalcium phosphate with a composite hydrogel that contained gelatin, glycerol, and hyaluronic acid [13]. The resultant biomaterial, when printed, had a cell viability of about 91% (the hydrogel had a low cytotoxicity), but the tricalcium phosphate gave it enough structural strength to serve as substitute bone tissue, for use in possible reconstructive surgery or other medical operations. These results show that, while it can be difficult to create a good biomaterial for use in bioprinting, it is not impossible. Most bioinks consist of a nutrient-providing base, living cells, and some polymer that can harden into the sample. Once the bioink is selected and the 3D model complete, the tissue can now be printed.

Types of Bioprinting

There are three main types of bioprinting, each with their own unique specialties and characteristics. The first type is inkjet-based bioprinting. Inkjet-based printing was the first type of bioprinting process created; in fact, the first inkjet bioprinters were simply commercial inkjet printers that were modified to use bioink. Theses printers encountered a few difficulties at first, because the cells would dry out when they were laid on the substrate. Scientists hurdled this problem by "encapsulating the cells in a highly hydrated polymer, [which] led to the development of cell-loaded hydrogels" [11]. Inkjet bioprinting, therefore, led the way to the rest of bioprinting that exists in the modern day, because the development of hydrogels is what allowed bioprinting to become more complex and larger. Modern inkjet bioprinters lay out the bioink in droplets, with each droplet being ejected by thermal or piezoelectric (acoustic) processes. Inkjet bioprinting has several advantages, including around a 50-micrometer resolution and a printing speed of up to 10,000 droplets/second; however, some limitations exist. Because of the size of the nozzle, only low viscosity bioinks can be printed, and these bioinks, to avoid clogging the nozzle, must have a cell concentration of less than 10^6 cells/mL [4]. Although inkjet bioprinting is quick and affordable, it cannot be used to print anything too strong or complex, because the printer simply cannot handle large amounts of cells.

Extrusion-based bioprinting, another type, is very similar to inkjet bioprinting; however, it is larger and more powerful. Extrusion bioprinting also squeezes bioink out of a nozzle; unlike inkjet bioprinting, which uses heat or sound to cause changes in internal ink pressure, extrusion-based bioprinting uses pneumatic pressure or mechanical force, such as a piston, to extrude the bioink from the nozzle.

Because more power can be delivered to the piston, extrusion-based bioprinting can handle bioinks with pressures up to 200 times higher than inkjet printers, as well as inks with higher cell concentrations [4]. With these factors in mind, extrusion printers can be used to create larger tissue constructs, even entire life-sized organs, because they have a higher volume of ink flow than inkjet printers. However, this increase in size comes with a decrease in resolution. Most extruders can only print with a resolution greater than 100 micrometers [11]. Also, the immense pressures in the nozzle can possibly damage some of the cells in the bioink, so care must be taken to ensure that the hydrogel is not too viscous for this to happen. In comparing inkjet and extrusion-based bioprinting, it becomes evident that inkjet bioprinting excels in creating fine details, while extrusion bioprinting is good at creating large assemblies. A third type of bioprinting has both these attributes, but it is a newer technology still in development.

Stereolithography

Stereolithography (SLA) is one of the newest types of bioprinting, being first developed and used in 1996. Unlike the other two types of bioprinting, stereolithography does not use a nozzle, which eliminates the concern for shear pressure and stress damaging the cells. Stereolithography uses special bioinks that contain photosensitive polymers. As shown in Figure 1, the stereolithograph uses a laser, often one of visible or ultraviolet light, and shines this laser at sections of the bioink, which is stored in a large vat. Where the laser hits, that local area of the bioink polymerizes and hardens, forming a solid, two-dimensional piece of tissue that contains whatever cells were in the bioink. After the laser is done with this whole layer, a platform under the new solid lowers down, allowing a new coat of bioink to flow over the top.

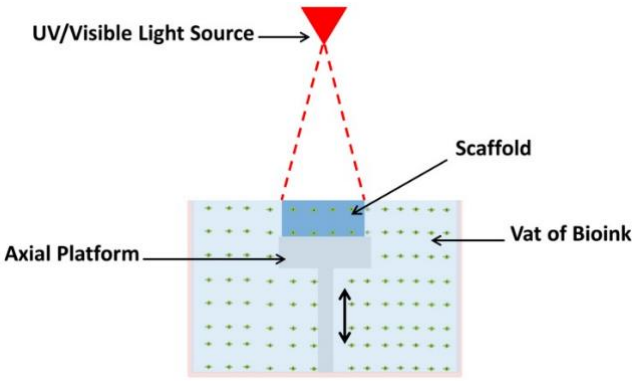

FIGURE 1 [11]
A diagram of a stereolithograph

As seen in Figure 1, this allows a construct to be built from the bottom up, layer by layer, but it has no potential to collapse on itself if it gets tall, because the solid is supported by the vat of liquid around it. A stereolithograph can also be designed to use not a single laser, but an array of mirrors to reflect little bits of light, to synthesize an entire layer instantly, instead of having a single laser trace the layer out [4]. This would also be programmed into the stereolithograph and be able to work with any CAD model.

Stereolithography is more complicated than the other types of bioprinting. This is due to several factors, but a main one is the presence of photoinitiators in the bioink. Photoinitiators are "chemical molecules that create reactive agents when exposed to light energy, which react with monomers of a material to then initiate the formation of polymer chains" [11]. Basically, photoinitiators are necessary to cause the polymerization of the bioink in a stereolithograph. Different photoinitiators react to different wavelengths of light. It so happens that photoinitiators that react to UV light can be more cytotoxic (toxic to cells) at high concentrations than visible light photoinitiators, so visible light stereolithography is more commonly used. In fact, after using an eosin-Y-based photoinitiator, the cell viability of NIH-3T3 fibroblasts was over 85% after five days [4]. Other alternative methods for polymerization include the use of a thiol-ene reaction (monomers that contain alkene or thiol groups), which eliminates the need for a photoinitiator: the monomers themselves are activated by light. This method exhibited cell viability of about 95% after three days [11], an even higher rate of survival. This reaction is only useful when the ratio of thiol to alkenes is 1:1; any higher, and the cellular microenvironment becomes cytotoxic. However, it still leads to better cell survival than bioinks that use photoinitiators, so it can be a way to extend the lifetime of a bioprinted construct, allowing for longer studies.

Stereolithography, like the other types of bioprinting, has some unique advantages and disadvantages associated with it. As previously discussed, the fact that stereolithography uses no nozzle eliminates any limits on bioink viscosity, because there is no shear stress associated with extrusion that the other types of bioprinting have. It can also be faster than other methods because the laser only needs to trace a path through the ink, instead of having a robotic arm lay down the ink droplet by droplet, layer by layer. Some disadvantages of stereolithography also involve limitations. To print something in a stereolithograph, the bioink needs to contain a compound that hardens when exposed to light. Combined with the need to make the construct not cytotoxic, there is a small range of bioinks that are useful, that is, small in relation to the bioinks available in other types of bioprinting. Also, the use of UV and near-UV light in stereolithography can cause damage to DNA if the bioink is subject to prolonged exposure to the light. However, alternatives such as the photoinitiator-free bioinks or visible light photoinitiators can be adequate substitutes. Another benefit of stereolithography compared to the other types of bioprinting is its relatively cheaper price [14]. This lower price allows for it to be implemented for different uses. Stereolithography has many possible applications in the

medical field. It can be used to create tissues for use in surgeries or other medical procedures. It can also be used to build microenvironments that simulate these organs, for use in *in vitro* studies such as drug testing and disease modelling. These simulated cellular environments have been used extensively in the field of cancer research.

CELL ENVIRONMENT RECREATION IN BREAST CANCER RESEARCH

One area of research that has benefited greatly from bioprinting and stereolithography techniques is in breast cancer research. Breast cancer is a heterogeneous disease responsible for the highest cancer-related mortality rates in women. In many cases, death results from distant metastases to other organs and tissues, with bone being one of the most common and prominent sites of occurrence [15]. Metastasis is the development of the cancer at a distance from the primary site and this is often referred to as metastatic breast cancer or stage IV breast cancer [16]. Metastasis is one of the deadliest consequences of breast cancer, with bone being one of the primary sites of occurrence and insufficient 3D biomimetic models currently exist to replicate this process in vitro. In vitro refers to the technique of performing a given procedure in a controlled environment outside of a living organism, while in vivo is inside of a living organism. Metastasis has proven difficult to study in individual patients, and thus, much work has focused on developing experimental systems to model this process in vitro [15].

Although two-dimensional culture models have been widely employed to understand breast cancer microenvironments over the past several decades, the two-dimensional models still exhibit limited success. Strong evidence from studies conducted by Frontiers in Bioengineering and Biotechnology as well as Applied Materials and Interfaces support that three dimensional, physiologically relevant culture models are required to better understand cancer progression and develop more effective treatment [17]. A three-dimensional microenvironment provides a physical barrier to processes such as spreading, proliferation, invasion, and migration that is not outstandingly present during culture on two dimensional surfaces [17]. This is important because it allows for more physiologically relevant models of the cell to be studied.

Microenvironments

Frontiers in Bioengineering and Biotechnology published a review article in 2018 that summarizes how cancer microenvironments are defined and what emerging technologies can be utilized to better mimic native-like breast cancer microenvironments. To most effectively understand how bioprinting is useful in recreating an accurate representation of cells affected by breast cancer an understanding of the microenvironment is needed first. The breast cancer microenvironment is a combination of cells within the tumor and its stroma, extracellular matrix, and surrounding signaling molecules. A tumor's stroma is defined as the supportive tissue and associated blood vessels composed of tissue-derived stem cells, adipose tissue, endothelial cells, and fibroblasts. The role for the stroma in cancer is undisputed and the stroma is demonstrated to have tumor-promoting qualities [17]. Adipose tissue, or fat, is an anatomical term for loose connective tissue. Its main role is to store energy in the form of fat, although it also cushions and insulates the body. Endothelial cells refer to cells that line the interior surface of blood vessels and lymphatic vessels, forming an interface between circulating blood and the rest of the vessel wall. Fibroblasts are cells in connective tissue which produce collagen and other fibers [17]. Ultimately, the ability to design and reengineer the tumor matrix allows the evaluation of the individual contributions of tumor-associated extracellular matrices while providing a platform to identify and test anti-cancer therapeutic strategies by accurately modeling extracellular matrix proteins.

With new technologies emerging in the field of tissue engineering, there are many different approaches in bioprinting models to better mimic native-like breast cancer microenvironments that were discussed in the review by Frontiers in Bioengineering and Biotechnology. The first bioprinted model is the natural matrix which is primarily composed of naturally derived extracellular matrix proteins. This model accurately mimics adhesion properties, variable stiffness, and secreted extracellular matrices found in vivo of actual patients. However, each model does have disadvantages and one issue with the natural matrices is batch-to-batch variability of the materials which can make drawing general conclusions difficult. Other microenvironment models being developed are synthetic matrices and composite matrices. As the name implies with synthetic matrices it is composed of synthetic polymers and the composite matrices are a combination of both synthetic and natural materials. Both models are much more tunable than the natural matrices and produce and allow for more factors in the cell to be altered and controlled based on the type of experiment that is being performed. A downside of these models is cytotoxicity, essentially poisoning other natural cells, which adds another variable to the study of the microenvironment that potentially could interfere with medicines being tested [17].

Looking past the types of models it is also important to consider the method of bioprinting that is best suited for the recreation of breast cancer microenvironments. In general, there is no best bioprinting technology, but choosing a desirable method depends on the limitation of the system and available biomaterials. Due to their complex organization of cells and varying permeability, diffusion barriers for mass transport and drug delivery varies. Thus, careful design of the architecture is required to accurately model breast cancer extracellular matrices. Of the three-

dimensional bioprinting methods, extrusion based bioprinting shows the most potential for precise architecture control [17].

Case Study: Metastasis Studies

ACS Applied Materials and Interfaces conducted a study in 2016 on bioprinting a cell-laden bone matrix for breast cancer metastasis. The goal of this study was to observe if their created microenvironment provided a suitable model with which to study the interactive effects of cells in the context of an artificial bone microenvironment and serve as a valuable tool for the investigation of post-metastatic breast cancer progression in bone. In their experiment they used tabletop stereolithography equipped with three-dimensional printer interface software to test a variety of bioink solutions. The bioink solutions were composed of gelatin methacrylate (GelMA) and nanocrystalline hydroxyapatite (nHA) to mimic bone tissue [15]. The criteria developed to measure the effectiveness of their bioprinted models was if osteoblast proliferation was inhibited, breast cancer cell growth was stimulated after co-culture, and if co-cultured cells also increased vascular endothelial growth factor secretion of breast cancer cells and decreased activity of osteoblasts [15]. These criteria were chosen because they were deemed the most important aspects in mimicking a real case of breast cancer metastasis. Osteoblasts are cells that secrete the matrix for bone formation and are an important consideration when analyzing the effectiveness of a culture of three-dimensional printed cells.

To evaluate the viability of osteoblasts embedded in three-dimensional bone matrix after bioprinting, four cell-laden samples were used, and breast cancer cells were seeded on the surface of the matrix [15]. The proliferation of co-cultured cells was then compared with those of corresponding mono-cultured cells which were used as a control. What was found was that the channels and pores in the bioprinted matrices were clear and well-defined and the addition of nHA did not alter the hydrogel function. So, the results found from the synthesis and characterization of GelMA were a success in creating an accurate environment. The next way they measured the effectiveness of the bioprinted model was measuring if there was an increase in vascular endothelial growth factor secretion of breast cancer cells. The conclusions they found were that compared to the mono-culture control, vascular endothelial growth levels of the co-cultured groups increased by 42% and 37% after two weeks. Also, vascular endothelial growth expression was increased when breast cancer cells were co-cultured with osteoblasts. After two weeks, the growth levels were 67% and 66% higher than breast cancer cells plus osteoblasts in monoculture for the bioprinted models [15]. This result exactly aligns with the desired outcome of increasing the vascular endothelial growth levels in the bioprinted models.

In this study, a GelMA bioink, containing nHA and cells, was successfully prepared for the bioprinting of cell-laden bone matrices. Osteoblasts spread much more quickly in the low GelMA concentration hydrogels in vitro. When osteoblasts were co-cultured with breast cancer cells, the osteoblast proliferation was slowed, and breast cancer cell growth was increased after co-culture. Meanwhile, co-cultured cells also increased vascular endothelial growth secretion of breast cancer cells. These phenomena demonstrated that the three-dimensional matrices provided an appropriate microenvironment. Matrices of 10% GelMA + nHA and 15% GelMA + nHA were found to be the most suitable for studying the interactions of osteoblasts and breast cancer cells in vitro [15]. From these results, the bioprinting of cell-laden bone matrices can be a valuable tool for investigating breast cancer bone invasion and metastasis.

Case Study: Drug Resistance

In addition to studying the mechanics and biological processes of breast cancer, researchers can also use three-dimensional models to study drug-resistant properties. A team of researchers, led by Ying Wang, did this exact study. In the breast cancer microenvironment, adipose-derived mesenchymal stem cells (ADMSC) play a major role in promoting cancer progression and metastasis. Wang's team conducted a study to test the effectiveness of ADMSC on the drug resistance of breast cancer, using a 3D construct to conduct a study that more accurately simulated a real breast cancer tumor.

Cancer drug resistance is a large obstacle that prevents chemotherapies from being effective. Several studies that Wang's team looked at demonstrated that, when exposed to common drugs, ADMSC can secrete fatty acids and IL-6 and IL-8 cytokines, all of which protect tumor cells from chemotherapy [18]. In response to this, his team chose to create several cancer constructs, each with different amounts and layouts of tumor cells and ADMSC. They used an extrusion bioprinter to lay down several disc constructs, each with a circle of 21PT hydrogel (which contained breast cancer tumor cells) surrounded by a ring of ADMSC. Some of these discs then had varying thickness of ADMSC added as to and bottom layers, and lone breast cancer cells were used as a control. The team then incubated each construct in a solution containing doxorubicin (DOX), a common chemotherapy drug. They used different concentrations of DOX to test how that affected the survivability of the cells. Additionally, the team tested the effects of a LOX inhibitor. LOX is an amine oxidase secreted by cancer cells that plays a role in breast cancer migration and metastasis [18]. Wang's team also wished to study the effects of LOX on the drug resistant properties of breast cancer, which was previously unknown. Therefore, they applied a LOX inhibitor to another set of bioprinted constructs to see if that changed the overall cell death.

The team's results show several interesting factors. First, they looked at how the ADMSC and different

concentrations of DOX affected cell survivability. Their results are shown below, in Figure 2.

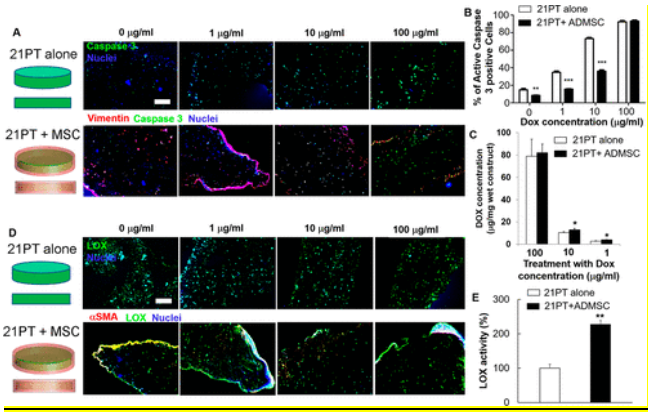

FIGURE 2 [18]
Apoptosis and LOX expression in response to various DOX doses

Part A of Figure 2 shows the staining of cleaved Caspace-3, which marks cells that have been killed. Part D shows the staining of LOX, which was released by the cells both in and out of the presence of DOX. Looking at Part B, which is a graph of the concentration of DOX after incubation, shows that with the ADMSC, a lower percentage of cells exhibited cleaved Caspace-3 at low doses of DOX [18]. When the DOX was available in high concentration, it penetrated the ADMSC and still was able to kill almost the same amount of cancer cells.

Next, the team looked at how different thicknesses of ADMSC affected the effectiveness of DOX. As seen in part B of Figure 3 (below), the thicker layers of ADMSC increased the number of living cells present.

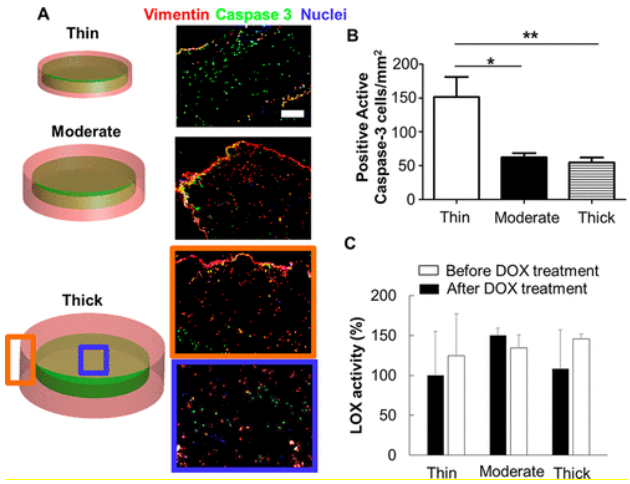

FIGURE 3 [18]
Effect of ADMSC layer thickness on DOX response

Another interesting find is that LOX secretion experienced no significant change, despite the different thicknesses of ADMSC.

Finally, the team added a LOX inhibitor to the constructs. The inhibitor had several effects, including decreasing certain gene expression, and lowering the stiffness of the bioprinted construct by weakening the intracellular bonds between the cells. Graphs of these effects can be seen in Figure 4 below.

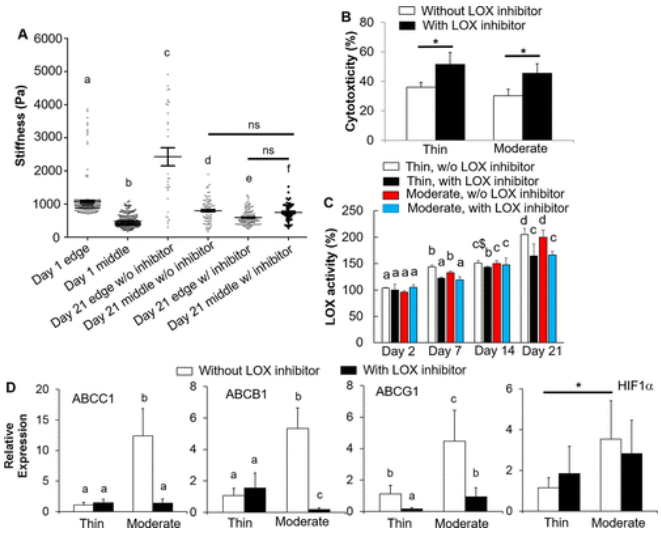

FIGURE 4 [18]
Effects of LOX inhibitor on bioprinted construct stiffness and toxicity

An important effect of the LOX inhibitor is that it increases the cytotoxicity of the microenvironment. Because it prevents LOX from being produced, which helps neutralize DOX, the inhibitor therefore increases the potency of the DOX toxin.

Overall, this study showed that ADMSC generally increases the drug resistant properties of breast cancer tumors, and LOX inhibitors can be used to help weaken breast cancer tumors. While this study could have been conducted in a two-dimensional environment, the use of bioprinting enabled Wang's team to study the effects of ADMSC and DOX in an environment very similar to a real breast cancer tumor in a person. Because this study showed these effects in an accurate manner, scientists can use the results to develop new methods of treatment. Specific to this study, drugs or some technology could be created that targets ADMSC, because reducing ADMSC allows the tumor to be affected by standard chemotherapies. These drugs could also incorporate LOX inhibitors, which were shown to weaken tumors.

Impact and Importance

These case studies show that bioprinting has the potential to revolutionize the field of cancer research. Earlier,

it was discussed that bioprinting can be difficult from an economic perspective, because the bioinks need to be sturdy and biocompatible. Both studies did successfully model breast cancer; the mechanics of metastasis and properties of drug resistance, respectively. It would not be economically feasible to do these studies repeatedly, or print more of the tumor models, because each model used complicated organic and inorganic compounds, and the process of 3D printing all the different variations does take up time and money on its own. However, the results of the studies help to justify, if not fully justify, the cost put into the studies. The experiments demonstrate that bioprinted, three-dimensional constructs serve as accurate models for cellular microenvironments, and can allow the scientific community to uncover new knowledge about how cancer works and how to treat it. With this knowledge, scientists can then develop better treatments that can target different parts or developmental stages of tumors, which can increase overall survivability and quality of life for individuals with cancer.

CLOSING REMARKS

Three dimensional bioprinting is conquering the medical research field. A recent breakthrough in the market, stereolithography, is simplifying the bioprinting industry. With the advanced technologies that it offers, such as more precise printings with faster production times [19], engineers can recreate the cell environments that make up the human body to study the infection, expansion, and remission of common diseases. With these environments, they can also test how the diseases respond to medications and other treatments. It is currently being implemented for cancer research and the outcome can potentially be lifesaving. A long-term possibility of this research could be the discovery of the cure for cancer. Soon enough, advances in the process and its technologies will allow medical experts to better understand how cancer spreads and progresses, as well as how to prevent and/or treat it.

Outside of the use for cancer research, bioprinting has a variety of other possibilities too. In the future, fully functioning organs can be created for implantation in human patients. With the ability to 3D print not only tissues, but also organs that function just as naturally as grown ones do, reliance on organ donation would decrease substantially. The countless number of lives that could be saved in one day alone using this technology render it invaluable. Furthermore, stereolithography could be implemented in regenerative medicine. Engineers can generate live tissue environments that can then be inserted into human patients where they will behave as part of a live tissue. If done effectively, the number of full organs needed could be diminished.

The cancer research market just got a new method of researching, which could potentially transform the industry. Before the creation of 3D bioprinting, the cure for cancer may have seemed like an unachievable goal. However, with the efforts of engineers, researchers, and doctors alike, that once unattainable dream may soon become reality.

SOURCES

[1] "All Cancers." International Agency for Research on Cancer. January 2019. Accessed 02.07.2019. http://gco.iarc.fr/today/data/factsheets/cancers/39-All-cancers-fact-sheet.pdf

[2] "Cancer Statistics." National Cancer Institute. 04.27.2018. Accessed 02.27.2019. https://www.cancer.gov/about-cancer/understanding/statistics

[3] R. Cagan, P. Meyer. "Rethinking Cancer: Current Challenges and Opportunities in Cancer Research." Disease Models & Mechanisms. 04.01.2017. Accessed 02.27.2019. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5399574/

[4] S. Vijayavenkataraman, W. Yan, W. Feng. "3D Bioprinting of Tissues and Organs for Regenerative Medicine." Advanced Drug Delivery Reviews. 07.04.2018. Accessed 01.16.2019. https://www.sciencedirect.com/science/article/pii/S0169409X18301686

[5] "NIH Sustainability Mission." National Institutes of Health. Accessed 03.26.2019. https://nems.nih.gov/Sustainability/Pages/sustainability.aspx

[6] R. Hajar. "Animal Testing and Medicine." Heart Views. 03.2011. Accessed 03.26.2019. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3123518/

[7] C. Chua, W. Yeong, J. An. "Special Issue: 3D Printing for Biomedical Engineering." Materials. 02.28.2017. Accessed 02.06.2019. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5503334/

[8] J. Prince. "3D Printing: An Industrial Revolution." Journal of Electronic Resources in Medical Libraries. Accessed 03.05.2019. https://www.tandfonline.com/doi/citedby/10.1080/15424065.2014.877247?scroll=top&needAccess=true

[9] G. Qi, H. Jie, L. YangJie, et al. "Three-Dimensional Bio-Printing." Science China. May 2015. Accessed 03.06.2019. https://link.springer.com/content/pdf/10.1007%2Fs11427-015-4850-3.pdf

[10] W. Jamróz, J. Szafraniec, et al. "3D Printing in Pharmaceutical and Medical Applications – Recent Achievements and Challenges." Pharmaceutical Research. 07.11.2018. Accessed 01.17.2019. https://link.springer.com/content/pdf/10.1007%2Fs11095-018-2454-x.pdf

[11] Ž. Kačarević, P. Rider, S. Alkildani, et al. "An Introduction to 3D Bioprinting: Possibilities, Challenges and Future Aspects." Materials. 11.06.2018. Accessed 02.13.2019. https://www.mdpi.com/1996-1944/11/11/2199/htm

[12] E. Bishop, S. Mostafa, M. Pakvasa, et al. "3-D Bioprinting Technologies in Tissue Engineering and

Regenerative Medicine: Current and Future Trends." Genes & Diseases. 12.2017. Accessed 03.22.2019. https://www.sciencedirect.com/science/article/pii/S2352304217300673

[13] H. Kang, S. Lee, I. Ko, et al. "A 3D Bioprinting System to Produce Human-Scale Tissue Constructs with Structural Integrity." Nature Biotechnology. 02.15.2016. Accessed 03.22.2019. https://www.nature.com/articles/nbt.3413

[14] Z. Wang, R. Abdulla, B. Parker et al. "A Simple and High-Resolution Stereolithography-Based 3D Bioprinting System Using Visible Light Crosslinkable Bioinks." Biofabrication. 12.22.2015. Accessed 03.26.2019. https://iopscience.iop.org/article/10.1088/1758-5090/7/4/045009/meta#bfaa0bfdbib26

[15] X. Zhou, W. Zhu, M. Nowicki et al. "3D Bioprinting a Cell-Laden Bone Matrix for Breast Cancer Metastasis Study." ACS Applied Material Interfaces. 10.21.2016. Accessed 1.17.2019. https://pubs.acs.org/doi/10.1021/acsami.6b10673

[16] "Metastatic Breast Cancer." breastcancer.org. 10.16.2018. Accessed 03.01.2019. https://www.breastcancer.org/symptoms/types/recur_metast

[17] J. Belgodere, C. King. "Engineering Breast Cancer Microenvironments and 3D Bioprinting." Frontiers in Bioengineering and Biotechnology. 05.24.2018. Accessed 01.18.2019. https://www.frontiersin.org/articles/10.3389/fbioe.2018.00066/full

[18] Y. Wang, W. Shi, M. Kuss, et al. "3D Bioprinting of Breast Cancer Models for Drug Resistance Study." ACS Biomaterials Science and Engineering. 11.15.2018. Accessed 01.18.2019. https://pubs.acs.org/doi/10.1021/acsbiomaterials.8b01277

[19] C. Kengla, A. Kidiyoor, S. Murphy. "Bioprinting Complex 3D Tissue and Organs." Kidney Transplantation, Bioengineering and Regeneration. 2017. Accessed 03.06.2019. https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/stereolithography

ADDITIONAL SOURCES

E. Petcu. "3D Bio-Printing: An Introduction to a New Approach for Cancer Patients at the Interface of Art and Medicine." University School of Medicine Gold Coast Campus. 03.26.2016. Accessed 02.07.2018. http://web.b.ebscohost.com/ehost/pdfviewer/pdfviewer?vid=12&sid=753c2d04-032b-4bcb-abb4-b5c325406d22%40sessionmgr104

M. Crawford. "Creating Valve Tissue Using 3D Bioprinting." The American Society of Mechanical Engineers. 05.2013. Accessed 02.05.2019. https://www.asme.org/engineering-topics/articles/bioengineering/creating-valve-tissue-using-3d-bioprinting

R. Suntornnond, J. An, C. Chua. "Bioprinting of Thermoresponsive Hydrogels for Next Generation Tissue Engineering: A Review." Macromolecular Materials and Engineering. 10.12.2016. Accessed 01.16.2018. https://onlinelibrary.wiley.com/doi/full/10.1002/mame.201600266

Thayer. S., Orrhult. S., Martínez. H. "Bioprinting of Cartilage and Skin Tissue Analogs Utilizing a Novel Passive Mixing Unit Technique for Bioink Precellularization." Chalmers University of Technology. 04.13.2018. Accessed 02.17.2019. https://www.jove.com/video/56372/bioprinting-cartilage-skin-tissue-analogs-utilizing-novel-passive

ACKNOWLEDGMENTS

We would like to thank our writing instructor, Rachel McTernan, whose feedback helped us change and edit our paper as needed. We would also like to thank Sreyas Ravi, who sent us several useful resources on bioprinting. We would finally like to thank our co-chair, Nolan Smith, who guided us throughout the process and proofread our paper before final submission.