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3D Bioprinting: Manufacturing the Human Heart

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ABSTRACT

Every day, the organ shortage crisis takes away the lives of 17 patients (Health Resources & Services Administration, 2022). The number of patients who require organ transplants significantly exceeds the number of possible donors, and consequently, most patients pass away while waiting for a matching donor organ. To address this issue, 3D bioprinting was suggested as a method by which patients could receive a functioning replica of their own biocompatible organ. This manuscript will cover in depth the materials—the bioink—and the printing methods that are widely used by various researchers in the bioprinting field. More specifically, this manuscript will analyze and compare natural bioinks—alginate and collagen—and synthetic bioinks—Gelatin methacryloyl and polycaprolactone. It will then introduce the two major bioprinting methods—extrusion-based printing and inkjet-based printing—and analyze the advantages and disadvantages of each method. Finally, the manuscript will highlight the successful applications of 3D bioprinting in cardiology using the aforementioned bioinks and printing methods.

Introduction

The last resort for most organ dysfunction or failure is organ transplantation. To this end, though it entails high risks and unpredictable outcomes, in the case of success, the procedure is by far the most effective approach to acquiring a functional body system (Kotton, et al., 2015; Beyar, 2011). Organ transplantation is one of the revolutionary fields in medicine and since the first successful human kidney transplantation, in 1954, the field remains a priority and the center of attention in the world of medicine (Kupiec-Weglinski, 2022). However, the disparity between demand and supply remains an issue for the domain. Despite the number of transplants doubling during the recent three decades, the number of patients on the transplant waiting lists has surged by 600%. According to UNOS, in 2021, despite a total of 41,354 transplant operations taking place in the U.S., 6,564 patients on the waiting list passed away, and the other 116,566 patients on the list continued to await in their critical conditions (Kupiec-Weglinski, 2022).

In response to this shortage of organs, researchers have been exploring various methods since the 1900s. One potential solution, xenotransplantation, or the act of transplanting an organ of another species, is under development to produce a viable alternative for human organs (Reardon, 2022). The recent pig-to-human heart transplant has brought this branch of organ transplantation to the spotlight. Although the patient died two months after the operation, the surgery was a groundbreaking advancement in consideration of the fact that it was the first pig-to-human heart transplant, with the 57-year-old patient having shortly survived without cardiopulmonary bypass assistance (Wang, 2022).

Experts suspect that pigs are the suitable donor species for xenotransplantation to humans due to their organs' anatomical similarity to humans as well as high availability from a sizable litter size and a rapid growth rate (Wang et al., 2022). In addition, current technological tools, such as engineered nucleases, TALEN, and CRISPR-Cas9, allow for the genetic modification of the species, a key aspect in reducing immune rejection (Wang et al., 2022). However, there is a wide range of complications involved in xenotransplantation, including transplant rejection, where the recipient's immune system attacks the organ, and xenozoonosis where an animal-

based disease is transmitted from the donor to the recipient (Ekser & Cooper, 2010). In the case of the pig heart transplantation, the pig genome was manipulated in such a way, xenoantigens such as α-1,3-galactosidase and other genes associated with immune responses against the organ, including cytidine monophosphate-*N* -acetylneuraminic acid hydroxylase (CMAH) and β-1,4-N-Acetyl-Galactosaminyltransferase 2 (B4GalNT2) were deleted (Wang et al., 2022). The removal of these specific genes has not adversely affected the organism's health and was projected to minimize rejection from the human host (Wang et al., 2022).

However, host rejection has yet to be completely eliminated for a truly successful case of xeno-transplantation, and immunosuppressive therapy remains underdeveloped in the field (Wang et al., 2022). Aside from the fact that xenotransplantation is still under development, there are critical ethical issues involved. Most importantly, protestors of xenotransplantation condemn that the source of the organs is animals, often genetically engineered and kept in laboratory conditions away from their natural settings (Rollin, 2020).

An alternative to autotransplantation is organs generated from stem cells. One key aspect of stem cells, specifically embryonic stem cells, a type of stem cell, is that they are pluripotent, or that they can develop into any cell in the human body, including cells in the ectoderm, endoderm, and mesoderm layers (Romito & Cobellis, 2016). In the past, the source of stem cells, embryonic and fetal stem cells, caused controversy over its usage and hindered research and advancement in the field of biotechnology (Weiss & Troyer, 2006). Fortunately, an alternative source of stem cells was discovered: umbilical cords (Weiss & Troyer, 2006). Multipotent stem cells can be retrieved from this structure post-birth without harming the mother and the newborn's baby.

Another source of stem cells, multipotent stem cells, despite their limited outcome range in comparison to pluripotent stem cells, could transform into a variety of cell types. However, the insufficient information regarding umbilical cord blood donation has led to its decline in countries like Brazil (Debiazi Zomer et al., 2021). In recent years, stem cells derived from umbilical cord blood have provided solutions to bone marrow disorders and inborn errors of metabolism (Weiss & Troyer, 2006). Theoretically, stem cells have the potential to generate an organ (Cascalho & Platt, 2006). However, although the idea of employing clones to evade attacks from the host immune system is appealing, there is currently limited knowledge of the fetal microenvironment that is required to grow organs (Cascalho & Platt, 2006).

3D Bioprinting

To circumvent the limitations mentioned in the introduction, 3D bioprinting was proposed. A key aspect of 3D bioprinting that differentiates it from other methods is its accuracy, as it allows for full artificial control in its formation. 3D Bioprinting employs the conventional 3D printing method of layering—arranging the bioink, the core material, in appropriate patterns to mimic actual tissues and organs. Bioinks can be found in various types, ranging from natural biomaterial-based bioinks, synthetic-biomaterials-based bioinks, cell aggregate/pellet-based bioinks, commercial bioinks—such as Derma-matrix and Novogel—to composite bioinks, or bioinks with bioactive molecules (Gungor-Ozkerim et al., 2018).

In addition to a wide array of ink sources, there is also a wide range of different printing techniques available: current technologies included fused deposition modeling (FDM), direct ink writing (DIW), inkjet bioprinting, selective laser sintering (SLS), stereolithography (SLA), and laser-induced forward transfer (LIFT), with DIW and inkjet bioprinting being the more commonly employed method in cell printing (Gopinathan & Noh, 2018). Two main approaches to 3D bioprinting organs are the cell-scaffold-based method and the scaffold-free cell-based approach (Gopinathan & Noh, 2018). The former uses biomaterial to print 3D structures, on which live cells can grow to replace the artificial mold. In other words, the biomaterial will simply serve as the skeletal guideline for cell growth before it biodegrades (Gopinathan & Noh, 2018). The material must be biocompatible as well as cytocompatible with the cells (Gopinathan & Noh, 2018). The scaffold-free cell-based approach utilizes the direct printing of cells that closely follow conventional embryonic development (Gopina-

than & Noh, 2018). When selecting bioinks, printability, biocompatibility with live cells, In situ gelation, viscoelasticity, tissue regeneration, biodegradation, the permeability of essential nutrients and waste, and shear thinning are all crucial factors to consider (Gopinathan & Noh, 2018). While natural bioinks can improve biocompatibility and biodegradation properties, synthetic ones can increase precision and control over variables (Gopinathan & Noh, 2018).

Bioink

Natural Bioink: Alginate and Collagen

Currently one of the most popular natural bioink options on the market is alginate, a polysaccharide extracted from bacteria and brown algae (Shah et al., 2020). Alginate is composed of β-D-mannuronic acid M units and α-L-guluronic acid G units, and a high G unit concentration can enhance the stiffness of the material (Shah et al., 2020; Kim et al., 2016). In 3D printing, the material can create an independent, free-standing scaffold or use a crosslinker pool for support (Ozbolat & Hospodiuk, 2016). Although alginate lacks cell adhesion sites, the bioink's cell viability can be improved through the use of an adhesive ligand, type I collagen, or oxygenation (Caliari & Burdick, 2016; Kim et al., 2016; Ozbolat & Hospodiuk, 2016).

Some of alginate's characteristics that enable the material to be a suitable candidate for organ printing are its affordability, dissolution and gelation properties, and fitness under physiological conditions of neutral pH and 37 degree Celsius (Caliari & Burdick, 2016; Ozbolat & Hospodiuk, 2016; Aarstad et al., 2017). However, the extreme hydrophilic properties of alginate may lead to the alginate matrix trapping the cells instead of degrading through time (Caliari & Burdick, 2016; Ozbolat & Hospodiuk, 2016). Therefore, although it offers excellent mechanical properties, a high content of alginate is not compatible with the growth and proliferation of live cells (Ozbolat & Hospodiuk, 2016). The results of a 2016 study—Applications of Alginate-Based Bioinks in 3D Bioprinting—revealed alginate's excellent biocompatibility and printability. The study also demonstrated that alginate's low cellular adhesion and slow degradation properties could be addressed through the use of Arg-Gly-Asp adhesion peptides for adhesion and the addition of oxidized alginate and sodium citrate for degradation (Axpe & Oyen, 2016).

Another commonly used natural bioink is collagen hydrogel, often composed of type I collagen, a protein type that accounts for 90 percent mass of connective tissues in mammals (Gelse, 2003). Type I collagen rearranges its molecular structure into fibrils, creating the collagen hydrogel, and a greater speed in this reorganization process facilitates a higher printing accuracy (Olegovich Osidak et al., 2020). As the abundance of the material in the body indicates, collagen is highly biocompatible and can be easily accessed compared to other materials like decellularized extracellular matrix (Sánchez-Cid et al., 2022; Wang et al., 2021). In addition, collagen's biocompatibility allows for cell adhesion and differentiation (Wang et al., 2021). On the other hand, collagen is often used in only small concentrations, only up to 10 mg/ml, due to its poor mechanical properties from a low Young's modulus and viscosity (Yoon et al., 2016; Izadifar et al., 2018). According to Diamantides et al., the most appropriate way to address this issue is to increase the storage modulus of the bioink before printing for extrusion-based bioprinting (Diamantides et al., 2017).

In a 2022 study by Pablo Sánchez-Cid et al, the team combined collagen with chitosan to improve collagen's low mechanical properties, poor thermal stability, and rapid degradation rate (Sánchez-Cid et al., 2022). Chitosan was chosen due to its high biocompatibility, biodegradability, and low toxicity, as well as other properties that made the material a suitable candidate for the hydrogel (Sánchez-Cid et al., 2022). Most importantly, the addition of chitosan helps create covalent bonds in the hydrogel that increases the mechanical properties and stability of the hydrogel (Sánchez-Cid et al., 2022). The study concluded that the combination

of collagen and chitosan can potentially be applied to scaffolds in 3D bioprinting, as the mixture retained the excellent biocompatibility of collagen and high stability of chitosan (Sánchez-Cid et al., 2022).

Synthetic Bioink: Gelatin Methacryloyl (GelMA) and Polycaprolactone (PCL)

Gelatin methacryloyl (GelMA) is a widely used synthetic bioink in the tissue engineering field. Currently, GelMA has been successfully applied in the printing of the meniscus, skin, and bone (Bahcecioglu et al., 2019; Eke et al., 2017; Celikkin et al., 2017). The natural, unmodified form of GelMA is the standard gelatin, which is not only cost-effective and easily accessible but also non-antigenic and suited to cell adhesion with the help of peptide motifs like arginine-glycine-aspartic acid, RGD (Van Den Bulcke et al., 2000). Due to GelMA's similarity to natural extracellular matrices (ECM), biocompatibility, and durability, hydrogels that contain gelatin are ideal for tissue engineering (Demirci et al., 2016).

The crosslinking of gelatin through various chemicals—such as glutaraldehyde, carbodiimide, and N-Hydroxysuccinimide—prevents the addition of cells to the gel during its formation, results in a long production time, and encourages the use of hazardous chemicals (Kilic Bektas & Hasirci, 2020). On the other hand, photocrosslinking with ultraviolet radiation or visible light allows for speed and more control, in addition to the ability to load cells during gel formation (Kilic Bektas & Hasirci, 2020). Gelatin can be transformed into its photocrosslinkable form, GelMA, through methacrylation (Kilic Bektas & Hasirci, 2020). A 2019 study by researchers Cemile Kilic Bektas and Vasif Hasirci demonstrated that during its application in corneal stroma engineering, GelMA hydrogels demonstrated high stability, biocompatibility, and transparency (Kilic Bektas & Hasirci, 2020).

GelMA hydrogel can be molded into a wide range of forms—micro/nanospheres, micro/nanofibers, micro/nanofibers—and used to create 3D tissue scaffolds (Piao et al., 2021). However, the low viscosity of GelMA leads to low printability (Piao et al., 2021). Still, low temperatures and the addition of modifiers that can increase viscosity, including alginate, can alleviate this issue (Piao et al., 2021). In addition, due to the high degradability of the material, GelMA has to be used in conjunction with less degradable materials for long-term application (Piao et al., 2021). For tissue engineering, rigid structures, including bone regeneration grafts, the relatively weak GelMA hydrogel has to be supported by more durable materials like polylactic acid (Piao et al., 2021).

Polycaprolactone, or PCL, is another synthetic bioink prevalent in the 3D bioprinting field which was counterintuitively chosen due to its slow biodegradation rate (Ghorbani et al., 2017). It was this property that makes PCL a suitable candidate for preventing the softening or collapse of a printed tissue or organ (Ghorbani et al., 2017). In addition, the material has been authorized to use in the human body by the United States Food and Drug Administration (Manoukian et al., 2017). PCL is appropriate for the production of firm yet flexible structures like the trachea (Gao et al., 2017; Townsend et al., 2018). One drawback of PCL is its hydrophobic property, which decreases the material's compatibility with live cells (Xu et al., 2019). Still, the addition of a hydrophilic substance, like collagen can help reduce its hydrophobicity (Xu et al., 2019). In the production of a tracheal replacement, the combination of PCL and collagen was able to offset the lack of biocompatibility of PCL as well as the rapid biodegradability of the collagen (She et al., 2021). As a result, the printed scaffold was able to feature both biocompatibility and stability (She et al., 2021).

Bioprinting Method

Extrusion-Based Printing

One of the most widely used 3D printing methods is extrusion-based printing. In this method, a pneumatic actuator, a piston, or a screw extrudes the bioink from a nozzle (Derakhshanfar et al., 2018). In pneumatic-driven based 3D printing, air pressure is the driving force, in piston-driven based printing, it is mechanical force, and in screw-driven based printing, it is the rotation of a screw (Derakhshanfar et al., 2018). In extrusion-

based printing, the dispersion and position of the bioink are controlled by actuators, which are responsible for guiding the motion of the nozzle based on the instructions from a control signal (Placone & Engler, 2017).

To create a design of the three-dimensional model, the exact guidelines for the structure must be defined using a computer-aided design (CAD) software, and the design must then be divided into flat, printable layers that can be stacked on top of one another using a slicing program (Placone & Engler, 2017). The spacing between the layers must be carefully chosen in order to find a balance between the risks of delamination and the separation of layers (Placone & Engler, 2017). For extrusion-based printing, the bioink must be modified to be compatible with the method, and the size and temperature of the nozzle and printing rate must be calibrated to complement the bioink's characteristics (Placone & Engler, 2017).

The strengths of extrusion-based printing are its precision and high controllability through the CAD software, which allows for the production of highly intricate designs (Guo et al., 2017). Most importantly, the layer-by-layer design provides high structural integrity, and there is a wide range of solidification methods, including pH and temperature change and photocrosslinking, for the printed structure (Landers et al., 2002; Fedorovich et al., 2009). In addition, extrusion-based printing is affordable and has a relatively rapid production rate (Placone & Engler, 2017; Guo et al., 2017).

Nevertheless, there are multiple drawbacks to extrusion-based printing that must be properly addressed for desired results. First, as the bioink is extruded by an external force, the cells may become more vulnerable to destruction (Placone & Engler, 2017). As a result, the printing process must achieve the perfect balance between speed—to limit the cells' exposure to harsh conditions, such as ultraviolet radiation—and precision—to prevent cell destruction (Blaeser et al., 2015). Second, the printed material may experience limitations in nutrient and waste exchange. Therefore, fluid dynamics must be taken into consideration in the design of the CAD models, and external structural components, such as channels and microvasculature, can be added to support diffusion (Placone & Engler, 2017). Regardless of the advantages and disadvantages of extrusion-based printing, the choice of bioink is the determining factor in achieving the desired scaffold design. Various characteristics like rigidity or whether the hydrogel allows for the embedding of cells should be considered in this choice.

Inkjet-Based Printing

Inkjet-based printing is another commonly used in the 3D bioprinting field. There are two main types of inkjet-based printing: continuous inkjet printing and drop-on-demand inkjet printing. The latter is more adapted to the printing of biomaterials, since continuous inkjet printing involves an inkjet circulation system that maximizes the use of the bioink but contaminates the biomaterial in the recovery process (Li et al., 2020). In contrast to continuous inkjet printing, which relies on an electric charge for the placement of the droplets, drop-on-demand inkjet printing ejects the droplet on demand through an ejection signal (Li et al., 2020). As a result, in addition to not requiring any electric charge and ink circulation systems, the droplets can be printed in an accurate and efficient manner (Li et al., 2020).

Methods of inkjet printing include thermal inkjet, piezoelectric inkjet, electrostatic inkjet, and electrohydrodynamic inkjet. Thermal inkjet printing, in which heat bubbles from the bioink push the ink out in form of droplets, is fast, cost-efficient, and convenient (Li et al., 2020). However, the small diameter of the nozzle limits the use of bioinks with high viscosity, which could lead to clogging, and increases the pressure on cells, in addition to heat (Cui et al., 2010). Piezoelectric inkjet printing uses piezoelectric actuators to eject the droplets (Li et al., 2020). While this method provides high controllability and a wide selection of nozzle diameters, it is costly and prone to cell damage from sonification (Li et al., 2020). Electrostatic inkjet printing uses an electrostatic plate to eject the droplets (Li et al., 2020). While it is not prone to cell destruction from heat or sonification, it only allows the use of a small nozzle (Li et al., 2020). Lastly, electrohydrodynamic inkjet printing uses electric voltage to eject the droplets (Li et al., 2020). This method is not a drop-on-demand inkjet printing method and therefore does not allow for the production of a single droplet at a time (Li et al., 2020). However, it offers a

high resolution and allows the use of high-viscosity ink, unlike drop-on-demand methods that are prone to clogging, and the nozzle size can be larger than the ink, which reduces pressure on cells (Li et al., 2020).

The inkjet-based printing method prints microdroplets at high speed, which is ideal for printing individual cells, while extrusion-based bioprinting prints a gel string. This makes the inkjet-based printing method, which uses an individual droplet form of the bioink instead of a continuous gel strand, produce a 3D bioprint with higher resolution and easier maneuverability (Liberski et al., 2010). As a result, the inkjet-based printing method helps synthesize not only intricate designs and complex geometries for scaffolds but also allows the placement of cells in specific locations (Liberski et al., 2010). Furthermore, one print head can print more than one type of bioink through multiple nozzles, and with such a method, Xu et al. successfully created multi-cell heterogeneous tissue (Xu et al., 2013). However, inkjet-based printing is prone to splashing, which can lower the accuracy and resolution of the process (Li et al., 2020). The splashing effect can be reduced with slower speed and the selection of a bioink with an appropriate viscosity, density, and surface tension (Li et al., 2020).

Discussion

This manuscript examined a wide range of bioinks utilized in three-dimensional bioprinting and then followed up with an analysis of the two major techniques that are widely used in the field. The conclusion after extensive comparison is that there is no single bioink and printing method appropriate for all forms of bioprinting. The following summarizes the main findings of the bioinks and printing methods.

Table 1. Advantages and Disadvantages of Bioinks

Bioinks	Advantages	Disadvantages
Alginate	• Affordability • Dissolution and gelation • Fitness under physiological conditions • Stable mechanical properties • Excellent printability	• Lacks cell adhesion sites • Extreme hydrophilic properties that could lead to the trapping of cells • High content of alginate not compatible with the growth and proliferation of live cells
Collagen	• Biocompatibility • High accessibility • Cell adhesion and differentiation • Low toxicity	• Unstable mechanical properties • Poor thermal stability • Rapid degradation rate

Gelatin methacryloyl (GelMA)	• Affordability • High accessibility • Non-antigenic • Cell adhesion • Biocompatibility	• Low viscosity • Low printability • Rapid degradation rate
Polycaprolactone (PCL)	• Slow biodegradation • Biocompatibility • Stability • Flexibility	• Hydrophobicity • Lack of biocompatibility

Table 1 compiles data from multiple studies and summarizes the various advantages and disadvantages of alginate, collagen, gelatin methacryloyl (GelMA), and polycaprolactone (PCL).

Table 2. Advantages and Disadvantages of Bioprinting Methods

Bioprinting Method	Advantages	Disadvantages
Extrusion-based Printing	• High precision • Rapid production rate • High controllability • High structural integrity • Wide range of solidification methods • Affordability	• Cells susceptible to damage during extrusion due to air pressure or mechanical force • Limitations in nutrient and waste exchange

Inkjet-based Printing	• High resolution • Easy maneuverability • Can produce intricate and complex designs • Multiple nozzles in one print head • Ideal for printing individual cells • Affordability (thermal inkjet-based printing)	• Limitation in nozzle size and bioink viscosity (thermal inkjet printing and electrostatic inkjet printing) • Cells susceptible to damage due to heat (thermal inkjet printing) or sonification (piezoelectric inkjet printing) • Splashing

Table 2 compiles data from multiple studies and summarizes the various advantages and disadvantages of extrusion-based printing and inkjet-based printing.

The first major comparison was between natural and synthetic bioinks. Naturally, the first pioneers of this novel field of 3D bioprinting turned toward using familiar materials that were already widely used in the science field. One of the natural materials they turned to was collagen. Though more commonly associated with the beauty industry, collagen is one of the most utilized natural bioink on the market. However, the material also has its drawbacks, most of which can be generalized to almost all natural bioinks. As this manuscript demonstrated, natural bioinks, including collagen, have superior biocompatibility but inferior printability, at times leading to a compromise in the whole structural integrity. Nevertheless, researchers can improve their printability through the addition of other chemicals that could enhance the structure's stability and durability. On the other hand, to demonstrate the variability in natural bioinks, this study analyzed alginate, an exception in the drawbacks of natural materials. In contrast to traditional natural bioinks, alginate demonstrated excellent printability and stable mechanical properties. However, it lacked in its compatibility with live cells.

Similarly, as polycaprolactone (PCL) demonstrated, the majority of synthetic bioinks provide excellent structural integrity but lack biocompatibility. This can lead to the conclusion that synthetic bioinks are more suitable for structures that demand strong mechanical properties, while natural bioinks are appropriate for structures that are designed to support cell growth and proliferation. Aside from alginate, another exception to this trend is gelatin methacryloyl (GelMA), which is widely used for its biocompatibility but often mixed with other materials for enhanced viscosity and printability. As this selection of bioinks demonstrates, there is no one, versatile bioink, whether natural or synthetic, that is ideal for the production of all forms of three-dimensional biological structure. Along with the consideration of affordability and accessibility, a particular blend of bioinks should be chosen, based on how well-suited their characteristics are to the final product and how effectively the separate bioinks can reduce one another's weaknesses and enhance their strengths.

In the case of comparing bioprinting methods, the inkjet-based printing method prints the bioink in the form of microdroplets, while extrusion-based bioprinting prints it in a continuous strand. The usage of individual droplets in inkjet-based printing offers a high resolution, helping create complex designs and print individual cells. In addition, inkjet-based printing can simultaneously print multiple types of bioinks. On the other hand, although it offers a lower resolution, extrusion-based printing still allows for precision through its layer-by-layer printing method and is superior in terms of price and convenience. In both types of printing, printing conditions must be regulated to minimize exposure of cells to stressful conditions, such as mechanical force, extremely high temperatures, etc.

Conclusion

Currently, 3D bioprinting can successfully print relatively simple structures. Researchers from Swansea University have developed an artificial bone matrix that can later combine with and replaced by the natural human bone tissues, and a different team from the University of Nottingham has developed a bone scaffold created from polylactic acid and alginate for adult human stem cells to grow on and replace through the degradation of the scaffold (Fu et al., 2013; Howard et al., 2008). For the skin, a research team from the Wake Forest School of Medicine has developed a printer for printing skin cells directly onto a burned area, and the company Organovo has successfully printed a multilayered skin model (Albanna et al., 2019; Retting & Nguyen, 2018). Most recently, in the summer of 2022, 3DBio Therapeutics reconstructed a patient's ear by utilizing the cartilage of the patient as the bioink for their company's bioprinter (Rabin, 2022).

On the other hand, printing internal organs that involve complex structures and mechanisms, including the heart, has seen less advancement. Still, a wide range of approaches has helped establish the groundwork for a promising future in which organ shortage is an issue of a distant past.

Eman Mirdamadi and his team used Freeform Reversible Embedding of Suspended Hydrogels (FRESH) printing, a technique in which a soft bioink like alginate was printed into a thermoreversible gelatin microparticle support bath through extrusion-based printing, to print a full-size adult human heart model (Mirdamadi et al., 2020). The FRESH method was also applicable to another soft bioink—collagen type I (Mirdamadi et al., 2020). Although the printing process has to be sped up for the cells to survive and large-scale cell culture technologies have to be improved for the embedding of large amounts of cells into the model, the study has helped achieve both the texture and size of a full-size human heart (Mirdamadi et al., 2020).

Beyond the prospect of printing a viable organ, more feasible applications of this technology include creating models for surgery planning and training. Extensions of the heart, such as primary cardiac tumors, ventricular septal defects, and the aortic arch, have already been printed to help plan surgery and train surgical residents (Schmauss et al., 2013; Costello et al., 2014; Chen et al., 2018). Through micro-computed tomography, providing cross-sectional images of the heart, the team was able to replicate the anatomical structure, down to the characteristics unique to an individual (Lee et al., 2019). Additionally, the cardiac ventricles that incorporated human embryonic stem cell-derived cardiomyocytes demonstrated synchronized contractions and wall thickening during contraction (Lee et al., 2019).

A team of researchers led by Dr. Kai Zhu printed functional cardiac tissues using a gold nanorod (GNR)-incorporated gelatin methacryloyl (GelMA)-based bioink (Zhu et al., 2017). In the development of novel pediatric neonatal human cardiac progenitor cell (hCPC)/cartilage extracellular matrix cardiac patch (cECM), GelMA allowed for the printing of hPC and cECM, and the patches did not exhibit significant degradation in vitro over 21 days, and when attached to rat hearts, these patches lasted for 14 days and showed signs of vascularization (Bejleri et al., 2018). In a study by Dr. Zhan Wang and his team, they used a supporting PCL scaffold for cardiac tissues to mature into functional cardiac muscles with synchronous contraction (Wang et al., 2018). Through inkjet-based printing, in combination with alginate, functional cardiac pseudo tissues, and through extrusion-based printing, myocardial constructs, heart valves, and blood vessels have been printed (Xu et al., 2009; Alonzo et al., 2019),

Despite these recent advancements in the field of 3D bioprinting, there exists an array of challenges and hurdles which must be overcome before a fully functional heart can be printed on a manufacturing scale. Humanity has yet to discover large-scale cell proliferation methods for cells to be embedded in the scaffolds, and the impact of different printing methods and cell culture and environment cannot be fully appreciated in the status quo. Nevertheless, the numerous breakthroughs that highlight the strengths of various bioinks—natural and synthetic—and printing methods all point toward a favorable future for widespread 3D bioprinting and ultimately organ manufacturing.

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