3D Bioprinting: From the Functional Restitution of Organs and Tissues to the Modeling of Chronic Degenerative Diseases
Bioimpressão 3D: da Restituição Funcional de Órgãos e Tecidos à Modelagem de Doenças Crônico-Degenerativas

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ABSTRACT

In order to overcome the long wait of patients in queues to receive a donated organ and the advancement of chronic degenerative diseases, regenerative medicine introduced 3D bioprinting. This study aims to expose the possibilities arising from the use of 3D bioprinting in organ transplantation and in the modeling of chronic degenerative diseases. A bibliographic review was carried out using the PubMed and SciELO databases, published between 1993 (due to its relevance) and 2023, no language restriction. By exploring the techniques, it was observed that biomodels created in vivo and in vitro, especially from induced pluripotent stem cells (iPSCs), the main target of this study, mimicked the architecture and physiology of bone, liver, heart tissue and even neural progenitor cells, which impact on the modeling of Parkinson’s and Alzheimer's diseases. Despite the existing challenges to reproducing functional organs on a clinical scale, 3D bioprinting is a promising technology that promises to revolutionize the field of regenerative medicine and resolve, in a few years, these obstacles that cause high morbidity and mortality.

Keywords: Bioprinting; Stem cells; Regenerative Medicine; Organ transplantation.
RESUMO

A fim de contornar a longa espera de pacientes em filas para receber um órgão doado e o avanço das doenças crônico-degenerativas, a medicina regenerativa introduziu a bioimpressão 3D. Este estudo tem por objetivo expor as possibilidades advindas do uso da bioimpressão 3D no transplante de órgãos e na modelagem de doenças crônico-degenerativas. Elaborou-se uma revisão bibliográfica utilizando as bases de dados PubMed e SciELO, publicadas entre 1993 (devido à sua relevância para o estudo) e 2023, sem restrição de idioma. Ao explorar as técnicas, observou-se que biomodelos criados in vivo e in vitro, sobretudo a partir de células-tronco pluripotentes induzidas (iPSCs), principal alvo deste estudo, mimetizaram a arquitetura e a fisiologia dos tecidos ósseo, hepático, cardíaco e até mesmo células progenitoras neurais, as quais impactam na modelagem das doenças de Parkinson e Alzheimer. Apesar dos desafios existentes para que se possa reproduzir órgãos funcionais em escala clínica, a bioimpressão 3D é uma tecnologia promissora que promete revolucionar o campo da medicina regenerativa e resolver, em alguns anos, esses entraves causadores de grande morbimortalidade.

Palavras-chave: Bioimpressão; Células-Tronco; Medicina Regenerativa; Transplante de Órgãos.

INTRODUCTION

The first documented transplants date back to the year 1869, when the Swiss surgeon Jaques-Louis Reverdin performed the first successful skin transplant attempt in Paris. In this process, he extracted a segment of skin from a patient’s right arm, ensuring the preservation of epithelial cells, and then used it to cover the wounded area of the patient's left arm (FARIÑA-PÉREZ, 2010). In the 19th century, there were attempts at solid organ transplants, but most procedures were unsuccessful due to immunological rejection of the transplanted organ (SCHLICH, 2011). In 1902, French surgeon Alexis Carrel revolutionized the medical field with the creation of the vascular suture technique. This method, named Carrel's Technique, played a crucial role in the evolution of transplant surgery, facilitating the anastomosis of the donor organ vasculature with the recipient vasculature (AIDA, 2014). The first successful solid organ transplant was performed in 1954 by American surgeon Joseph Murray, who transplanted a kidney between identical twins (WATTS, 2011).

Patients suffering from severe diseases or organ failure are given a second chance at life through transplants. With the new organ, quality and life expectancy increase, followed by a reduction in the need for support, invasive or limiting therapies (GRINYO, 2013). However, the scarcity of donated organs represents a significant challenge faced by transplant medicine. Nowadays, the number of donors is vastly inferior to the number of patients on waiting lists. As a result, countless individuals die while waiting for a compatible organ (WATSON; DARK, 2012). This problem is what drives the
development of research in regenerative therapies, with 3D bioprinting as the main agent. Technological advancements like this promise robust and effective solutions to face such challenges (CHIMBO, K. M. O. et al, 2016).

The 3D printing of biological tissues is an emerging technology that revolutionized regenerative medicine (MANDRYCKY et al., 2016). It can be defined as the overlapping, layer upon layer, of cell-loaded biomaterials and growth factors with the aim of obtaining constructions that mimic the physiological and mechanical properties of human tissues (MOGHADDAM et al., 2021). This technique enables not only the creation of accurate models for the research and development of new treatments for existing chronic-degenerative diseases but also the production of tissues and organs on demand, supplying the deficient number of donors. This method presents fewer risks of rejection since it uses the patient's own cells to construct the biomodel (LEBERFINGER et al., 2019). Studying the proliferation of tumoral cells and pathophysiological mechanisms of other affections, developing new drugs and replacing the use of animals for clinical trials are other advantages of using printed biomodels (OLIVEIRA et al., 2017; RESTAN PEREZ et al., 2021).

Although research in bioprinting is indeed recent, there has been progress at impressive rates. As part of this rapid advancement, we can cite the historic milestone achieved in 2018 when the first bioprinter was sent to space aboard Russia's Soyuz MS-11 spacecraft, used for printing living tissues under microgravity conditions (KAI CHUA, 2019). Recently, in March 2023, the American company Redwire launched, on mission CRS-27, materials for printing a meniscus in microgravity (O’NEILL, 2023) to study the process of cellular maturation and research new bioprinting techniques, without risk of collapse of the printed structure. Here on Earth, researchers are already able to reprogram mature cells and induce them into a state of pluripotency with the aim of creating versatile bio-inks, capable of being bioprinted to originate any tissue in the body (SHUKLA; GAO; KIM, 2022). In addition, neural cell implants have been bioprinted in an attempt to reduce the effects of chronic-degenerative diseases in the elderly (YEFROYEV; JIN, 2022).

This study aims to explain the workings of this new technology, presented as the great innovation of regenerative medicine. Its characteristics will be addressed, as well as its current and future applications, both in the development of biomodels that mimic organs and in the modeling of currently incurable diseases, such as Alzheimer's and
Parkinson's disease. Additionally, it discusses the challenges and perspectives for its large-scale clinical use.

**METHODOLOGY**

This article presents bibliographic research that explores the use of 3D bioprinting in organ transplantation and in the modeling of chronic degenerative diseases.

The databases used were PubMed and Google Scholar, with the keywords “3D bioprinting”, “Stem cells”; “Regenerative Medicine” and “Organ transplantation”. The articles selected for this study cover a time span from 1993 (due to its relevance to the research topic) to 2023. Language restrictions were not applied during the selection process, allowing for a comprehensive analysis of available literature on the subject.

**DISCUSSION**

The creation of a functional human tissue biomodel through 3D bioprinting is a complex task. Numerous variables must be considered to create a model capable of accurately mimicking human physiology. The choice of stem cells, as well as the components of the bioink, the 3D bioprinting method, and the post-printing maturation process depend on the type of tissue to be created. From the construction of autologous cartilage grafts to the creation of cancer cell biomodels for chemotherapy drug testing, the 3D bioprinting process can be divided into three important stages: pre-processing, processing, and post-processing (OLIVEIRA et al., 2017; FAN et al., 2022).

**PRE-PROCESSING**

**Biomodel**

Pre-processing involves obtaining or creating the three-dimensional model of the tissue to be replicated, to achieve a satisfactory and efficient clinical result, and selecting, collecting, and expanding the cells that will form the bioink (ZHANG et al., 2021). Magnetic resonance imaging (MRI) and computed tomography (CT) are methods capable of reproducing anatomical structures such as heart valves and coronary arteries with extreme precision (SUN; WEE, 2022). With modeling software, such as SolidWorks and CAD, researchers can create functional anatomical structures. Although the vascularization of artificial tissues is a significant obstacle, with the mentioned softwares, researchers were able to design a network of blood vessels ideal for more efficient nutrient
and waste transport (ZHANG et al., 2021; LEE; WAI YEE YEONG, 2018; SKELDON; LUCENDO-VILLARIN; SHU, 2018). In addition, ultrasound, echocardiography, and 3D scanning are other means to obtain a three-dimensional digital model (HONG et al., 2017).

Subsequently, the three-dimensional construction is sliced into multiple 2D layers, which overlap to form the biomodel, and sent to the printer in STL (Standard Triangle Language) or g-code files (YILMAZ; TAHMASEBIFAR; BARAN, 2020). Despite the existence of numerous tools to acquire a three-dimensional digital model, each has its advantages and disadvantages. The speed of obtaining and processing data, the resolution of the scanning of the structure to be replicated, the costs for its handling, and the presence or absence of ionizing radiation are factors considered in the planning of bioprinting (HONG et al., 2017).

**Perspectives on Stem Cells**

The success of the biomodel depends not only on the choice of appropriate biomaterials for the creation of the extracellular matrix (ECM) and cell hosting on scaffolds. The selection of cells is a significant factor. The largest cell source comes from stem cells, capable of achieving a specific phenotype depending on the growth factor added to the bioink or the maturation process in a bioreactor (ZHANG et al., 2021). With these, it is possible to reproduce the ideal microenvironment for recreating tissues, from liver to brain. They appear to be a plausible alternative to address the deficient number of organ transplants performed, which limits the life expectancy of many patients on waiting lists (SKELDON; LUCENDO-VILLARIN; SHU, 2018). Another significant hurdle in organ donation is the immunogenic effect triggered by the transplanted organ, even under immunosuppressive therapy. By using the host's stem cells, the chance of such an event tends to zero. However, their acquisition is limited to a few existing sources, requiring cultivation to multiply before the tissue is printed (ESWARAMOORTHY; RAMAKRISHNA; RATH, 2019).

Human mesenchymal stem cells (hMSCs), embryonic stem cells (ESCs), and human amniotic fluid-derived stem cells (hAFSC) are a few examples. In addition to these, cells with a previously determined phenotype are also used. Among these are osteoblasts, chondrocytes, adipocytes, human umbilical vein endothelial cells (HUVEC), or progenitor endothelial cells (EPC) are some options (ZHANG et al., 2021). Due to their wide range of uses, human mesenchymal stem cells (hMSCs) are among the
preferred for the bioprinting process. They promote physiological balance in mesenchymal tissue through immunosuppression and neutralization of reactive oxygen species and differentiate into bone, neural, and cartilaginous cell lineages (Snyder et al., 2015). Another factor contributing to the use of hMSCs is the ease of obtaining them, as they can be isolated from adipose, placental, and endometrial tissues, amniotic fluid, Wharton's jelly, and dental pulp (THARAKAN; KHONDKAR; ILYAS, 2021). A significant highlight is their multipotency, meaning they have the capacity to differentiate into various types of cells found in the adult body, broadening their use in various fields (CHOI et al., 2023). As an example of their potential, mesenchymal stem cells derived from adipose tissue were bioprinted and induced to differentiate into dopaminergic neurons for the modeling of neurodegenerative diseases. The resulting tissue expressed cellular markers and electrical activity characteristic of the nervous tissue found in the patients from whom the cells were collected (RESTAN PEREZ et al., 2021).

Human amniotic fluid stem cells (hAFSC) have a high proliferative capacity, excellent angiogenic response when compared to other cell types, and irrelevant immunogenicity, a fundamental characteristic when bioprinting is applied directly in vivo (TARASSOLI et al., 2018). Researchers were able to print ESCs in alginate and later differentiate them into cells similar to human pancreatic islets (SKELDON; LUCENDO-VILLARIN; SHU, 2018). These cells are derived from the blastocyst of an embryo, having the ability to originate any cells in the human body. However, their use in bioprinting is accompanied by high incidences of teratomas, making in vivo transplantation unfeasible due to the host's immunological rejection (MOGHADDAM et al., 2021). Departing from the usual, Wang et al. (WANG et al., 2020) developed a 3D biomodel of a glioma, a malignant brain tumor, to study the angiogenesis mechanism of the extensive abnormal vasculature formed by this tumor and thus understand its means of in vivo dissemination. They used glioma stem cells (GSC) as the basis of the study.

Pluripotent stem cells (PSCs) have infinite proliferative capacity, giving rise to cells from the three germ layers, making them the richest and most sought-after sources for regenerative medicine (YAMANAKA, 2020). However, the debate regarding the bioethics of using embryonic stem cells, with the consequent destruction of the human embryo, has made their use in biomodels difficult. To circumvent this discussion, induced pluripotent stem cells (iPSCs) lead the studies in regenerative medicine allied to 3D
bioprinting, becoming the focus of major achievements in the field (ABOUL-SOUD; ALZAHRANI; MAHMOUD, 2021).

iPSCs are cells that originated from the reprogramming of the genetic code of a patient's own adult somatic cells, avoiding the need to use embryos for their attainment and reducing the risk of rejection to almost zero. Their collection is not invasive, as skin fibroblasts can be used, and their differentiation potential is virtually unlimited. These factors make iPSCs the main candidates for disease modeling. Through nuclear reprogramming and reactivation of suppressed genes, with the use of stimulating factors such as Nanog, Lin28, Sox2, Klf4, Oct4, and cMyc, it was possible to force the differentiation of patient fibroblasts into pluripotent cells. For the transfection of the genetic code to be possible, the stimulating and transcription factors use lentiviral or retroviral vectors, adenoviruses, messenger RNA (mRNA), and recombinant proteins for this purpose. From this, they can be induced to differentiate into cardiac muscle cells, blood and intestinal cells, for example (ABOUL-SOUD; ALZAHRANI; MAHMOUD, 2021; SHUKLA; GAO; KIM, 2022; YEFROYEV; JIN, 2022).

An in vitro study observed the potential of iPSCs in modeling neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases, after they originated microglia, oligodendrocytes, and astrocyte cells. The bioprinted structure was able to accurately mimic the cytoarchitecture of the damaged area, perfecting the new synaptic connections that formed. A 3D biomodel can be implanted at the focus of the neurodegenerative disease to replace damaged tissues or model the disease microenvironment. The product of iPSC bioprinting retains its power of self-replication and induction of multilineage in situ, having flexibility to originate the tissue of interest through pre-determined stimulus (YEFROYEV; JIN, 2022; CROOK; TOMASKOVIC-CROOK, 2020). iPSCs can originate hepatocytes, cholangiocytes, endothelial cells, and Kupffer cells, replicating vascularization and cell-cell interaction, to form functional bioartificial livers, which will serve as a future solution for the scarce number of donors (OLGASI; CUCCI; FOLLENZI, 2020). It has become possible to differentiate this cell type into cardiac progenitor tissue, allowing the creation of spheroids, transplantable patches, and bioprinted hearts, as well as contractile cardiomyocytes, endothelial cells, fibroblasts, and smooth vascular muscle cells (CHO et al., 2021). The non-immunogenic potential of iPSCs, already mentioned in this work, was extended when Japanese researchers managed to generate "universal donor" iPSCs by inactivating the human leukocyte
antigen (HLA) system, making their transplantation into humanized mice possible without generating an immune response on the part of the host. Furthermore, they are excellent cells for testing new drugs with the aim of predicting their response and toxicity to the drug. Thus, it is possible that pharmacological treatments and the remodeling of diseases are personalized to serve each patient, based on their own physiology (VAN DEN BERG et al., 2019).

Despite being the subject of significant research in 3D bioprinting and leading the future of regenerative medicine, this type of cell presents some drawbacks, such as the accumulation of chromosomal alterations transmitted after each division, including mutations in TP53, and genetic instability (DOSS; SACHINIDIS, 2019). Cho et al. (CHO et al., 2010), by using proteins to induce pluripotency without genetic modification, bypassed the probability of inherent oncogenicity in the method that uses viral vectors to introduce exogenous genetic material into the somatic cell. Because of this and other advancements in the use of induced pluripotent stem cells, the prospects for their use, along with 3D bioprinting, are positive in the field of regenerative medicine, making disease modeling feasible and enabling the creation of artificial organs to meet the great demand that exists (SHUKLA; GAO; KIM, 2022).

PROCESSING

Bioink

The core of the processing is the preparation of the bioink used to make up the biomodel, originating from the combination of the biomaterial solution, the cells previously collected and expanded in a culture medium, and growth factors which will induce the cellular phenotype. When choosing the ideal bioink, factors such as porosity, mechanical resistance, cell adhesion, and cross-linking technique should be considered (YEFROYEV; JIN, 2022). The biomaterial, or ink, encapsulates the cell bodies and may be composed of natural polymers, such as gelatin, collagen, alginate, and hyaluronic acid (HA), or synthetic polymers, like polyethylene glycol (PEG) (FREEMAN et al., 2022), polycaprolactone (PCL) (CUI et al., 2021), and polylactic acid (PLA) (ANTICH et al., 2020).

Natural origin polymers present extreme biocompatibility, hydrophilicity, and biodegradability, not releasing toxic degradation products or inducing immunogenicity. They are the ones that most closely resemble biological tissue (TAMAY; HASIRCI, 2021). Furthermore, they easily promote cell adhesion through the presence of adhesion
molecules, such as integrins (THARAKAN; KHONDKAR; ILYAS, 2021). On the other hand, synthetic polymers present certain challenges, such as low biocompatibility, toxic degradation products, and loss of mechanical properties during degradation. However, their chemical specifications can be adapted to meet the desired application. Some bioinks mix natural and synthetic polymers to obtain unique characteristics, being called hybrid biomaterials (GARCIA et al., 2022). Studies have shown that the physicochemical characteristics of the biomaterial in which the stem cells will be cultivated can determine the function and guide cell differentiation. For example, stem cells in rigid ECM differentiated into osteogenic lineage, whereas elastic substrates induced differentiation into neuronal lineage (ESWARAMOORTHY; RAMAKRISHNA; RATH, 2019).

Collagen molecules, used as an extracellular matrix in the bioprinting of cartilaginous tissue, showed low antigenic capacity, high resistance, and biomimicry. They achieved great success in replicating the ECM, providing mechanical resistance, and allowing the structural rearrangement of cells and signaling molecules (LEE et al., 2019). It was found, through a study on nerve tissue regeneration in rodents, that the flexible property of a collagen scaffold allowed the development of nerve conduits with different characteristics for different sections of a nerve pathway, as well as assisting growth factors in axonal development, stimulating nerve regeneration and muscle reinnervation (POONGODI et al.; 2021). However, its high viscosity imposes some difficulty for the printing process (FAN et al., 2022).

The sodium alginate, extracted from marine algae, and chitosan, obtained from chitin, promote good adhesion and proliferation, intercellular biocompatibility, and incomparable biodegradability. In a study for the printing of cartilage and bone tissue, chitosan showed an incredible ability to provide vascular endothelial growth and multiplication of keratinocytes and osteoblasts, while alginate, after its cross-linking, showed high resistance to compressive forces (ZHANG et al., 2021; THARAKAN; KHONDKAR; ILYAS, 2021). Another material used as ECM is nanocellulose. This has been able to not only reproduce the collagen fiber network of human tissue but also provide a hydrophilic and resistant environment (HONG et al., 2017). Bioinks based on hyaluronic acid reduce the shear forces suffered by the cells during printing and have high cytocompatibility (PETTA et al., 2020). Moreover, their physicochemical characteristics can be changed to optimize the bioprinting process. For this reason, they are the most used in the elaboration of organoids (MUTHU PARKKAVI SEKAR et al., 2023). Skardal
et al (SKARDAL et al., 2010), in an in vitro assay, used fibroblasts associated with gold nanoparticles and gelatin along with hyaluronic acid to create functional vascular structures.

When the bioink has reinforcing mechanisms, such as gold or silver, to drive cellular functions, it is called high-performance bioink. In a work by Tharakan and colleagues (THARAKAN; KHONDKAR; ILYAS, 2021), this concept was explored in more depth. In this case, gold nanocomposites were added to the bioink to enhance the functionality of the cardiomyocytes and assist in the electrical conduction of bio-printed cardiac muscle tissue. Many other materials are used to compose high-performance bioink. The use of aligned poly-ε-caprolactone nanofibers directed the distribution of neural stem cells (NSCs) and, in the presence of laminin and RGD (arginylglycylaspartic acid), promoted greater cell elongation and formation of axonal connections. With the help of graphene foams in a 3D culture, NSCs were able to grow and maintain an active proliferative state (LEI; WILLERTH; FERNANDES, 2021).

The decellularization involves acquiring an acellular extracellular matrix (aECM) through the removal of cellular components from biological tissues by degrading or solubilizing their membranes. The result is an ECM that will serve as a natural scaffold to support the cells that will proliferate there. They are able to replicate the host tissue with extreme accuracy, preserving chemotaxis and perfusion functions, and are ideal for neovascularization and cell expansion (EDGAR et al., 2020; VERNENGO et al., 2020). Despite having low mechanical properties, the decellularized extracellular matrix (dECM) is a biomaterial that promotes tissue regeneration and repair. Materials such as gelatin and polyethylene glycol can be mixed with dECMs to improve their extrusion capacity through the printer nozzle and structural stability (KHATI et al., 2022).

The polyamide, popularly known as nylon, provides excellent biocompatibility. A study showed that the combination of polyamide 66/nano-hydroxyapatite has high potential for biological activity and treatment of bone defects, making it an excellent choice for such an application. Extracted from corn and potatoes, polylactic acid (PLA) provides shine, transparency, and low viscosity, allowing high cell viability without shape restrictions (BAENA et al., 2019). A study using iPSCs demonstrated the success of ethylene glycol hydrogel for the development, proliferation, and differentiation of these cells, as well as excellent delivery of differentiation and reprogramming factors. This
hydrogel also induced the differentiation of the three germ layers, as well as the formation of teratoma in the long term (LIU et al., 2020).

The characteristics of the printer, such as the print head or the method used to materialize the three-dimensional construction, limit the choice of bioink due to its viscosity, surface tension, and crosslinking method. The viscosity and other physicochemical properties determine the print resolution (SKARDAL et al., 2010). The higher its resolution, the more cell structures can be grouped in the same tissue volume, ensuring greater success during cell expansion (TASHMAN; SHIWARSKI; FEINBERG, 2022).

The ideal bioink should have not too high viscosity and good mechanical resistance, allowing the cells of the initial layers to withstand the weight of the subsequent layers, thus preventing the structure from collapsing. Bioinks with shear thinning exhibit high success rates in post-processing of the biomodel. This characteristic allows, during the extrusion of the bioink through the printer nozzle, its viscosity to temporarily increase, avoiding damage to cell bodies. After its deposition, the viscosity returns to normal, ensuring the strength of the construct (SKELDON; LUCENDO-VILLARIN; SHU, 2018). In addition, bioinks need to be biocompatible to promote the interaction of the printed tissue with the host without, however, being immunogenic, and not releasing toxic byproducts after its biodegradation. It is their responsibility to protect cells during the stressful printing process, allowing cell growth after several cycles in a bioreactor (HONG et al., 2017).

**Growth factors**

For the expansion, differentiation, and maintenance process to occur, the correct chemical signaling is necessary before, during, or after bioprinting. The molecules responsible for this are the signaling molecules solubilized in the bioink, making it bioactive (TRIPATHI et al., 2022). Growth, differentiation, and transcription factors, genetic code encoders, among others, can be added to it to originate and maintain the specific cell type to meet the purpose of the study. In some cases, they act as conductors in inflammatory processes for tissue repair, accelerating healing and rehabilitation (SOMAN; VIJAYAVENKATARAMAN, 2020). All these molecules are grouped into what can be called growth factors. Examples include transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF) and fibroblast growth factors (FGF) (WANG et al., 2021).
In vivo and in vitro models of scaffolds composed of decellularized cartilage extracellular matrix (DCECM) and bone marrow-derived mesenchymal stem cells (BMSCs) had their chondrogenic capacity amplified when transforming growth factor β-3 (TGF-β3) was added to the bioink formulation. While connective tissue growth factor (CTGF) and TGF-β3 induced bone marrow mesenchymal stem cells to regenerate and simulate the structure and function of a bioprinted intervertebral disc, originating cells from the nucleus pulposus and the fibrous ring and demonstrating satisfactory biomechanical mimicry (YANG et al., 2021; FREEMAN et al., 2021). VEGF and bone morphogenic proteins (BMPs) were loaded into the bioink to form a biomodel capable of inducing bone tissue proliferation in fracture scenarios. When acting on BMSCs obtained from a swine donor, they directed cell differentiation and promoted angiogenesis. In addition, hydroxyapatite (HA) nanoparticles were added to regulate the release of these factors in the correct timeline and amount throughout the osteogenesis process. The correct release of these factors, in exact quantity and at the right time, is another variable that must be executed excellently, imitating, as much as possible, the organic tissue to be replicated to guide cell differentiation effectively (FREEMAN et al., 2021; FREEMAN et al., 2020). During the composition of the bioink, some materials like bioactive glass (BG) can be used to enhance the carrying of growth factors within the extracellular matrix (HEID; BOCCACCINI, 2020).

Scaffolds

For the process to materialize the digital file sent to the printer, most researchers choose to use scaffolds (Figure 1) in the bioprinting process. This is the name given to the bioink after it has been printed (THARAKAN; KHONDKAR; ILYAS, 2021). Scaffolds are temporary structures whose function is to host the chosen cells so that their differentiation, proliferation, and extracellular matrix secretion is possible (ABDOLLAHIYAN et al., 2020). The mechanical properties of scaffolds must accurately mimic the physical and chemical microenvironment required by the cell type to be cultured (YEFROYEV; JIN, 2022). In addition, scaffolds have high porosity to allow good cell adhesion since cell bodies stay on their external surface (FAN et al., 2022). In a study, Zhou et al. (ZHOU et al., 2018) implanted bioprinted ears in human patients with microtia. Through CT, scaffolds were created that perfectly replicated the shape of the healthy ear. For three months, chondrocytes extracted from the patients were cultured inside PLA/PGA (polyglycolic acid) scaffolds until the final shape established in pre-
processing was achieved and could be implanted. In the construction of cartilaginous tissue, highly elastic hydrogels can be used to mimic the mechanical characteristics of natural cartilage. In some cases, other materials, such as thermoplastic, are used to compose the scaffold, forming a hybrid structure. Another example of a hybrid structure comes through nanocellulose fibers or bacterial cellulose nanocrystals. Thanks to their structural similarity with extracellular matrices, these materials assist in the mechanical resistance and biocompatibility of the scaffold (WANG; WANG; XU, 2020).

Some scaffolds may carry exosomes in their composition. These are vesicular arrangements secreted by cells, consisting of proteins and messenger RNA, whose function is to promote intercellular communication, regulate immune response, and mediate host-parasite interaction, in addition to carrying growth and chemical signaling factors. It was reported that alginate scaffolds carrying exosomes were superior in collagen production, skin regeneration, and angiogenesis when compared to scaffolds without exosomes. In addition, they promoted proliferation of Schwann cells, better myelin synthesis and, thus, nerve transmission and motor function were enhanced (POONGODI et al., 2021; ARAÚJO et al., 2016).

Despite all the advantages mentioned, some obstacles are present in this form of structural arrangement. The structural stability of the hydrogel depends on the viscosity of the bioink and, consequently, on the factors that influence it (ABDOLLAHIYAN et al., 2020). Furthermore, cell positioning in the scaffolds brings some inconveniences that can hinder the establishment of the physiology of the printed tissue. The imprecision with which cells will be positioned and the low print resolution are inherent disadvantages of this method of structuring the biomodel. The solution to this problem was achieved through layer-by-layer printing, which is capable of positioning cells with extreme spatial precision, causing the cell clusters to have a higher density compared to the use of scaffolds (ZHANG et al., 2021; ESWARAMOORTHY; RAMAKRISHNA; RATH, 2019).
3D Bioprinting Techniques

After creating and exporting the 3D biomodel to the printer, culturing and expanding the chosen stem cells, and subsequently composing the bioink with the addition of growth factors, the biomodel can finally be printed and sent to the bioreactor for maturation. There are numerous types of bioprinting used to achieve the final biomodel. However, the main ones include inkjet-based, laser-assisted (LAB), and extrusion-based bioprinting (Figure 2). Each presents different ways of cell positioning, each with its advantages and disadvantages. Additionally, the choice of the correct method to print the biomodel depends on its purpose and the inherent printability. This can be defined as a parameter used to compare the characteristics of the designed and printed biomodel and, subsequently, to assess the factors that led to the difference between them (FU et al., 2021). Moreover, it quantifies the ability to form and maintain scaffolds from the used bioink (NAGHIEH; CHEN, 2021).
In extrusion-based bioprinting, a continuous filament, fibers or droplets of bioink are extruded from the cartridge, through a nozzle or syringe, by the application of pneumatic or mechanical forces, by compressed air or by a piston or worm screw, respectively (CUI et al., 2020; DAVOODI et al., 2020). This method reproduces structurally stable constructs, in the form of hydrogel scaffolds, with precision in cell positioning and high versatility in biomaterials (NAGHIEH; CHEN, 2021; TIAN et al., 2021). Extrusion-based bioprinting showed excellent results in reconstructing tissues with graded cell architecture, such as bone. Extrusion methods with alginate were able to accurately reconstruct hydroxyapatite and osteochondral cell gradients (MERVE KUZUCU et al., 2021).

Moreover, it is preferred for the printing of skeletal muscle due to the way the printed fibers are deposited, in an almost exclusively parallel pattern of this method, mimicking the native tissue architecture (FORNETTI et al., 2023). Although widely used, some obstacles may be present. Low resolution and printing speed limit its fidelity to certain organic tissues and implementation in the clinical setting (DAVOODI et al., 2020).

Depending on the printability of the chosen biomaterial, problems such as nozzle clogging and cell destruction may be present. However, following the latest technological advances, machine learning has been used in conjunction with the extrusion-based printer to predict and circumvent factors that may cause cell inviability during printing, such as nozzle diameter, the biomaterial used, extrusion temperature, in order to increase printability (TIAN et al., 2021).
With inkjet-based bioprinters, the amount of bioink droplets deposited is controlled by thermal, piezoelectric, or electrostatic mechanisms, which eject them onto receiving substrates, through thermal or acoustic pressure. Great alternative due to the low cost, fast printing speed, and high resolution. Up to 1,000 drops are placed every second in layers 20 to 100 μm high (LU et al., 2023). The droplets formed by this technique are more uniform, allowing better control over cell deposition (TANG; RICH; CHEN, 2020). After the formation of complex patterns, made possible by the nature of the method, the liquid hydrogel undergoes gelation, becoming suitable to receive another layer of bioink (CHEN et al., 2021). The instant heating generated in the process does not damage the stem cells wrapped in the biomaterial, since there is a rise of approximately 4 to 10 degrees Celsius (MEMIC et al. 2017).

Despite the mentioned benefits, some disadvantages are inherent to the process, such as ink viscosity, which must be well controlled so that the nozzle does not clog, and the emergence of tension and shear forces, which could damage the cell bodies if not avoided (TANG; RICH; CHEN, 2020). The inkjet technique is widely used in the printing of liver and blood vessels. In these examples, vascular endothelial cells were added to the gelatin and alginate hydrogel to replicate the extremely delicate and complex hepatic microvascular environment (MING et al., 2023).

The mechanism of laser-assisted bioprinting consists of the transfer of cells, DNA, and any other biological material contained in a thin layer of bioink, to a receiving substrate through concentrated laser beams (ZHANG et al., 2021). The moment the laser hits the surface of the energy absorption layer, located above the bioink, a pressure microbubble is formed that propels a droplet of ink onto the collection substrate, positioning cell over cell (OLIVEIRA et al., 2017). This method has incredible resolution and precision in cell positioning (HAKOBYAN et al., 2020). As there is no contact with the bioink, it avoids recurrent problems in other printing methods, such as nozzle clogging, contamination, and tension and shear forces that could potentially cause mechanical damage to the cells (CHEN et al., 2021). In the absence of a nozzle, higher viscosity inks can be printed. Depending on the frequency of the laser beam, hundreds of droplets can be fired in a single second. Also, the laser settings can be changed to deposit numerous or just a single cell on the substrate (BETZ; HO; GASTON, 2020). Despite its incredible properties, the cost of printing is high, the preparation time is longer compared to other techniques, and the method is still under development, which leaves room for
variations in the properties of the generated droplets (ZHANG et al., 2023; YILMAZ; TAHMASEBIFAR; BARAN, 2020).

**POST-PROCESSING**

Upon completion of the biomodel printing, it is necessary for it to undergo a final stage before it can be used for the desired purpose. This involves its maturation in a bioreactor (figure 3). Mechanical bioreactors are systems designed to provide a controlled and dynamic environment, capable of simulating physiological conditions of the body in vitro, and promoting maturation and functionality of bio-printed tissues (PLUNKETT; O’BRIEN, 2011). They work by providing nutritional support, adequate temperature and pH conditions, oxygen, elimination of carbon dioxide (CO2), as well as allowing the application of mechanical and electrical stimuli, facilitating cellular differentiation and organization (MANDENIUS, 2016). In the literature, various types of bioreactors have been employed in the cultivation of bio-printed tissues, with multiple models often combined into one to better meet cellular needs (MARTIN; WENDT; HEBERER, 2004; BANCROFT; SIKAVITSAS; MIKOS, 2003; GOODWIN et al., 1993; LARISSA BUENO TOFANI, 2020; MATZIOLIS, 2011; BHISE et al., 2016). Thus, the choice of the appropriate bioreactor for each application depends on the cell type, the complexity of the tissue, the size of the sample, and the objective of the study (MAZZIERO, 2021).

Rotating drum bioreactors (RDB) use a rotating cylinder to provide a controlled and dynamic environment for cell cultivation and the maturation of three-dimensional tissues (BANCROFT; SIKAVITSAS; MIKOS, 2003). They are suitable for the cultivation of shear-sensitive cells, becoming a valuable tool for tissue engineering and the development of regenerative therapies, and can cultivate different types of tissues, including cartilaginous, bone, and muscle tissues (GOOCH et al., 2001; SIKAVITSAS; BANCROFT; MIKOS, 2002). The printed scaffolds are submerged in the culture medium (ZHANG et al., 2021) and, through centrifugal force, the drum mixes and evenly distributes nutrients and oxygen to the cells. Due to their mechanics, they can generate turbulent flow in some cases, which is not suitable for some cultures. In this case, the drum wall should be corrugated to reduce swirling (BUENO et al., 2004).

The Rotating Wall Vessel (RWV) bioreactor is a special type of bioreactor. Initially conceived by NASA (National Aeronautics and Space Administration) in the 1980s with the objective of simulating microgravity conditions and investigating cell behavior in space (GOODWIN et al., 1993), this system employs the rotation of the wall,
typically made from transparent polymers or glass, which keeps cells suspended in a culture medium. In this way, the RWV enables three-dimensional cell growth and the production of more physiologically relevant tissues, similar to those found in living organisms (LARISSA BUENO TOFANI, 2020). The rotation of the bioreactor wall is adjustable, allowing the optimization of cultivation conditions according to the specific needs of each cell type, reducing shear and turbulence (GOODWIN et al., 2015). This condition mitigates potential cell damage and death, simultaneously increasing the viability and functionality of cells (LARISSA BUENO TOFANI, 2020).

Bioreactors with compression, tension, and torsion stimuli aim to replicate the mechanical forces that tissues experience in vivo, promoting appropriate cell differentiation and organization (MATZIOLIS, 2011; SCAGLIONE et al., 2010). Such stimuli can be applied cyclically or statically, depending on the specific application and the needs of the tissue under study (SCHULZ; BADER, 2007). Moreover, bioreactors can be equipped with force and deformation sensors, allowing real-time monitoring of tissue mechanical properties and optimization of cultivation conditions, if necessary (NEIMAN, 2010). This approach has been particularly promising in the cultivation of tissues such as cartilage, tendons, and ligaments, which are naturally subjected to mechanical forces in vivo and depend on these stimuli for development and maintenance of function (WANG et al., 2014; WOON et al., 2011; MOUTHUY et al., 2022; SPANGENBERG et al., 2021).

Microfluidic bioreactors are miniaturized systems that allow the cultivation of bioprinted tissues under highly controlled conditions. These bioreactors are fabricated using microfabrication techniques, such as photolithography, and are designed to manipulate fluids at the micro or nanoscale (PAN; WANG, 2010). The main advantage of these bioreactors is the ability to integrate multiple functions into a single device, such as nutrient perfusion, application of mechanical and electrical stimuli, and co-culturing of different cell types (ZHANG, et al. 2016). The microfluidic platform has been explored for the development of organ-on-chip systems (CHLIARA; ELEZOGLOU; ZERGIOTI, 2022), replicating functions and architecture of various human organs, such as the heart, lung, liver, intestine, and brain, in microfabricated devices, paving the way for innovative applications in personalized medicine and in the evaluation of efficacy and safety of new drugs (SUN et al., 2022; SUNG et al., 2018; BHISE et al., 2016).
Perfusion bioreactors provide a continuous flow of culture medium through the interconnected pores of scaffolds, via a perfusion pump, to continuously offer cells nutrition, oxygenation, and metabolite removal (KAZIMIERCZAK; PRZEKORA, 2022). In this class of bioreactor, nutrients are continuously supplied. Cell metabolism products are removed from the system using filters, membranes, or centrifugation. They allow precise control of cultivation conditions and the maintenance of cell viability and functionality throughout the perfusion process, providing a stable and suitable environment for cell growth (GRAYSON et al., 2009). This results in higher productivity, cell survival, and product yield compared to other cultivation systems (GOMES et al., 2003; HOLTORF et al., 2005). However, the speed of the flow can increase the risk of shear stress and cell damage. Thus, it is preferable to rely on simulation methods to better meet the needs of each structure (MARTIN; WENDT; HEBERER, 2004). Computational fluid dynamics software has been extensively developed in recent years and offers an efficient way to calculate flow fields, shear stresses, mass transport, and oxygen in three-dimensional structures (WILLIAMS; SAINI; WICK, 2002).

Figure 3 - (A) Rotating Drum Bioreactor. (B) Rotating Wall Bioreactor. (C) Compression, Traction, and Torsion Stimulus Bioreactor. (D) Microfluidic Bioreactor. (E) Perfusion Bioreactor.

Source: author’s collection (2023).
CONCLUSION

This article provides a detailed analysis of the crucial role of 3D bioprinting in regenerative medicine, specifically in the creation of organs to address the shortage of donors and in the modeling of diseases previously considered incurable. Furthermore, it explains the critical steps in creating a biomodel that accurately mimics organic tissue. Despite all the challenges to be overcome, and considering all the examples given in the text, 3D bioprinting holds great prospects and promises to solve not only the problems discussed in the article but also many others that could be resolved through regenerative medicine.

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