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WHITE PAPER

RUSHING TO PRINT: RECENT ADVANCEMENTS IN 3D BIOPRINTING

Imagine being able to print personalized replacement skin, bone, muscles or even organs. 3D bioprinting promises to do just this. Not only this, but bioprinting technology provides the opportunity to generate high-throughput and relevant 3D-models to study the progression of diseases and test the efficacy and safety of therapeutics – speeding up drug discovery, screening, and ushering in a new era of personalized medicine.

For organ printing to become a reality, each stage of this workflow (Figure 1) needs to be carefully developed and refined. However, working with biological materials comes with a deluge of unique challenges. In this article, we explore these challenges and the way research teams across the globe are rising to them with innovative solutions at every step.

THE BODY'S BLUEPRINT

Whether you're building simple structures or complex organs, to 3D print you must understand what your object looks like. Visualizing tissue architecture at the high resolution needed to 3D print is one of the first challenges of bioprinting. Typically, researchers use medical imaging data – from x-rays, MRI scans, CT scans, ultrasounds, and optical microscopes – to guide the creation of a digital file.

This digital file, containing the blueprint of your 3D model, is sliced into two-dimensional graphic files that can be used as a set of instructions for a 3D printer. Due to the limitations of medical imaging data and the complex architecture of vascular systems that supply organs like the heart, 3D modelling at these fine resolutions can be tricky. Some researchers are turning to nature to fill in any gaps – with leaf venation systems serving as inspiration for modelling microvascular networks[1].

As computers get more powerful, algorithms become more advanced, and the resolution of medical imaging technology improves, the ability to print more sophisticated biomaterials will open up a new realm of possibilities. However, while a detailed set of instructions is a great start, the printer – and its ink – must too be optimized.

A BIOPRINTER NEEDS BIOINK

Bioink is the name given to the printable material used for bioprinting. It consists of various biologics including cells, media, serum, genes, and proteins. The selection of the right biomaterials and cell types - as well as combining them in a way that satisfies the demands of printability, biocompatibility and cell functionality - is an epic challenge.

Adding further complexity to the formulation of bioinks is the range of bioprinting technologies available (Table 1); with each printing technique placing a unique set of demands on the characteristics of the bioink.

Possibly the most common form of 3D bioprinting is the extrusion-based method (Table 1). The ideal bioink must be extruded into

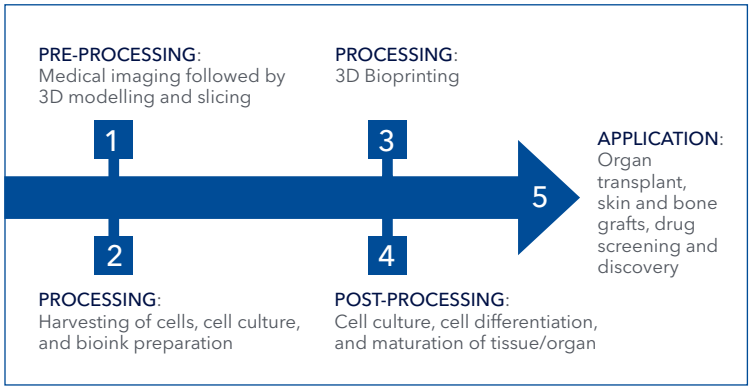

FIGURE 1. Outline of a typical 3D-bioprinting workflow

a stable 3D structure, while protecting the cells from shearing or other external forces, and provide the perfect environment for remodeling into the target tissue. However, as many of us know, biological materials don't always co-operate.

For example, collagen makes up over 30% of the total body protein, forms fibrous networks that strengthen the structural integrity of the extracellular matrix, and promotes cell adhesion, growth, signaling, and direct tissue development[2] - making it an excellent biomaterial for 3D printing. But, if you try to print it into the airspace, it just forms a puddle.

A recently published method, developed by researchers at Carnegie Mellon University, overcomes the barriers imposed by collagen by printing into a bath of hydrogel[3]. This unique technique - namely Freeform Reversible Embedding of Suspended Hydrogels (FRESH) - allows collagen to solidify before the gel is removed. Functional collagen scaffolds, that can be seeded by the right cells for maturation have been produced using this method.

Other research groups have refined the bioink itself - yielding full-scale reconstructions of ears, blood vessels, cartilage, and bone segments. By combining two reinforcement techniques, advanced bioinks, known as Nanoengineered Ionic-Covalent Entanglement (NICE) bioinks, generate much stronger structures than previously possible[4].

One of the other major challenges in 3D bioprinting is the lack of control over cellular functions. Growth factors, which are a special class of proteins, can direct cellular fate and functions. Consequently, growth factors – such as those available from Bio-Techne – have been incorporated into bioinks to help promote processes such as vascularization and the development of tissues; including neural, heart, and cartilage tissues[5].

PRINTER TYPE	DESCRIPTION	RESOLUTION CAPABILITIES	BIOINK CHARACTERISTICS
Inkjet	Uses surface tension to hold the bioink at the nozzle of the printer head and then employs a thermal, mechanical piezoelectric, or acoustic piezoelectric strategy to force droplets out in a controlled fashion.	Medium-High	• Low viscosity • Rheopectic behaviour • Non-fibrous nature • Medium surface tension • Rapid gelation kinetics
Extrusion based	Continuously forces viscous, usually cell-dense, bioink out of the nozzle in a controlled manner using either a pneumatic or mechanical mechanism.	Low	• Shear thinning • Thixotropic behavior • Low surface tension • Low adhesion • Rapid gelation • Shape retention
Low Induced Forward Transfer	All use a process called laser-induced forward transfer (LIFT). LIFT employs a high-powered laser, directed through a transparent glass, into an energy-absorbing layer of a metal – such as gold or titanium. Laser pulsations cause the energy-absorbing layer to transfer energy to the bioink – which is released in a highly controlled manner.	High	• Adhesion to the intermediate layer • Low surface tension • Viscoelasticity • Absorption kinetic energy • Rapid gelation
Stereolithography	Precise movement of UV light directed at a bath of liquid, cell-dense biopolymer causes photoactivation – resulting in the cross-linking of macromolecules and ultimately, the development of tissue architecture.	High	• Undergo photopolymerization • Use of light absorber • Use of photo-initiators with low toxicity • Stability and high mechanical strength • Retention of uniform cell distribution

TABLE 1. Descriptions of the 4 major types of 3D-bioprinting technologies and their required bioink characteristics. Partly re-drawn from Jamieson et al. (2021)[6]

READ THE FINE PRINT: HIGH-RESOLUTION 3D-PRINTING

Once the bioink has been formulated, the 3D printer can begin construction. Following the blueprint, the printer deposits successive layers of bioink to build up your final product… if only it was that simple! There are actually four major types of bioprinters - inkjet droplet, extrusion, laser droplet, and stereolithography – and each come with their own variations (Table 1).

Bioprinting technologies are not only getting faster, but their resolution, accuracy, reproducibility are also rapidly improving. For example, high-throughput bioprinting technology that prints highly resolved tissue organoids for drug discovery and screening on 96-well plates can now be done in just 30 minutes[7].

Moreover, just this year, a new method – known as fast hydrogel stereolithography printing (FLOAT)[8] - with the potential to vastly speed-up printed organs was announced. By refining stereolithography (Table 1), tiny models of 3D printed organs with vascular networks were created – with speeds up to 50 times faster than the industry standard.

And there's even more! Researchers are moving into the 4th dimension – time. By using responsive materials and cells, 4D-printed structures that can grow or even change functionality over time and in response to their environment are possible. These dynamic 4D constructs could eliminate the need for additional surgeries in fields such as pediatrics – where 4D printed transplants could grow and change as a child ages[9].

WHAT COULD 3D-BIOPRINTING DO FOR YOU?

DRUG TESTING AND HIGH-THROUGHPUT SCREENING

Getting a drug to market is a competitive, expensive, and challenging feat. Demanding preclinical laboratory and animal testing followed by four phases of labor-intensive and costly human clinical trials, drug development can take approximately 10 years at a price tag in excess of $2.5 billion[10]. Not to mention, the high failure rate of drug candidates – with only one in ten that make it to clinical trials getting to market[10].

Since bioprinted tissues have many features in common with native tissues - including multiple cell types, cellular density and architectural features – could they offer a more relevant model that also saves valuable time and resources?

Indeed, bioprinted tissues can be used in place of living subjects or 2D cell culture models during the early stages of drug development – providing a more ethical, high-throughput and cost-effective solution.

Even benchtop versions of the most complex organs in our bodies are being developed. Exciting advancements in printed brain organoids are set to revolutionize our understanding of

disease progression and drug development for various disorders including brain tumors, schizophrenia, and Alzheimer's.

For example, a research team at Northeastern University in Boston and the Rensselaer Polytechnic Institute were able to bioprint a 3D brain model containing blood vessel cells connected to all major cell types found in the human brain[11]. In the center of the structure, the researchers placed tumor stem cells derived from patients with brain tumors.

By laser scanning the model to gain an understanding of the cellular structure, researchers were able to reconstruct tumor morphology and growth to see how it responds to drug treatments. Notably, they demonstrated that a commonly used chemotherapy drug – temozolomide – was ineffective against glioblastoma tumors.

A CHANGE OF HEART: ORGAN TRANSPLANTS

While printing organs that can be successfully transplanted into humans remains firmly in the future, exciting developments in 3D bioprinting are keeping this goal in sight. With hundreds of thousands of people anxiously sitting on organ transplant waiting lists, printed organs could eventually give hope to many.

Researchers at the Carnegie Mellon University, have brought us one step closer to printing complex, full-sized organs using collagen as a scaffold. By combining FRESH (see section: A Bioprinter Needs Bioink) with MRI data of a human heart, the team successfully reproduced patient-specific anatomical structures using collagen and heart cells3. This included functional parts, like a heart valve and a small beating ventricle.

SAVING ONE'S SKIN: 3D PRINTED SKIN GRAFTS

While some researchers have focused on printing living skin with blood vessels for skin grafts, others are developing handheld 3D printers that deposit sheets of skin to cover burn wounds. Printed skin has been previously limited in its application by the difficulty associated with generating a vascular system to circulate blood. But, recent advancements in 3D printing have overcome this challenge – allowing printed skin with blood vessels to be successfully grafted onto mice[12].

Elsewhere, by dispensing bioink composed of mesenchymal stroma cells, a hand-held technology covers wounds with a uniform sheet of biomaterial, strip by strip[13]. As the cells differentiate, they promote skin regeneration and reduce scarring.

WHO SAID MILLENNIALS NO LONGER PRINT?

While the younger generations may be sending classic ink printers the way of the Dodo, the only way is up for 3D bioprinting. But, while we may be seeing exciting advances at an ever-increasing pace, this is still a new and emerging field – leaving plenty of opportunity for innovation!

Whether you're culturing stem cells, creating a bioink, or maturing tissues post-printing, Bio-Techne have a full range of products to support your 3D-bioprinting workflow. As well as culture media tailored for the growth of a range stem cell types, Bio-Techne offer a full suite of GMP or RUO grade small molecules, growth factors and differentiation factors that can be used for culturing, bioink formulation, cell differentiation and growth.

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