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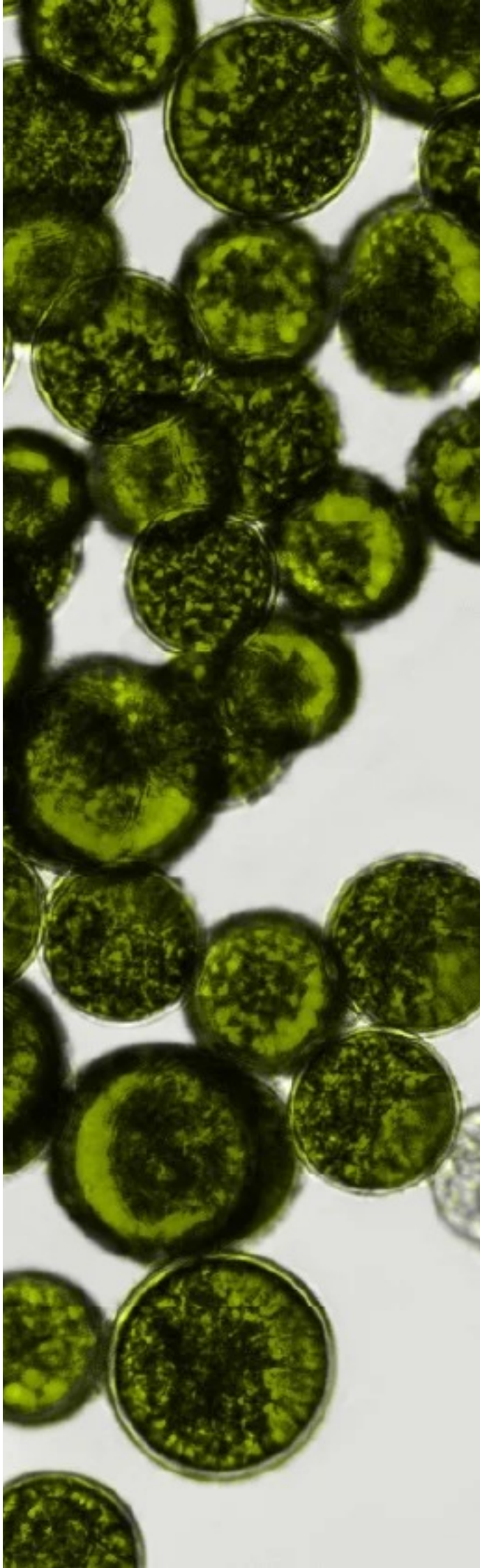

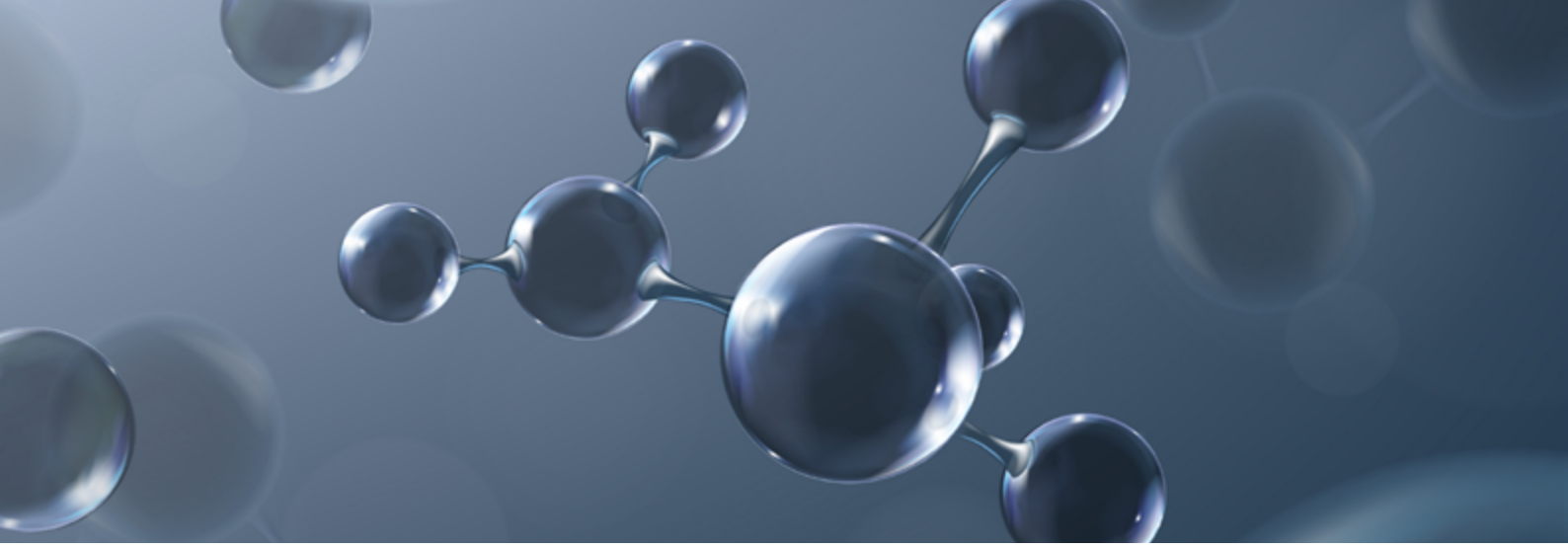

Foreword

Welcome to the latest edition of our Industry Focus eBook, where we explore the dynamic and rapidly evolving biotechnology field. This sector sits at the intersection of biology and technology, delivering revolutionary solutions reshaping the landscape of medicine, manufacturing, and beyond. From advanced organ regeneration techniques to next-generation gene therapies, the promise of biotechnology has never been more exciting or more essential.

One of the most inspiring frontiers in this field is regenerative medicine. In Harnessing Biotechnology to Engineer Tubular Organs for Regenerative Medicine, we explore how researchers use biotechnology to develop lab-grown. These functional tubular organs could one day transform the treatment of organ failure.

3D printing is also playing a vital role in the biotech revolution. **3D Printing in Healthcare: From Surgical Tools to Organ Transplant Breakthroughs** highlights how additive manufacturing enables everything from patient-specific surgical instruments to creating complex biological tissues, bringing new possibilities to personalized medicine.

The design and optimization of bioreactors is crucial to producing life-saving therapies. **Bioreactor Design and Control in the Biopharmaceutical Industry** explores the engineering innovations driving more efficient and scalable biologics production.

In neuroscience, **Bioprinting breakthrough: Tech platform creates 3D neural tissues in which neurons and glia connec**t, introducing a fascinating advancement that mimics real brain tissue, potentially opening new doors for studying neurodegenerative diseases and testing treatments.

Genetic therapies are advancing at an incredible pace. **Foam technology revolutionizes gene therapy, boosting efficiency and safety** and presenting an inventive approach that could enhance delivery methods. In contrast, Breakthrough in gene editing: Enhanced virus-like particles promise a new era in genetic disease treatment examines a promising new tool that could make gene editing safer and more precise.

This eBook offers a comprehensive look at the innovations shaping modern biotechnology. We hope you enjoy this journey into the science that redefines what's possible for human health and healing.

Investigating microbial metabolisms with Thermo Scientific MS, GC and Fluorometry Systems

Making sense of the complex metabolic activity of microbial communities is no mean feat. Understanding the stoichiometry and reaction kinetics of this activity typically requires precise quantification of reactants, metabolic products, and the composition of the microbial community itself. Researchers at the universities of Delft and Wageningen in the Netherlands used mass spectrometry, gas chromatography and fluorometry systems from Thermo Scientific to do just this; helping them to gain insight into the effects of pH and product inhibition on chain-elongating microorganisms in sequencing batch bioreactors.[1]

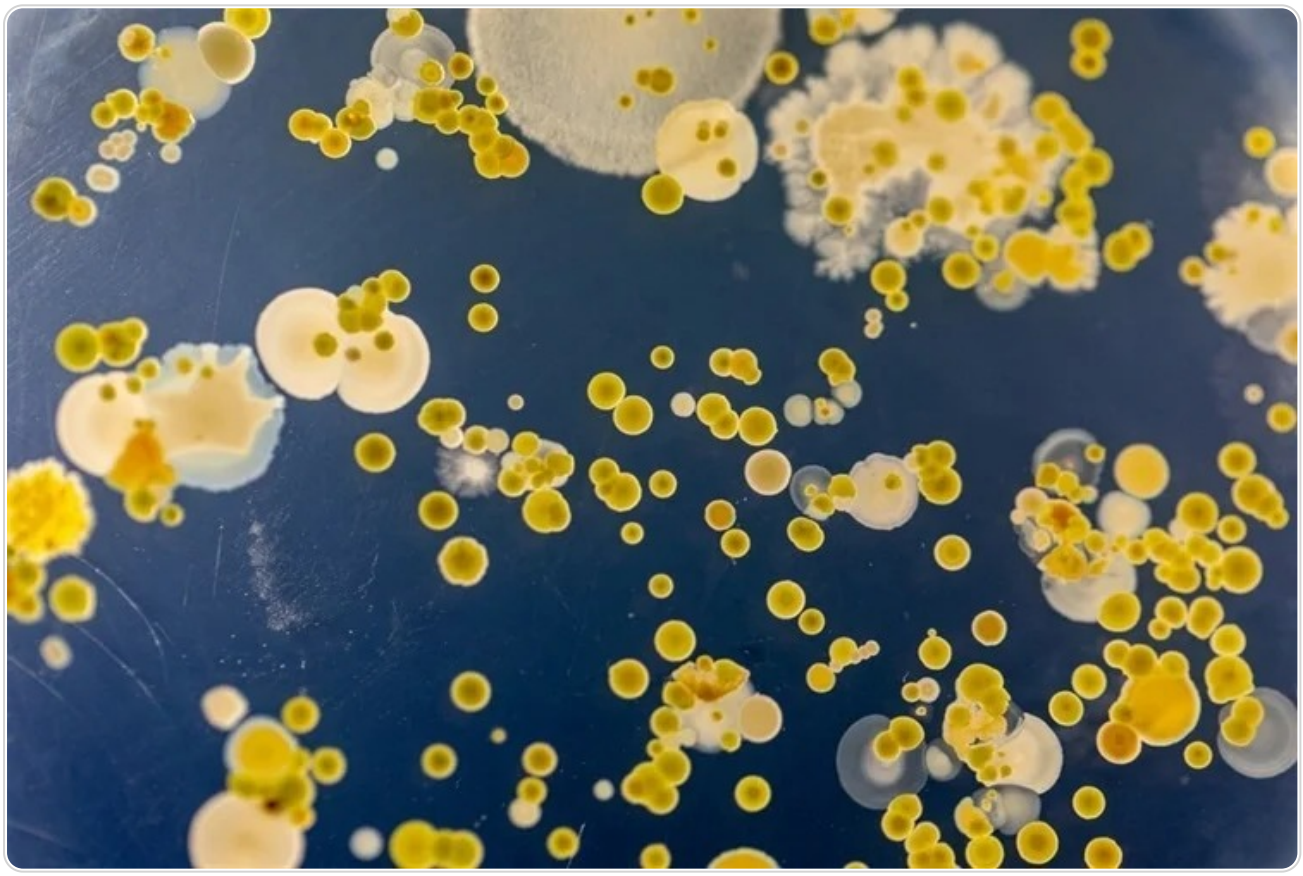

Image Credit: RattiyaThingdumhyu

Sequencing batch reactors

Sequencing batch reactors are one of the simplest types of bioreactors, each consisting of a single tank in which a full cycle of anaerobic processing is carried out by a community of microorganisms. This cycle consists of four sequential steps (hence "sequencing" bioreactor): feeding, reacting, settling, and decanting.[2] Sequencing batch reactors are predominantly used

to process wastewater from both municipal and industrial sources. In this capacity, sequencing batch reactors are a relatively new technology – however, their simple configuration and high efficiency in removing suspended solids and reducing biological oxygen demand (BOD) makes them a promising low-cost approach to the effective treatment of dangerous wastewater.

Processing wastewater using sequencing batch reactors has benefits beyond removing contaminants from wastewater and making it safe to return to the water cycle. By harnessing the metabolic power of microorganisms in sequencing batch reactors, researchers are hopeful that it may be possible to convert complex industrial and agricultural waste effluent into recoverable microbial "products". In the transition away from a petroleum-based society, the microbial production of useful chemicals from renewable resources in this way has gained significant interest.[3,4]

Such techniques would have potentially enormous ecological and economic benefits. However, the influence of environmental conditions on the stoichiometry and kinetics of many types of reaction that occur in sequencing batch bioreactors remain elusive.

Investigating microbial carboxylic acid chain-lengthening reactions

A team of researchers from the universities of Delft and Wageningen in the Netherlands set out to investigate the effects of environmental factors on a specific type of reaction in a sequencing batch bioreactor: the elongation of short-chain fatty acids through reversed β-oxidation. Processed by microorganisms (in particular *Clostridium kluyveri* related species), the carbon "backbones" of short chain fatty acids such as butanoic acid, present in complex wastewater streams, are elongated. Product of this metabolic processing is medium-chain carboxylic acids such as hexanoic acid and octanoic acid (also known as caproic acid and caprylic acid, respectively).

Many medium-chain carboxylic acids are valuable compounds. Hexanoic acid, for example, can be used as an antimicrobial agent, as a corrosion inhibitor, and as a precursor for a number of flavors, fragrances, solvents and fuels.[5]

By tuning the conditions within a reactor, the composition and behavior of microbial communities can be changed in order to suppress or enhance certain reaction types. The team of researchers aimed to explore the influence of environmental factors (in particular, pH and product inhibition) on carboxylic acid chain-lengthening reactions within a sequencing batch reactor in order to help uncover the fundamental aspects of these reactions.

A window into the microbial realm

Sequencing batch bioreactors typically play host to a dynamic community of competing (and cooperating) microbial species. Untangling the complex metabolic processes within a bioreactor and quantifying a particular class of reactions can be challenging.

At the least, it's generally necessary to keep a detailed inventory of the reactor's inputs and outputs, and to monitor the presence of specific reactants and products in solution within the reactor. In addition, characterizing the microbial population itself can provide valuable insight into the roles of various microbial species.

Rather than processing real waste streams, the team of researchers created an experimental setup whereby process parameters and microbial conversions could be closely monitored within two 1L batch reactors, each operating for 48 days.

A Thermo Scientific PRIMA BT benchtop mass spectrometer was used to carry out in- and off-gas analysis of the reactors. Designed for process development laboratories, the PRIMA BT uses scanning magnetic-sector technology to deliver accurate, precise and stable on-line gas analysis. Physicochemical modeling enabled off-gas measurements to be converted into bioreactor-specific respiration rates of H_2, N_2, CO_2, and CH_4.

Concentrations of butanoic acid (a short chain fatty acid), hexanoic acid (a medium chain carboxylic acid) and ethanol (an electron donor for chain-lengthening reactions) were all measured using a Thermo Scientific Trace 1300 gas chromatograph equipped with an injector maintained at 180 °C. The trace 1300 series is a compact GC system designed to deliver extremely fast results at a low cost of ownership. The unique modular design enables researchers to build only the configuration they need, minimizing costs compared to traditional GC systems.

The team processed samples from the bioreactor into microbial pellets in order to carry out genomic analysis. Prior to DNA sequencing, a Thermo Scientific Qubit 4 fluorometer was used to provide rapid quantification of DNA within the pellets, ensuring that sufficient DNA was present before sending samples off for amplicon sequencing. Providing results in under 3 seconds and requiring as little as 1 µL of sample, the Qubit 4 is unparalleled for simple and rapid DNA/RNA quantification.

Through careful control of process parameters and precise monitoring of reactants, products and microbial populations; the researchers were able to elucidate the effects of varying pH

and product inhibition on chain-lengthening reactions within the bioreactors. Their results provided insight into the ways microorganisms deal with energy losses associated with product inhibition, showing that the chain-elongating reactions rely on a balance between substrate uptake and product inhibition.

Thermo Scientific develops world-leading analytical solutions which enable researchers to solve complex challenges in biotechnology. To find out more about the Thermo Scientific systems used in this research – or any of our other analytical systems – get in touch with Thermo Scientific today.

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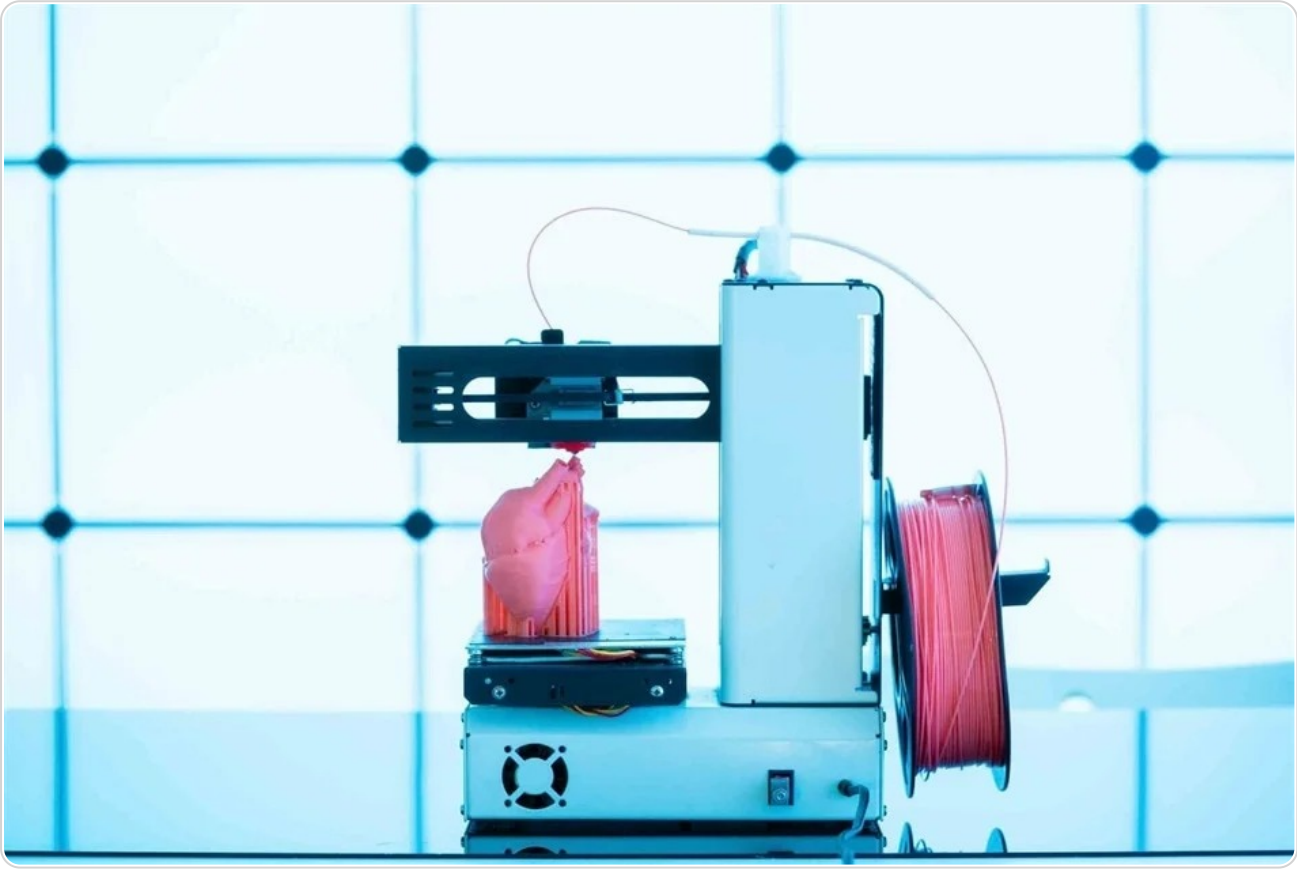
Harnessing Biotechnology to Engineer Tubular Organs for Regenerative Medicine

Tubular organs refer to the body's hollow, tube-like structures, which are vital for critical bodily functions such as blood circulation (via blood vessels), digestion (via the intestines), as well as breathing (via the trachea and bronchi). Such tubular tissues and tissue structures are also crucial for sustaining essential processes in human health, such as nutrient absorption and oxygen transport.[1]



Biotechnology to Engineer Tubular Organs for Regenerative Medicine" />*Image Credit: luchschenF/Shutterstock.com*

Introduction

Given the susceptibility of these tubular systems to disease and trauma, much research has been focused on the engineering of tubular organs, using techniques from tissue engineering and regenerative medicine, thereby seeking to develop functional organ replacements for those that are damaged or failing. With this medical innovation, it serves as a viable alternative to conventional organ transplants, which are hindered by limited donor availability and immune rejection issues. [1-3]

Moreover, patient-specific solutions are addressed in tubular engineering through the use of advanced technologies, such as 3D bioprinting and tissue scaffolding. These technologies allow for the customization of vascular grafts to match the individual patient's anatomy and physiological needs.[2] This personalization minimizes complications and enhances graft integration, promoting more successful long-term outcomes.

Technologies and Techniques for Tubular Organ Engineering

Tissue Scaffolding

One of the fundamental techniques in tubular organ engineering is the use of tissue scaffolding. Biodegradable scaffolds are designed to imitate the natural structure of tubular organs, providing a temporary framework that supports the attachment and growth of cells. Over time, as cells proliferate and form functional tissues, the scaffold gradually degrades, leaving behind the newly formed organ without any foreign materials.[4,5]

3D Bioprinting

3D bioprinting, also known as additive manufacturing, is a cutting-edge technology used to fabricate tubular organs with high precision. It often involves either extruding material through a nozzle or using photo-crosslinking from a liquid precursor. In extrusion-based printing, a liquid material is dispensed from a nozzle onto a platform, creating a single layer, whereby after the solidification of that layer, additional layers are applied to form a 3D object. By carefully controlling the placement of these materials, 3D bioprinting allows for the creation of complex, functional tubular organs that mimic the natural architecture and function of the body.[1]

Stem Cell Therapy

Stem cells play a crucial role in tubular organ regeneration due to their ability to differentiate into various types of cells required for functional tissues. In regenerative medicine, stem cells are introduced into the scaffolds or damaged areas, where they grow and differentiate into the necessary cell types, such as endothelial cells for blood vessels or epithelial cells for airways, thereby aiding in the repair or creation of tubular organs.[6]

Decellularisation and Recellularisation

Another important technique in tubular organ engineering is decellularisation and recellularisation. In this process, a natural organ is decellularised, which removes all of the original cells, leaving behind only the extracellular matrix, which acts as a biological scaffold. The resulting scaffold can then be recellularised with the patient's cells, reducing the risk of immune rejection while creating a functional organ that closely mimics the original tissue's

properties.[1,7,8]

Applications in Regenerative Medicine

Vascular Grafts

Engineered blood vessels, or vascular grafts, are being explored in cardiovascular treatments, such as bypass surgeries and vascular disease therapies. These grafts are designed to restore blood flow in patients suffering from blocked or damaged arteries. As reported by the University of Edinburgh, researchers from their School of Engineering, in collaboration with Heriot-Watt University, used a 3D printer with a rotating spindle to create tubular grafts from a water-based gel.[9,10]

These grafts were then reinforced using electrospinning, which produced nanofibers coated in biodegradable polyester. Testing revealed that the synthetic vessels are as strong as natural ones and can be produced in diameters ranging from 1 to 40 mm for diverse applications. In addition, their flexibility enables smooth integration into the body.[9]

Urinary and Digestive Systems

Significant advancements have been made in engineering tubular organs for the urinary and digestive systems. In the urinary system, bioengineered ureters and bladders can be used to replace damaged tissues. In contrast, in the digestive system, efforts are being focused on creating tissue-engineered intestines to treat conditions such as short bowel syndrome. [11,12]

Respiratory System

Progress is also being made in the engineering of tubular structures for the respiratory system, such as the trachea. Bioengineered tracheas have the potential to revolutionize treatments for airway diseases and conditions, for example, tracheal stenosis, by offering biocompatible, functional replacements that can restore normal airflow in patients. [13]

Challenges in Tubular Organ Engineering

Structural Complexity

One of the largest challenges in tubular organ engineering is replicating the complex structures and functions of natural organs. Tubular organs require specific mechanical properties, including flexibility and strength, to perform their functions effectively. Additionally, ensuring proper vascularisation and nutrient delivery within the engineered organ is critical to its long-term viability.[14]

Integration with the Host

Successfully integrating engineered organs with the patient's body is another major challenge. The organ must not only function properly, though also avoid triggering immune responses that could lead to rejection. [15]

Scaling for Clinical Use

Scaling up the production of engineered organs to meet clinical demand is a significant hurdle. Key challenges include inducing vascularisation, modulating the immune response, developing universal donor cell lines, and ensuring tissues replicate the in vivo environment with proper mechanical properties.[16]

In addition to the technical challenges of producing larger quantities of complex tissues, regulatory approval processes must be navigated to ensure safety and efficacy.[17] Overcoming these barriers will be essential for bringing engineered tubular organs into widespread medical practice.

Impact on Healthcare and Organ Transplantation

Advances in tubular organ engineering have the potential to transform healthcare by alleviating the organ donor shortage and significantly lowering transplant waiting times. This technology could lead to improved patient outcomes by providing engineered organs as an alternative to traditional transplants.[18,19]

Additionally, engineered tubular organs could enable more personalized treatment options tailored to individual patients. It would improve compatibility and lower the risk of immune rejection, offering a more effective and sustainable solution for transplant patients.[20]

Conclusion

The engineering of tubular organs offers transformative potential in regenerative medicine and organ transplantation by addressing donor shortages and improving patient outcomes.

Advances in tissue scaffolding, 3D bioprinting, and stem cell therapy are key to creating functional, patient-specific organ replacements. However, overcoming challenges in replicating organ complexity, ensuring biocompatibility, and scaling production is essential. Thus, continued research and collaboration are vital to bringing engineered organs into clinical settings.

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Further Reading

- All Biotechnology Content
- What is Biotechnology?
- Dissecting the Current Landscape of Biotechnology in Europe
- The Colors of Biotechnology; What do they mean?
- Importance of Biotechnology in Agriculture

More...

3D Printing in Healthcare: From Surgical Tools to Organ Transplant Breakthroughs

3D printing is still a relatively novel method of manufacture, and has already diversified massively in terms of printing methods, materials, and design possibilities, finding niche application in a range of fields, including healthcare and the life sciences.

3D printing is having a transformative impact on the way surgery and dentistry is performed, and how prosthetics and implants are designed, allowing the creation of custom, personalized items fit for the patient or the particular task at hand.

This article will explore the wide-ranging applications of 3D printing in healthcare, from creating surgical tools to facilitating organ transplants.

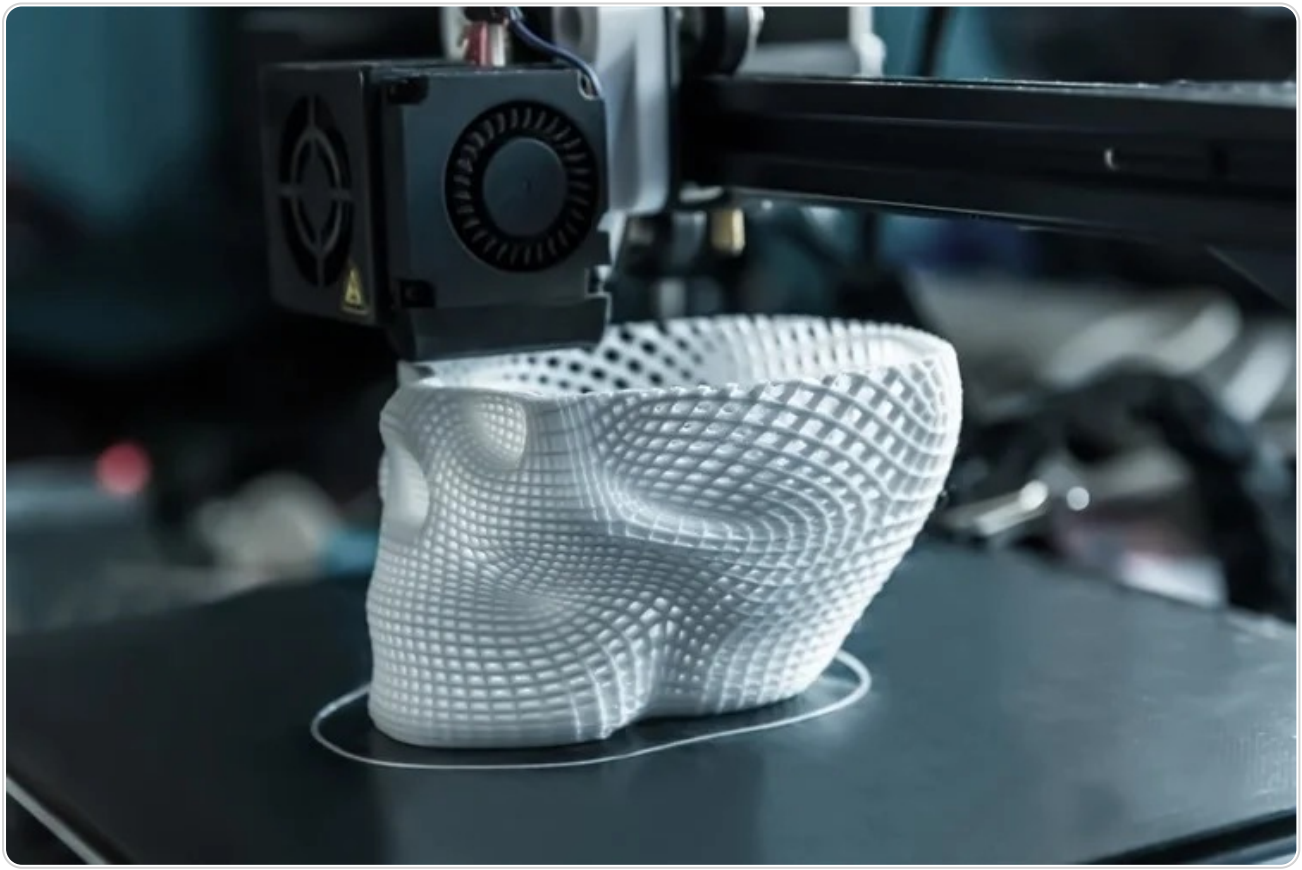

Image Credit: Olga Ilina/Shutterstock.com

Brief history of 3D printing technology

3D printing typically refers to an additive manufacturing process, i.e. one where material is added in successive layers or stages, rather than being removed from bulk material (subtractive) or directly molded to shape, as with materials such as thermosetting plastics.

One of the earliest forms of 3D printing was stereolithography, now more commonly termed resin printing, in which a UV laser is aimed in the desired pattern in a layer-by-layer manner at photopolymer resin, hardening it and transforming the liquid into a solid three dimensional structure.

Research into this technology was ongoing throughout the 1970s and patented in 1984, and is broadly utilized to produce custom manufactured parts. The type of resin employed can be adapted to purpose; for biocompatibility in cases of biological implant or prosthesis, for toughness and rigidity where required, and so on.

The term 3D printing was not actually coined until 1995, by Professor Ely Sachs, MIT, who worked on modifying inkjet printers to extrude a binding solution onto a powder bed, known as powder bed fusion 3D printing (of which there are many types: selective laser sintering, direct metal laser sintering, electron beam melting, etc.).

This method of printing evolved into many of the types perhaps more commonly used today, which employ a frame capable of moving an extrusion head in three dimensions above a platform, such as fused deposition modeling (FDM) 3D printing.

Now, there are over 18 methods of 3D printing, each with numerous modifications, allowing custom products to be manufactured in a broad range of materials, with differing degrees of ease and accessibility, quality, and suitability towards medical applications.

Innovations in surgical tools and equipment

3D printing is increasingly employed in the creation of surgical aids, including the design and production of accurate training models, specialized instruments, and scaffolds that aid in implantation or tissue repair.

One of the major advantages of 3D printing technologies is that iterative changes can be made to newly designed tools based on immediate feedback from surgeons and other medical

professionals; design changes can be implemented *in silico* and a new device printed overnight.

The facility of producing patient specific training models could potentially be revolutionary in terms of the way surgery is performed, as the highly particular details of a patient's internal organs, as ascertained from various scanning technologies, can be reproduced in detail.

This leaves fewer surprises for surgeons during surgery, and would massively assist in preparation for more complex surgeries.

Image Credit: belekekin/Shutterstock.com

Personalized prosthetics and implants

Some of the major issues with ordinary mass-produced prosthetics is surrounding abandonment; the user ceases to wear the prosthetic as they are uncomfortable, awkward, or unappealing aesthetically.

Bionic prosthetics, which are capable of coordinating roboting movement by muscle

contractions, must in particular be positioned and secured carefully in order to maintain their function and comfortable usability.

The custom sizing possible using 3D printing technologies allows much more comfortable prosthetics to be manufactured from biocompatible components, potentially in more complex designs and lower mass than traditional prosthetics.

In 2014 a conference was held at Johns Hopkins Hospital titled: Prosthetists Meet 3D Printers, in which medical and 3D printing experts met to discuss the state and future of 3D printing of prosthetics.

A broad range of collaborative efforts are currently underway with a view to utilizing 3D printing in prosthetics. For example, prosthetic devices are freely available to download and print at home on a number of dedicated websites, while many companies dedicated to producing prosthetic devices for particular markets have emerged.

For example, Openbionics is a UK based company that prints custom prosthetics, with superhero designs aimed at children, ones with specialized fittings for musicians, and so on.

Breakthroughs in 3D-printed organs

Various biomaterials can be laid down in an additive manufacturing method such as 3D printing to produce implantable scaffolds, tissues, and even whole new organs.

Bioinks containing living cells are deposited in a layer-by-layer manner to print the organ, typically employing a scaffold and/or natural polymers within the bioink, which harden and keep the cells in place; hydrogel polymers such as fibrin, gelatin, alginates, chitosan, and hyaluronic acids are typically employed. 3D printed organs such as this contain cells cultured from the patient, and thus are much more biocompatible than a donor organ.

There are several types of organ 3D printing, and the technology is still in its infancy. One of the earliest and most broadly employed methods is known as cell seeding, wherein a supporting scaffold is 3D printed from biocompatible materials and then seeded with cells that will propagate to fill the structure, potentially *in situ* in order to aid in wound healing.

Where custom organs are 3D printed they can be made to best suit the patient, not only in terms of biocompatibility but also in terms of shape and size; for example, adjusting the size of the heart valves to patient size.

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Using Process Mass Spectrometry for Multipoint Analysis in Biological Production

In recent years, the use of online process analytical technology (PAT) in the biotechnology industry has become a preeminent endeavor.

Since the start of the 1980s, numerous fermentation scientists have utilized the reliability of Thermo Scientific™ Process Mass Spectrometers to monitor the composition of gas streams into and out of fermenters and bioreactors.

When taking the first steps towards process control, it is not atypical for some to think that the only effluent required is the measurement of carbon dioxide and oxygen and that enough accuracy can be achieved by discrete measurement technology. It would be wrong to make assumptions.

Due to external biological factors – trees and people – that inevitably change the input to the instrument air system, the variability of sparge gas remains consistent. The all-pervasive twin-tower desiccant dryer systems will also either regurgitate or absorb CO_2 depending on their position in the regeneration cycle.

To facilitate an accurate pre-screening for potential contamination, only a precise comparison of effluent and sparge gas is appropriate. What is more, an accurate comparison is also necessary to calculate information in real-time regarding culture respiration and the availability of nutrients.

For suitable control, the minimum requirements are as follows:

- All gas components must be measured
- Automatic calibration of all components
- Flexible analysis schedules and techniques
- High accuracy is needed to calculate meaningful metrics
- Local support
- Measurement of sparge and effluent gases
- Operational reliability and simplicity

Figure 1. *Typical benchtop bioreactor. Image Credit: Thermo Fisher Scientific – Environmental and Process Monitoring Instruments*

Source: Thermo Fisher Scientific – Environmental and Process Monitoring Instruments

Magnetic Sector Analytical Performance	N_2 %mol Mean	N_2 %mol St Dev	O_2 %mol Mean	O_2 %mol St Dev	Ar %mol Mean	Ar %mol St Dev	C_2 ppm Mean	C_2 ppm St Dev
Day 1	78.0807	0.0028	20.9459	0.0026	0.9337	0.0003	396.84	1.31
Day 2	78.0767	0.0023	20.9494	0.0023	0.9342	0.0003	397.46	1.25
Day 3	78.0761	0.0024	20.9500	0.0023	0.9342	0.0003	397.34	1.28
Day 4	78.0798	0.0023	20.9469	0.0023	0.9337	0.0003	396.31	1.31
Day 5	78.0777	0.0030	20.9487	0.0028	0.9339	0.0003	396.76	1.34
Day 6	78.0741	0.0023	20.9518	0.0022	0.9344	0.0003	397.47	1.27
Day 7	78.0750	0.0023	20.9512	0.0022	0.9342	0.0003	397.23	1.30

Taking the first steps towards advanced process control

A fully instrumented fermenter is exhibited in the process diagram. In order to track a wide range of process variables in real-time, both liquid and gas-phase measurements are provided.

These data are input into the advanced process control (APC) system, which typically includes hybrid models comprised of neural (nonlinear) network models and formal (linear) models.

The APC system provides the set-points for numerous variables, which include sparge content and flow, control of nutrients and amino acids, in addition to the conventional variables of temperature, pressure, agitation and pH.

This diagram demonstrates the optimal situation for process scale-up and understanding, but there is a detailed amount of complexity and expense associated with this expansive technique.

The more common technique is to add a multi-stream mass spectrometer due to it offering a considerable amount of extra value with minimum risk and moderate cost. The Thermo Scientific™ Prima PRO has the capacity to monitor 60+ fermenters without compromising sterility.

For smaller-scale fermenters that are configured with 15 samples and six calibration ports, the Prima BT is a good bench-top solution. The complete gas composition measurements supplied by both models are both accurate and easily incorporated into the APC system.

Great improvements in process control can be rapidly achieved rapidly within one or two days of a start-up.

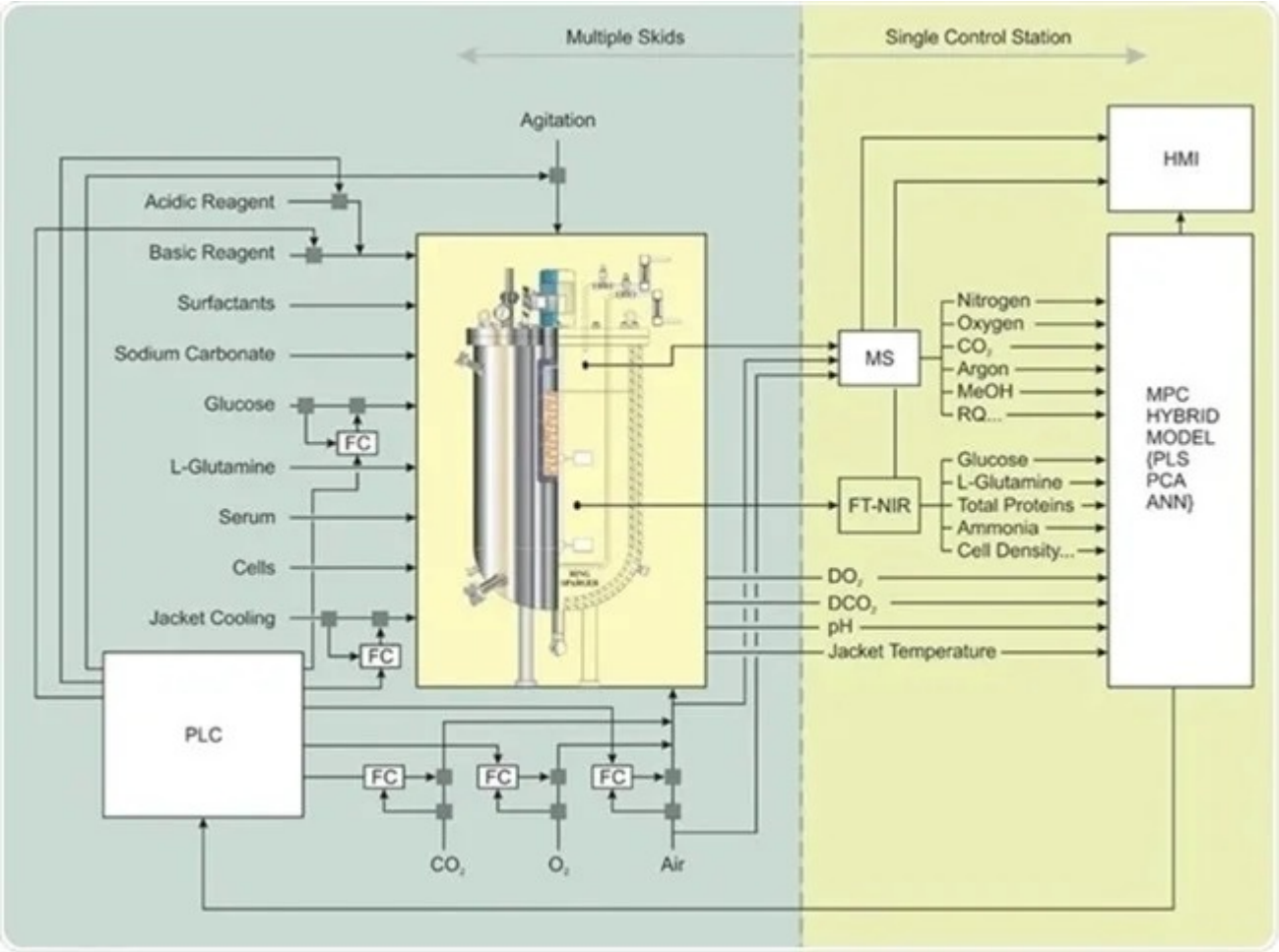

Figure 2. *Fully instrumented fermentor. Image Credit: Thermo Fisher Scientific – Environmental and Process Monitoring Instruments*

Supplying value during every stage of product development

Advanced instrumentation is necessary for the complicated manufacturing processes which are intrinsic to biotechnology in order to optimize the clear path to the final product. Mitigating risk throughout the scale-up process is the key to increasing profits.

The Prima PRO and Prima BT process mass spectrometers offer the speed and accuracy required to monitor process dynamics reliably and facilitate timely corrective action.

The Prima platform technology helps bring products to market more quickly, enhance yields and increase profits - and offers a fast return on investment, from research and development to the final product.

Acknowledgments

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Bioreactor Design and Control in the Biopharmaceutical Industry

Bioprocessing is an umbrella term that describes the research, development, manufacturing, and commercialization of products derived from or used by biological systems. For industrial purposes, bioprocessing is widely incorporated into pharmaceutical, nutraceutical, food, and beverage production processes.

Within the pharmaceutical industry, bioreactors refer to vessels and containers used to store microorganisms such as bacteria, cells, and algae. Typically, these organisms are used to produce certain biomolecules or biomaterials that can subsequently be incorporated into vaccines, medications, or genetic engineering tools[1].

Image Credit: FOTOGRIN/Shutterstock.com

Principles of bioreactor design

Several different types of bioreactors have been developed for pharmaceutical applications, the most common of which include stirred tanks, bubble columns, airlift, fluidized beds, and fixed bed bioreactors[2]. The design of each of these bioreactors depends on various factors, including the type of biocatalyst being used.

When a bioreactor is used to support cell culture growth, the continuous flow of media is essential to supporting the proliferation and survival of these cells. Stirred tank bioreactors, for example, are steel-based systems equipped with an impeller that provides mechanical agitation to homogenize media containing cells, antibodies, or enzymes within the system.

Other cell culture projects, such as those that rely on immobilized cells, may rely on fluidized bed bioreactors, in which media passes through the distributor to the cells. Within this system, velocity allows media to reach, subsequently suspend, and mix with the cells.

Within the biopharmaceutical industry, single-used bioreactors have become increasingly relied upon as they can range in size from 50 liters up to 2,000 liters[2]. Additional advantages of these systems include the ability to incorporate different filters, valves for pressure and flow control, and other ports for sensors to monitor them, particularly when used for large-scale production processes.

Control strategies in bioreactor systems

The biological nature of the microorganisms cultured in bioreactors leads to inherent variability and unpredictability. Despite these characteristics, precise control strategies, algorithms, and processes can be used to maintain bioreactor operations within desired ranges.

In almost all bioreactors, actuator levels can be controlled through various devices ranging from pumps, valves, and heaters to electric voltages and stirrer speeds. For large-scale industrial processes, proportional integral derivative (PID) controllers have been successfully used for electrical, aerospace, and mechanical single-input single-output linear systems[2]. Typically, PID controllers are used to control a single variable, such as the bioreactor's temperature or acidity, as they are not ideal for monitoring complex bioprocesses.

Since their initial development in the 1970s, distributed control strategies (DCSs) have enabled various advanced process control strategies to be controlled within a single framework. DCSs allow operators to supervise and control an entire industrial plant from a centralized control station while simultaneously gathering and storing data for control and process analytics.

As technology has advanced, control devices within DCSs have become more digital. For

example, many DCSs are now equipped with smart transmitters and actuators, each with its own microprocessor, which allows them to perform highly complex tasks ranging from autocalibration and signal conditioning to self-diagnosis on-site.

Technological advancements

Sensing technologies have become an essential aspect of modern industrial bioreactors. These sensors facilitate the non-contact and automated monitoring of numerous variables, such as pH, temperature, dissolved oxygen (DO), glucose, and lactate levels, that must be precisely maintained to ensure the sterility and quality of final products.

To monitor the pH of media, for example, porous glass electrode-based electrolyte-filled sensors, as well as optical property-based and electrochemical sensors, have been incorporated into industrial bioreactors. Maintaining temperature within a bioreactor with a precision of 0.5 °C or better is also optimal[2]; therefore, many bioreactors are equipped with temperature sensors such as thermocouples, resistance temperature detectors (RTDs), or thermistors.

Impeller speed can also be controlled and closely monitored in both single- and multiple-impeller bioreactors. Tachometers, for example, can be installed into bioreactors to sense whether desired impeller speeds are within the optimal range[3]. Importantly, uneven shear characteristics or energy dissipation of impellers can lead to the destruction of cells and microorganisms.

Challenges in bioreactor design and control

Pharmaceutical-grade industrial bioreactors are associated with numerous challenges due to the inherent complexity of biological systems housed within these devices, sterility requirements, as well as high process variability.

Products obtained from bioreactors are initially developed in smaller laboratory settings. Therefore, when scaling up these systems to meet the greater demand of pharmaceutical industry production requirements, physiological similarity must be achieved within the bioreactor. Although complete physiological similarity is not a practical goal, partial similarity in biomass growth, feeding, accumulation, and removal rates, as well as micro- and macronutrient concentrations[2], is often the goal of bioreactor designers.

Each type of biopharmaceutical-grade bioreactor is associated with different design and control challenges. Batch bioreactors, for example, experience fluctuating process conditions that require highly sophisticated control algorithms to maintain the integrity of batch reaction processes. Since each batch will begin with different initial conditions, quality control is essential to optimize process yields and titers.

Image Credit: FOTOGRIN/Shutterstock.com

Case studies

Cedarstone Industry, a Houston, Texas-based manufacturing company, offers a comprehensive range of bioreactors and fermenters for use at any stage of the biopharmaceutical development process.

To meet the highest industry standards, the Cedarstone Bioreactor / Fermentation Tank is equipped with temperature control sensors that maintain an accuracy of 0.2 °C, in addition to automated sensors that monitor pH, DO, stirring, aeration, defoaming, rehydration, and inoculation[3]. Four different types of ventilation devices can also be incorporated into these systems, depending on the chemical gas requirements, without compromising the sterility of the system.

Likewise, the Austrian technology company Zeta offers several magnetic mixers that have successfully been incorporated into different bioreactors to overcome some of the challenges associated with traditional mixing technologies. More specifically, the magnetic coupling technology of these products ensures the transmission of materials at a very high torque while reducing the risk of potential contamination to extremely low levels[4].

Future directions

As artificial intelligence (AI) has advanced at an unprecedented rate over the past several years, it has inevitably been incorporated into bioprocesses to enhance their performance and optimize their control for a wide range of applications. For example, deep learning approaches trained on anaerobic digestion (AD) sensor data have effectively predicted critical process parameters (CPPs)[5].

Artificial neural networks (ANNs) have also been explored for their ability to monitor, simulate, optimize, and control bioreactors. More specifically, ANNs appear to be ideal solutions for monitoring and optimizing the complexity of these systems while simultaneously identifying any errors to increase process reliability and performance[6]. In the near future, ANNs will likely be integrated with Internet of Things (IoT) devices and advanced sensors to transform modern bioreactors into smart, automated, and self-adaptive systems.

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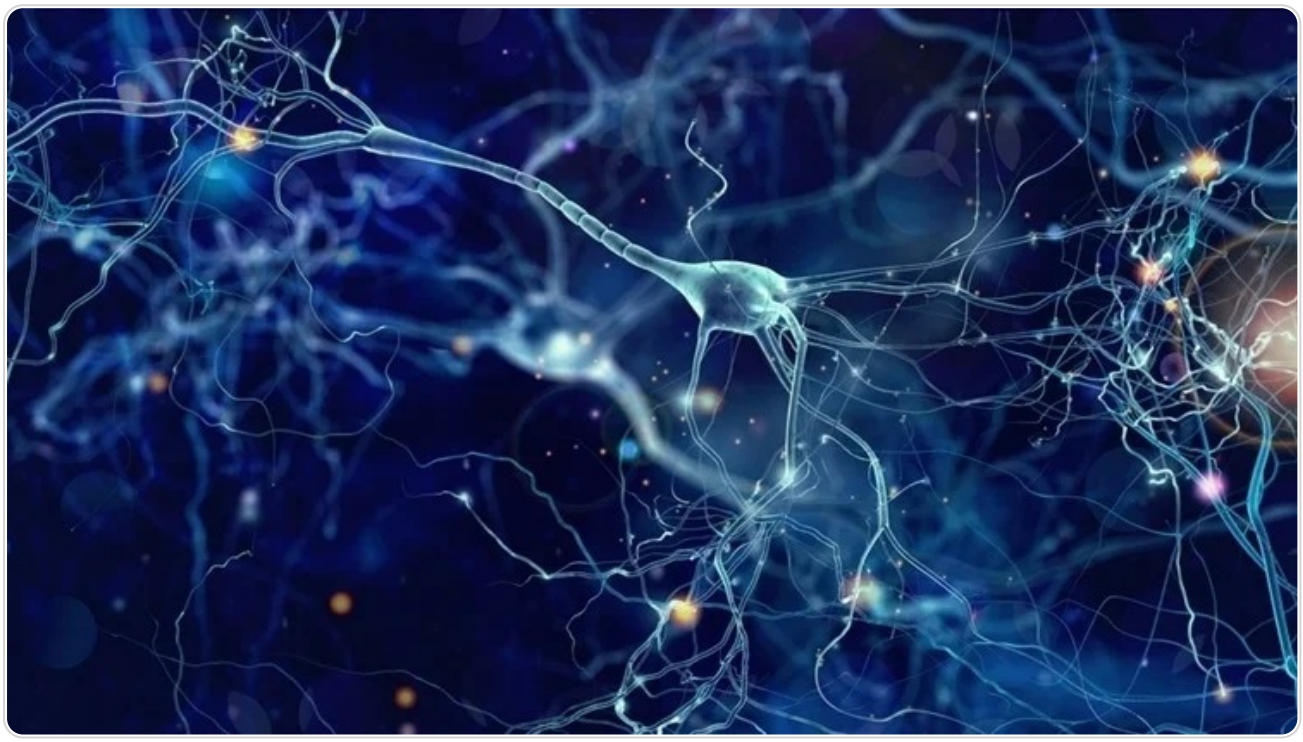
Bioprinting breakthrough: Tech platform creates 3D neural tissues in which neurons and glia connect

In a recent study published in *Cell Stem Cell*, researchers produced three-dimensional (3D) bioprinted human brain tissues, allowing for the creation of functioning neural networks that could simulate network activity in normal and pathological situations.



Study: 3D bioprinting of human neural tissues with functional connectivity. Image Credit: whitehoune/Shutterstock.com

Background

Understanding neural networks in the human brain is critical for understanding brain health and disease. However, animal-based models cannot effectively reproduce the human brain's high-order data processing due to variations in cell composition, neural networks, and synaptic integration. 3D bioprinting provides a more accurate method for creating human brain tissues by physically repositioning hydrogels and live cells inside a physiologically complicated cytoarchitecture. However, bioprinting soft tissues, such as the brain, is of concern since soft biomaterials cannot sustain intricate 3D architectures or rigid gels.

About the study

In the present study, researchers developed a 3D bioprinting platform to manufacture tissues

with defined human brain cell types in any desired dimension.

The team aimed to build layered neural tissues, including neural progenitor cells (NPCs) that generate connections inside and between the brain layers, maintaining the structure intact. They created a bioink for printing. They used fibrin gel to print the tissues. Methods for bioprinting include extrusion-based, laser-based, and droplet-based techniques. The extrusion three-dimensional bioprinting technique deposited gel in layers to simulate brain structures such as human cortex laminations.

The researchers selected a 50 mm thickness for every layer and built multi-layered tissues by placing the layers in a horizontal arrangement adjacent to one another. They designed 3D-printed brain tissues to be relatively thin but functional and multi-layered, with established cell compositions and desirable dimensions, and easily maintained and tested in a standard laboratory setting.

The researchers determined that 2.50 mg per mL fibrinogen and 0.50 to 1.0 U of thrombin were optimum concentrations for hydrogel formation, resulting in a gelation duration of 145 seconds, which allowed for 24-well plate printing. After six hours, most (85%) of the cells were viable and survived for seven days. The team created medial ganglionic eminence (MGE)-derived gamma-aminobutyric acid (GABA) and cortical (glutamate) progenitors from green fluorescent protein-expressing (GFP^+) and GFP^- human pluripotent stem cells (hPSCs) to investigate whether GABAergic interneurons and glutamatergic neurons form synaptic connections when inserted into printed tissues. Before printing, they combined the two progenitor populations in a 1:4 ratio to match the ratio of interneurons to cortical projection neurons in the cerebral cortex.

The researchers recorded electrophysiological data from tissues printed with GFP+ glutamatergic cortical progenitors, noncolored MGE GABAergic progenitors, and hPSC-derived astrocyte progenitors incorporated into glutamate neurons and GABA interneurons. The printed tissue was immunostained with an axonal marker, SMI312. They studied Alexander disease (AxD), a neurodegenerative disease caused by GFAP gene abnormalities, to investigate pathogenic mechanisms. They used live imaging of glutamate uptake by glutamate-sensitive fluorescent reporters (iGluSnFR) to investigate neuron-astrocyte interactions and neuron-glial connections in AxD.

Results

The printed neuronal progenitors developed into neurons within weeks, forming functional neural networks inside and across tissue layers. Printed astrocyte progenitors matured into astrocytes with complex processes to function in neuron-astrocyte networks. Conventional culture techniques could retain the 3D brain tissues, making them easier to investigate in

physiological and pathological settings. Cell viability declined with rising concentrations of thrombin at 2.50 mg/mL fibrinogen concentrations but remained unaltered at a constant concentration of 0.50 U fibrinogen, and cells aggregated at increased fibrinogen levels.

The bioprinted neural cells matured and retained tissue form, with GFP-expressing cells in one band transforming into microtubule-associated protein 2 (MAP2+) neurons a week after printing. The printed tissue maintained a stable configuration where neural progenitors multiplied and built neural networks. The neuronal subtypes established functional networks within the bioprinted tissues, with hPSC-derived MGE cells expressing NK2 homeobox 1 (NKX2.1) and GABA and cortical progenitors positive for forkhead-box G1 (FOXG1) and paired box 6 (PAX6). The bioprinted neural tissue constructions promote the growth of cortical glutamatergic neurons and GABAergic interneurons.

The researchers utilized a high-concentration potassium chloride solution to print tissues containing neurons and astrocytes, demonstrating functional connections. The astrocytes expressed glutamate transporter 1 (GLT-1), indicating maturation. The printed cortical and striatal neuronal bands remained intact 15 days after printing, and GFP and mCherry neurites developed towards each other. The printed human brain tissues might replicate diseased processes, with AxD astrocytes exhibiting intracellular GFAP aggregation. By 30 days, MAP2+ neurons and GFAP+ astrocytes exhibited complex morphology and synapsin expression.

Conclusion

Overall, the study findings demonstrated the ability of 3D printing to generate functioning brain tissues for simulating network activity in normal and pathological settings. The bioink-created tissues establish functional synaptic connections between neuronal subtypes and neuron-astrocyte networks in two to five weeks. The 3D platform provides a defined environment for studying human brain networks in healthy and pathological settings; however, it has limitations, such as the softness of the gel and the 50mm thickness of the printed tissues.

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Understanding dynamic microbial cultures with the Prima BT Mass Spectrometer

Researchers at Delft University of Technology, VU University Amsterdam and Radboud University in the Netherlands developed a new technique for monitoring biological rates in dynamic microbial systems using on-line measurements from a Thermo Scientific Prima BT benchtop mass spectrometer.[1]

Image credit: Shutterstock/ymd2881

Making sense of microbial worlds

Microbial communities are complex, dynamic systems in which the behavior and survival of each strain is inexorably linked to every other strain.[2] These complex microbial ecosystems can form intricate webs of metabolic activity in which the products of one strain's metabolic activity serve as fuel – or poison – for countless other organisms.

One of the fundamental tools that scientists use to make sense of these convoluted

communities is the enrichment study. In an enrichment study, a microbial community is exposed to certain selective pressures within a bioreactor. By controlling environmental factors such as temperature, light levels, pH or the availability of certain carbon sources; researchers can create conditions that are favorable to a certain strain within the culture. Over time, the culture will become "enriched" with whichever microbial strain is best adapted to the imposed conditions.[3]

Throughout the 20th and 21st centuries, enrichment studies have enabled the discovery and characterization of a huge number of new microorganisms. However, the majority of these enrichments have been carried out by imposing specific static conditions in either continuous or batch systems – holding the pH constant, for example, or inhibiting the availability of certain growth media. Recent work shows that imposing *dynamic* process conditions reveals additional microbial diversity compared to traditional chemostat or batch cultivation.[4–6]

The challenge of studying dynamic enrichment cultures

Done right, dynamic enrichments provide access to additional information on time-dependent evolution of the system, through analysis and comparison of each operational cycle.

But studying dynamic microbial systems is much more experimentally demanding than studying static ones. Cyclic changes in conditions (such as nutrient pulses) result in variable conditions within the bioreactor, and there is a certain response time before measurable parameters (such as dissolved oxygen concentration) respond to these changes. In order to gain insight into dynamic cultivation systems, researchers need to be able to examine the time-dependent properties of the system. This requires repeated measurement at short time intervals.

Enrichment studies often take place over the course of weeks or months, making short-interval manual sampling cumbersome and often impractical. Online data from liquid probes and off-gas analyzers is readily available – however, utilizing this data for detailed process characterization is often limited due to complicating physicochemical processes and inherent measurement noise.

A new mass spectrometry-based approach to on-line characterization of dynamic cultures

With a setup of eight 2.2 L bioreactors, the team of researchers demonstrated a novel technique for detailed, time-resolved analysis of dynamic cultures.

Each bioreactor in the study was equipped with four feed/effluent pumps and two acid/base pumps. pH and dissolved oxygen probes were placed in each reactor. At the heart of their measurement system, the researchers used a Prima BT process mass spectrometer for online monitoring of bioreactor in- and off-gas composition. Offering fast switching between sample streams without compromising sample quality, the Prima BT enabled the researchers to collect frequent measurements of gas composition: the in- and off-gas composition of all eight bioreactors was measured every three minutes.

Using mass spectrometry to measure continuously changing gas streams presents challenges. When gas composition remains constant, the Prima BT enables analysis of composition down to ppm levels. However, during dynamic bioreactor operation, the off-gas composition changes continuously – as a result, gas composition within the flow cell changes continuously throughout the measurement window. This typically requires a trade-off between accuracy (achieved by larger sample times) and analysis time. Because the Prima BT features a small flow cell, which is continuously analyzed at different mass/charge ratios, the researchers were able to achieve a strong compromise between the two: alternating between 10 seconds of stream flushing and 8 seconds of measurement per channel (2 seconds at 4 different mass/charge ratios), they were able to take measurements of each system in under 3 minutes with an accuracy of between 10-300 ppm depending on the gas compound mole fraction.

The team combined their rapid on-line mass spectrometry measurements with sophisticated automated data processing. Gas composition measurements were processed with a Particle Filter and kinetic process model, enabling accurate reconstruction of the dominant biological rates within the bioreactor.

This, in turn, enabled differentiation between the processes of storage compound production and biomass growth within the bioreactors.

The researchers' methods allow for accurate and time-resolved assessment of the functional behavior of long-running enrichment cultures without needing off-line samples. This work paves the way for new insights into process dynamics with minimal experimental effort.

Utilizing scanning magnetic-sector technology, the Prima BT bench top mass spectrometer offers powerful and precise gas real-time gas analyses. Features including 16-port rapid multistream sampling (RMS) and six-port automatic calibration manifold make it the ideal MS solution for biotechnology research and process development.[7] To learn more about the range of mass spectrometry systems available from Thermo Scientific, get in touch with us today.

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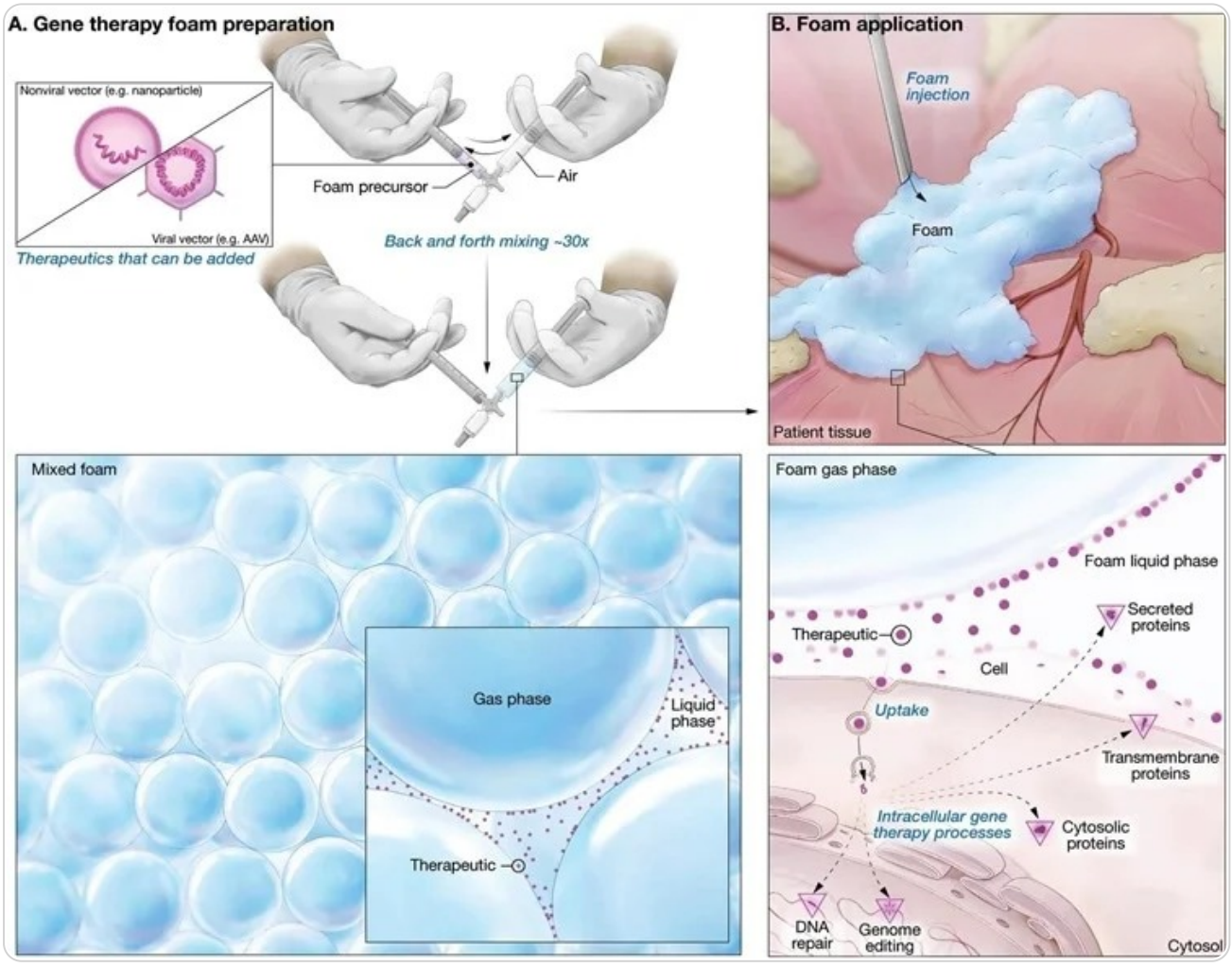
Foam technology revolutionizes gene therapy, boosting efficiency and safety

In a recent study published in the journal *Nature Communications*, researchers in the United States investigated the benefits of liquid foam as an alternative to conventional liquid-based gene delivery agents. They accessed the safety, practicality, and accessibility (including cost) of these novel vectors. Their findings highlight that a liquid foam comprised of methylcellulose and xanthan gum (both approved by the US Food and Drug Administration [FDA]) as being safe for human use) depicted transfection efficiency improvements between 2.9- and 384-fold over liquid-based approaches in nonviral gene delivery to murine model systems.

Together, they estimate that foam-based vectors can outperform liquid-based ones by preventing the leaking of the vector's DNA cargo to non-target cells (practicality), can reduce the cost of treatment by tenfold or more (cost and accessibility), and can shield the vector from the host immune system (safety), thereby preventing immune-system-mediated toxicity or oncogenesis (common concerns in conventional liquid vector-based approaches).

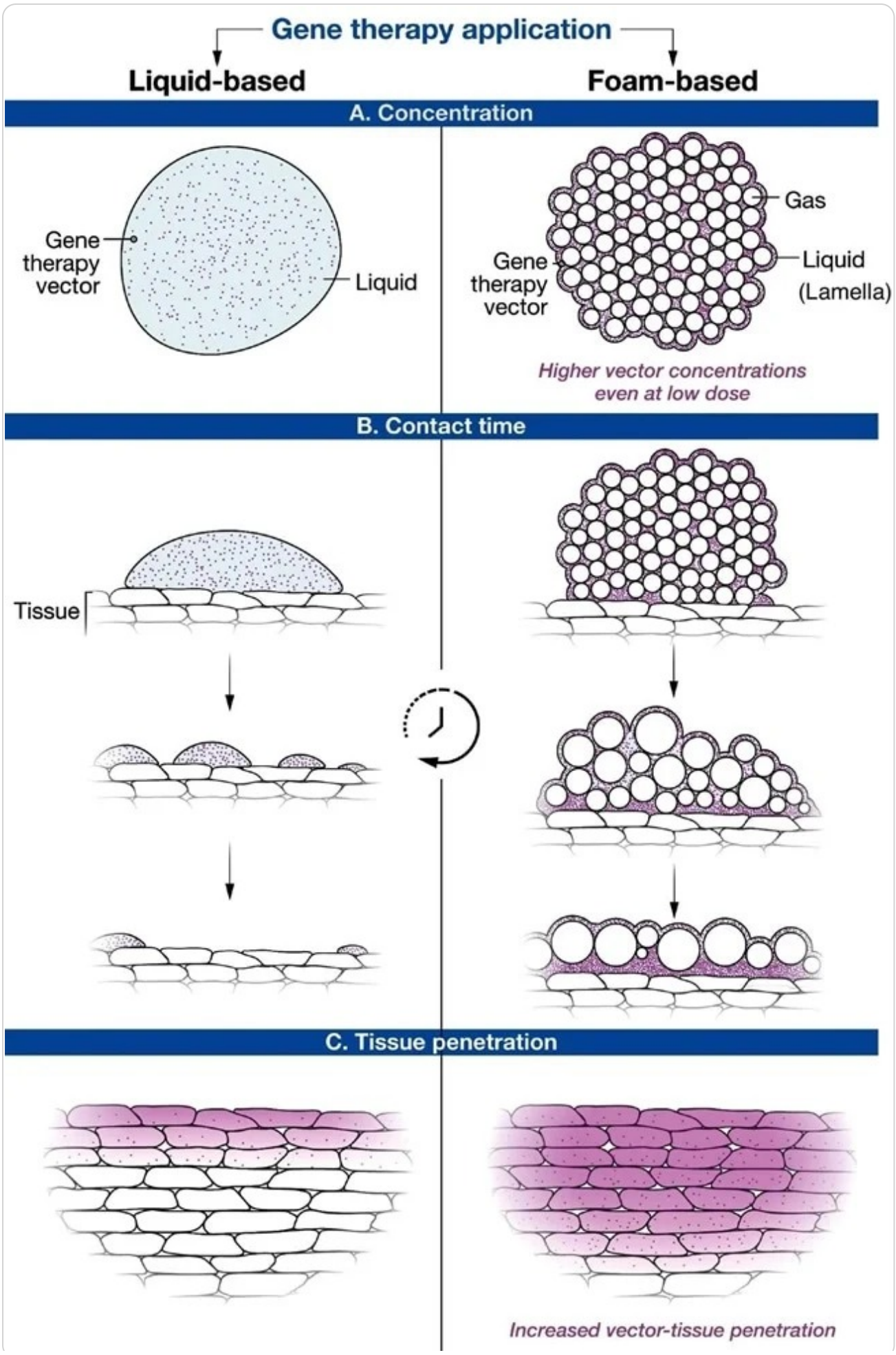


Schematic illustration depicting how gene therapy foam is freshly prepared and applied

*therapeutically to supply new genetic material or change existing DNA in cells. **A** Nonviral or viral vector (Therapeutic) is added to foam precursor in a syringe connected to a second syringe filled with air. The air and foam precursor are mixed by vigorously drawing the syringe plungers back and forth at least 30 times, creating a uniform microfoam consisting of gas bubbles separated by a network of interconnected liquid film structures called lamellae. Gene therapy vectors are concentrated in this liquid phase as the foam matures. **B** Once applied to tissue, the foam gradually deploys its therapeutic cargo and either supplies new genetic material or changes the endogenous DNA in the target cell. Study: Liquid foam improves potency and safety of gene therapy vectors*

What are foams, and how can they benefit medicine?

Foams are materials formed by the colloidal dispersion of packets of air trapped within liquid or solid layers (called 'lamellae'), with bath sponges and the head (froth) of beer being commonly observed examples. Medical researchers are taking a keen interest in foams (especially liquid-based foams) as drug-delivery systems due to their unique physicochemical properties – their large gas volumes interspersed by low-volume liquid lamellae ensure that their therapeutic payloads are concentrated in the lamellae. This, in turn, provides these materials with the advantages of high stability, sustained drug delivery at the target site, and low- to no leakage of the therapeutic agent to non-target tissue.


Gene therapy application
Liquid-based
Foam-based
A. Concentration
Gene therapy vector
Liquid
Gas
Gene therapy vector
Liquid (Lamella)
Higher vector concentrations even at low dose
B. Contact time
Tissue
C. Tissue penetration
Increased vector-tissue penetration

Schematic explaining the key advantages of foam as a gene delivery system in comparison to conventional liquid formulations. A *Foam is mostly gas, so the embedded vector particles become heavily concentrated in its liquid component, which ensures high-density exposure of target tissue to the gene therapy vector.* ***B*** *Foam remains at the application site longer, thereby enhancing the delivery of the gene therapy drug to the intended cells and minimizing unwanted off-target effects.* ***C*** *Higher vector density combined with longer contact time results in higher transfection rates and deeper tissue penetration.*

Given these advantages, a growing number of foam-based therapeutics (e.g., Varithena®, Uceris®, or Luxiq®) have entered the clinical market. Notably, these products have been validated as safe for human use (by the United States [US] Food and Drug Administration [FDA]). Encouragingly, clinical trials investigating efficacy comparisons between these foams and conventional liquid-based drug-delivery agents have revealed that the former outperforms the latter to such an extent that in most medical areas where foams are used, these novel drug-delivery agents have effectively replaced their liquid counterparts.

Despite these substantial advantages and their rising popularity across pharmaceutical and cosmetic industries, foam-based vector delivery agents have surprisingly not been investigated for clinical gene therapy applications. As the first wave of gene-correcting drugs begins to reach patients, the limitations of their liquid vectors become more apparent – liquid-based agents are prone to tissue leakage, oftentimes spilling over to non-target tissue.

This introduces many problems, including 1. Manufacturers deliberately increase drug concentrations (to account for leakage). This, in turn, 2. significantly increases the prices of these cutting-edge and extremely costly drugs, reducing their accessibility to the general public. 3. Delivery of these highly cell-type-specific gene therapies to non-target tissue has been known to cause immune-system mediated toxicity or oncogenesis, affecting the drug's safety.

About the study

In the present study, researchers investigate if foam-based drug delivery vectors' unique and often exceptional benefits extend to gene therapies, especially from the lenses of safety, efficacy, and cost/accessibility. They evaluate numerous FDA-approved foam candidates for their use as vectors, notably methylcellulose, sodium caseinate, and human serum albumin. To augment the stability and performance of these candidates, Xanthan gum was added to each.

They tested these potential drug delivery agents in the delivery of a nonviral vector (a modification of Moderna's COVID-19 mRNA vaccine, wherein the mRNA antigen was replaced with firefly Luciferase mRNA) in clinically meaningful *in vivo* murine model systems (four- to six-week-old female albino B6 mice).

> *"Cells were incubated with LNP suspensions or LNP foam for two hours, during which the culture dish was placed in a horizontal or tilted position. The horizontal setup is designed to compare gene transfer efficiencies in the absence of any liquid drainage, whereas the angled transfection mimics the more realistic scenario of patient tissue that is shifting in position and lacks defined borders that would prevent drainage of the applied therapeutics."*

Transfection efficacy estimates (measured using the intensity of Luciferase-induced bioluminescence) revealed that while all three foams outperformed their liquid counterparts across both horizontal and angled transfection mimics, the performance of Xanthan-augmented methylcellulose was exceptional. In the horizontal scenario, efficacy was estimated at 2.9 times that of liquid vectors. In the more realistic angled scenario, efficacy was an astounding 384-fold that of conventional liquid delivery agents.

> *"Furthermore, because methylcellulose foam stays in place, the transfections were spatially well-defined. This is vividly illustrated by our ability to inscribe text onto cells grown in a tissue culture plate while holding the dish vertically, which then appeared as an identical pattern of gene expression 24 h later."*

Following the selection of methylcellulose as the candidate foam of choice, researchers characterized its foam structure (bubble size, distribution, and rate of foam decay) using an automated Dynamic Foam Analyzer (Krűss Scientific DFA100FSM). They further estimated the dispersion of lipid nanoparticles (LNPs – the 'payload') visually using high-resolution confocal microscopy.

Subsequently, they used the aforementioned murine models to test foam's intraperitoneal benefits, its bioavailability and biocompatibility across multiple tissue types, and its potential for use as a carrier of viral gene therapeutic payloads (herein, Lentivirus [LV]).

Study findings and conclusions

In the first study to evaluate the potential benefits of foam-based drug delivery agents for gene therapy, researchers reveal that foam (herein, Xanthan-augmented methylcellulose) outperforms its liquid counterparts many times over. In *in vitro* horizontal models, methylcellulose depicted a close to 3-fold efficacy improvement over currently available liquid vectors. This observed foam advantage rose to 384-fold in more realistic, angled models. If even a 10-fold efficacy improvement could be realized, this would translate into substantial cost and time savings (gene therapy drugs are extremely expensive and complex to manufacture), significantly improving the general accessibility of these hitherto 'exclusive' clinical interventions.

> *"While gene therapy foam is clearly not suited for systemic infusion, the potential clinical applications of this foam platform are numerous and include improving the safety and potency of oncolytic virus therapy, enhancing vaccines, developing in situ gene therapy for gastrointestinal diseases (oral cancer, esophageal cancer, stomach cancer, colorectal cancer, autoimmune diseases that affect the digestive system), gynecological cancer, skin disease (in particular wound healing), mesotheliomas, cancers spreading to the peritoneal cavity, or any kind of in situ gene modification that requires topical application."*

Foams were also shown to persist longer at the target tissue site without any leakage commonly observed in liquid vectors, thereby further reducing the amount of therapeutic agent required for meeting dosage requirements and minimizing oncogenesis and autoimmune toxicity due to off-target events.

> *"...findings establish that liquid foam is a highly versatile delivery platform to enhance localized gene therapy. Incorporated into the clinical workflow, this platform could shift the paradigm on how topical gene therapy is applied for the treatment of a wide range of diseases."*

Journal reference:

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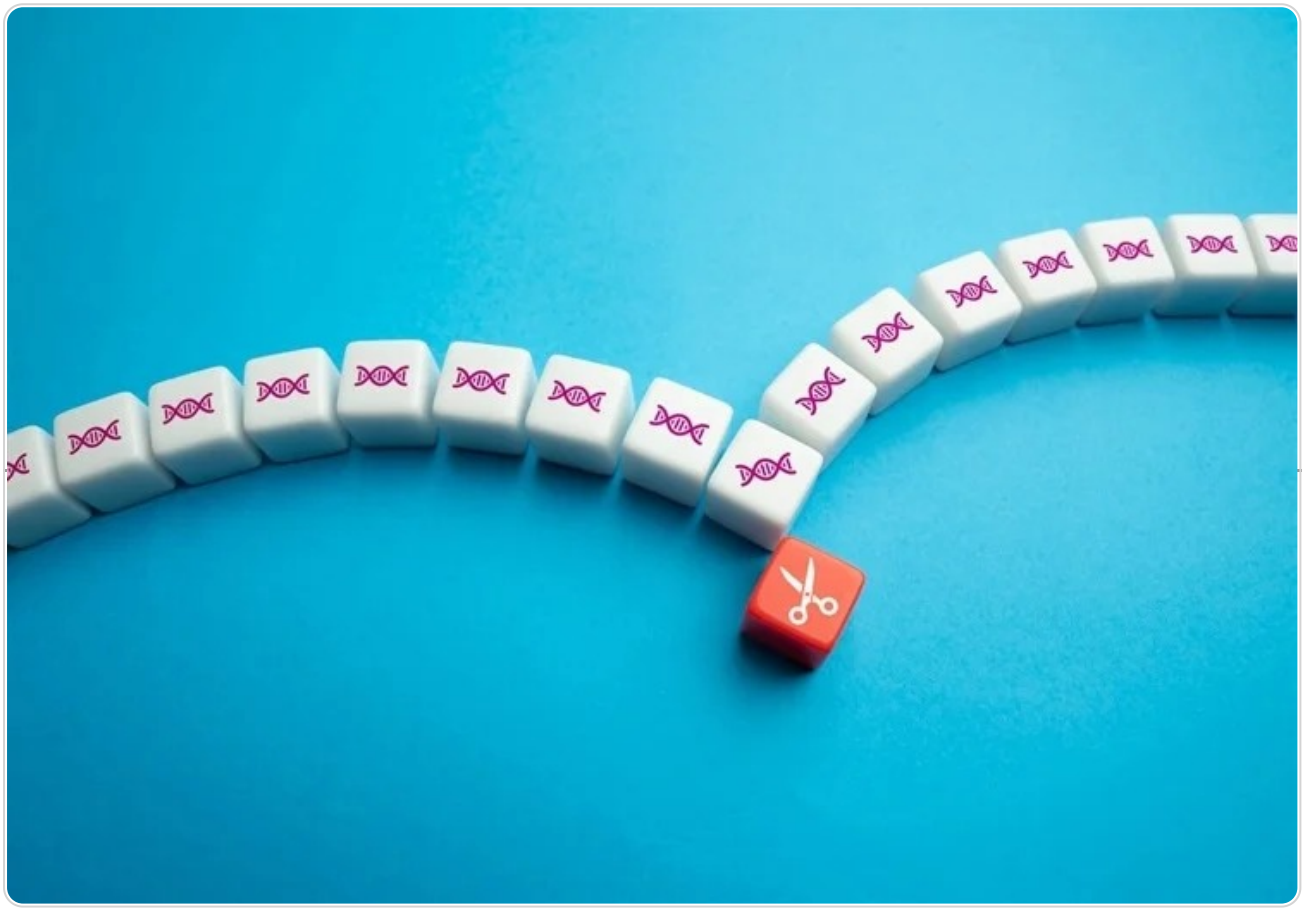
Breakthrough in gene editing: Enhanced virus-like particles promise new era in genetic disease treatment

In a recent study published in the journal *Nature Biotechnology*, researchers engineered prime editor (PE)-engineered virus-like particles (eVLPs) delivering PE proteins, PE guide ribonucleic acids (pegRNAs), and nicking single guide ribonucleic acids (ngRNAs) as ribonucleoprotein (RNP) complexes.



Study: Engineered virus-like particles for transient delivery of prime editor ribonucleoprotein complexes in vivo. Image Credit: Andrii Yalanskyi / Shutterstock

Background: The Promise of Prime Editing

Prime editing is a promising technology for changing genomic deoxyribonucleic acid (DNA) that has the potential to be used to cure genetic diseases in individuals. Prime editors are proteins that can replace a specific deoxyribonucleic acid sequence with another. PE systems necessitate three distinct nucleic acid hybridizations and are not dependent on double-strand deoxyribonucleic acid breaks or donor deoxyribonucleic acid templates.

Researchers must devise efficient and safe techniques to deliver prime editors in tissues in

the *in vivo* settings to fulfill PE's objective. While viral delivery techniques such as adenoviruses and adeno-associated viruses (AAVs) can transport PE *in vivo*, non-viral delivery techniques like lipid nanoparticles can sidestep these concerns by packaging PEs as temporarily expressing messenger ribonucleic acids.

Developing the PE-eVLP System

In the present study, researchers developed a prime editor-engineered VLP system to deliver prime editors, including ngRNAs and pegRNAs as ribonucleoproteins.

The team evaluated the PE-eVLP system in HEK293T cells, Neuro-2a cells, and Gesicle Producer 293T cells for cell culture tests. The researchers created v3 and v3b PE-eVLPs with 65- to 170-fold greater editing effectiveness in human cells than a previously reported base editor eVLP design. They used v1.2 prime editor-eVLPs with engineered PE guide ribonucleic acids (epegRNAs) and replaced the PE protein with PEmax2.

Optimizing for Efficiency: The v1.2 and v2.3 PE-eVLPs

The team used v1.2 PE-eVLPs with engineered pegRNAs to replace the prime editor protein with PEmax2, an enhanced PE that includes SpCas9 amino acid substitutions, an optimized linkage molecule between the RT domain, Cas9 nickase, nuclear localization signal (NLS) optimization, and codon optimization. They sought to identify mechanistic bottlenecks in v1 PE-eVLPs, solving the problem by relocating the nuclear export signals (NES) within the Gag protein and inserting three nuclear export signals before the site of protease cleavage of every Gag protein subdomain with two additional regions that could tolerate large insertions into the MMLV Gag-Pol.

The researchers observed that cellular mismatch repair (MMR) pathways can reduce PE efficiency and that avoiding or inhibiting MMR enhances PE efficiencies. To investigate this potential for eVLP-delivered primary editing systems, they used the v2.3 prime editor-eVLP system to insert additional close alterations at the HEK3 and Dnmt1 loci.

The researchers investigated whether the ngRNA could be packaged in the same or a different particle from the epegRNA to find the best all-in-one particle v3 PE3-eVLP system. They additionally examined whether these changes improved eVLP-mediated BE delivery. The transient introduction of PE via eVLPs reduced the capacity of v3 and v3b PE-eVLPs to facilitate in vivo prime editing in the mouse central nervous system.

Results: Advancements in PE-eVLP Technology

The research aims to create third-generation PE-eVLPs with clinically relevant levels of primary editing in the retina, protein expression restoration, and partial visual function rescue.

The researchers found and designed prime editors and engineered VLP architectures, resulting in a PE efficiency boost of 79-fold in murine neuro-2A (N2A)-type cells and an improvement of 170-fold in human HEK293T cells compared to v1 PE-eVLPs. One subretinal v3 PE-eVLP injection corrected a 4.0-bp Mfrp deletion in the rd6 murine retinal degeneration model (mean efficiency of 15%) and an Rpe65 mutation to partially release visual functions in the rd12 model (mean efficiency of 7.2%).

Key Innovations in PE-eVLP Design

The nuclear export signals promote Gag-cargo protein localization in the cytoplasm, and the p= four additional MMLV protease cleavage regions in the Gag protein enhance incomplete cleavage possibility, leading to the retaining of some Gag and nuclear export signals by PE cargo. In the human embryonic kidney 293T cells, inserting three nuclear export signals between the CA and p12 domains of Gag (nuclear export signal position number 5) resulted in the highest PE efficiency, generating v2.2 prime editor-eVLP.

The researchers used the v2.3 prime editor-eVLP technique to insert extra adjacent replacements at the human embryonic kidney (HEK3) and Dnmt1 loci, which enhanced primary editing efficiency in both cases. Inadequate epegRNA packing hampered PE-eVLP efficiency, whereas epegRNA supplementation increased PE-engineered VLP editing efficiencies by more than 8.0-fold. With v3 PE3-eVLPs, the researchers achieved 2.3% bulk cortex editing and 36% editing among GFP+ nuclei.

Conclusion: Future Directions for PE-eVLPs

Overall, the study findings showed that optimized PE-eVLPs enable transitory in vivo administration of prime editor ribonucleoproteins, improving safety and inhibiting oncogenic transgene integration. In both culture and in vivo, these virus-like particles transport PE RNPs into mammalian cells. Recent advancements in primary editing systems, including epegRNAs, PEmax design, and MMR avoidance, have resulted in better results. In vivo, the improved v3 and v3b PE-eVLPs systems corrected pathogenic deletions in the mouse retina and achieved editing levels equivalent to triple-vector AAV-PE systems in genetic blindness models. Next-generation PEs and enhanced eVLP systems will need further technical work.

Journal reference:

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