

Single-cell control: paving the way for bioprinted living tissues and organs

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The field of bioprinting, which merges biological sciences with advanced printing technologies, has the potential to revolutionise medicine by enabling the fabrication of living tissues and organs. Central to this innovation is the precise manipulation of single cells, as cellular organisation and function are fundamental to creating viable and functional tissues.

Recent advancements under the HOTBIOPRINTING project have introduced an exciting new technology that combines optical tweezers with bioprinting to capture and manipulate individual cells with high precision. The

integration of holography to generate an array of optical traps further enhances the bioprinting process, allowing for the accurate deposition of multiple cells to form complex tissue architectures.

This report examines the significance of single-cell manipulation in bioprinting, with a focus on the HOTBIOPRINTING project's contributions to single-cell capturing and printing technologies, their applications in tissue engineering, and the potential impact on organ generation and personalised medicine.

Introduction

Bioprinting has emerged as a revolutionary technology with the potential to address critical challenges in medicine, particularly in the areas of tissue engineering and organ transplantation. By using 3D printing techniques with biological materials—such as living cells, bioinks and growth factors—researchers can create functional, complex tissues that mimic the architecture and function of native organs. However, achieving the level of precision needed to replicate the intricacy of living tissues requires accurate control over the manipulation of single cells. The ability to deposit cells with high precision ensures that tissues develop the appropriate cellular interactions and architecture, ultimately enhancing their viability and function.

A recent development in the field, under the HOTBIOPRINTING project, introduces a cutting-edge approach that combines optical tweezers with bioprinting for single-cell manipulation. Optical tweezers use laser light to trap and manipulate cells at the single-cell level, offering unparalleled precision. Furthermore, holographic techniques are being employed to generate an array of optical traps for multiple-cell deposition, enabling the bioprinting of complex tissue structures. This report explores the importance of single-cell manipulation in bioprinting, with a specific focus on the innovations of the HOTBIOPRINTING project and their potential to improve the fabrication of living tissues and organs.

The role of single-cell manipulation in bioprinting

Single-cell manipulation plays a pivotal role in ensuring the proper organisation of cells within a bioprinted tissue construct. Tissues are composed of highly specialised cell types that are organised in precise patterns to perform specific functions. For example, the arrangement of endothelial cells in blood vessels, or the layering of keratinocytes in skin, is essential to maintaining the functionality of the tissue. In traditional bioprinting methods, such as extrusion-

based printing, it can be challenging to control the exact placement of individual cells within a 3D construct. However, with advances in single-cell manipulation technologies, including optical trapping, precise control over cell positioning can be achieved, enabling the creation of tissues with highly accurate cellular architectures.

By employing optical tweezers, researchers can capture and hold individual cells in specific locations with minimal mechanical force. This control over the positioning of cells is crucial for replicating the natural arrangement of tissues, such as the layering of cells in the skin or the alignment of muscle fibres in skeletal muscle. As a result, single-cell manipulation is essential for creating bioprinted tissues that not only resemble native tissues in structure but also function effectively.

Cellular interactions and functionality

Cell-to-cell interactions are fundamental for tissue development and function. Cells communicate through various signalling pathways, mechanical interactions and physical contacts. In engineered tissues, promoting the correct cellular interactions is essential to ensure that the tissue can perform its intended functions, such as secretion,

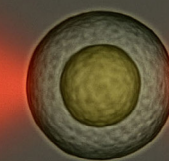
contraction or signal transduction. Single-cell manipulation enables precise control over cell positioning, facilitating optimal cell-to-cell interactions within the tissue.

In the context of the HOTBIOPRINTING project, optical tweezers are used to carefully place single cells in predefined positions. This enables the creation of tissues where cells are in close proximity to one another, allowing them to form functional cellular networks. The integration of holographic techniques to generate multiple optical traps further enhances this process by enabling the capture and placement of several cells simultaneously in a specific configuration. This approach promotes the formation of highly functional tissues, such as those required for organ regeneration, where complex cellular interactions are essential for tissue functionality.

Mimicking complex tissue structures

Native tissues and organs often exhibit intricate, hierarchical structures that are essential for their function. For example, in the heart, muscle cells are aligned in a specific orientation to facilitate coordinated contractions. In the brain, neurons form highly complex networks to enable communication and cognition. Replicating these complex structures

Single-Cell Manipulation for Bioprinting



Optical Tweezers for Precise Positioning

Figure 1: Single cell manipulation for bioprinting.

in bioprinted tissues is a significant challenge, particularly in large-scale tissue engineering. However, advances in single-cell manipulation, including optical trapping, allow for precise control over the deposition of cells, enabling the creation of more sophisticated tissue architectures.

The ability to manipulate single cells with high precision is critical for replicating the multi-layered and organised structures found in native tissues. By using optical tweezers to control the placement of each individual cell, researchers can ensure that the correct cellular architecture is achieved. The addition of holography to generate an array of optical traps further enhances this ability, allowing for the simultaneous manipulation of multiple cells to create more complex tissue structures.

The HOT-BIOPRINTING project: a new approach to single-cell manipulation

Optical tweezers for single-cell capturing and manipulation

The HOTBIOPRINTING project has pioneered the use of optical tweezers combined with bioprinting to capture and manipulate single cells. Optical tweezers utilise focused laser beams to generate a highly localised light field that can trap and manipulate microscopic particles, such as cells, with incredible precision. The ability to capture individual cells using optical tweezers is essential for single-cell bioprinting, as it enables researchers to position cells accurately within a 3D printing matrix.

In bioprinting applications, the laser's focused light is used to gently capture and hold a single cell in place without causing mechanical damage or disrupting the cell's viability. Once the cell is captured, it can be transported to the desired location within the bioprinted tissue construct, where it is deposited to form the tissue's structure. This level of precision allows for the creation of tissues with highly organised and functional cellular networks, essential for organ regeneration and other medical applications.



Figure 2: An AI driven robotic arm in a 3D bioprinting lab, precisely layering living cells to form functional human tissue. Adobe Stock © IM Imagery.

Holography for generating arrays of optical traps

A key innovation of the HOTBIOPRINTING project is the use of holographic techniques to generate an array of optical traps for the simultaneous manipulation of multiple cells. Holography allows the creation of multiple optical traps using a single laser beam by encoding complex light patterns into the beam. These light patterns create interference patterns that result in the formation of multiple optical traps at different locations in space. This technique enables the capture and manipulation of several cells simultaneously, making it possible to bioprint complex tissues with a higher level of precision and efficiency.

The ability to generate an array of optical traps provides a powerful tool for single-cell manipulation in bioprinting. By controlling the position and movement of multiple cells at once, researchers can rapidly build up layers of cells to form more complex tissue structures. For example, in creating vascular networks, multiple endothelial cells can be simultaneously trapped and placed in precise patterns to form capillary-like structures. This level of control over cell

placement is crucial for replicating the functional complexity of native tissues and organs.

Applications and potential in tissue engineering

The integration of optical tweezers and holography into the bioprinting process has vast implications for tissue engineering and regenerative medicine. By enabling precise control over single-cell placement, the HOTBIOPRINTING technology can be used to fabricate a wide range of tissues, from simple epithelial layers to more complex structures like vascularised tissues and organs.

One of the most promising applications of this technology is in the creation of personalised tissues and organs for transplantation. By using a patient's own cells, researchers can create custom tissue constructs that are immunologically compatible, reducing the risk of rejection. Additionally, the ability to print functional tissues with high precision may eventually lead to the development of bioengineered organs for transplantation, addressing the critical shortage of donor organs worldwide.

Challenges in single-cell bioprinting

Cell viability and functionality

One of the significant challenges in single-cell bioprinting is maintaining cell viability and functionality during the printing process. The application of optical tweezers and holographic techniques helps minimise mechanical stress on the cells, but other factors, such as temperature and medium composition, must also be carefully controlled to ensure that cells remain viable and functional after deposition. Ongoing research in the HOTBIOPRINTING project is focused on optimising these parameters to ensure the highest levels of cell viability and tissue functionality.

Scalability and tissue complexity

While the HOTBIOPRINTING technology holds great promise for fabricating small tissue constructs, scaling up the process to produce larger tissues or organs presents additional challenges. Larger tissues require the formation of vascular networks to ensure proper nutrient and oxygen delivery to the cells. Additionally, the creation of functional organs requires the manipulation of a wide range of cell types, each with specific requirements for growth, differentiation and integration. Overcoming these challenges will require continued innovation in single-cell manipulation and bioprinting technologies.

Conclusion

The ability to manipulate single cells with high precision is a cornerstone of bioprinting technologies, enabling the creation of tissues and organs that closely resemble their native counterparts. The HOTBIOPRINTING project has introduced a groundbreaking approach that combines optical tweezers and holography to capture and manipulate single cells, providing an unprecedented level of control in the bioprinting process. By enabling the precise deposition of cells in complex patterns, this technology has the potential to transform tissue engineering and regenerative medicine, offering new solutions for organ transplantation and personalised therapies. While challenges remain, ongoing advancements in single-cell manipulation hold the promise of a future where functional, bioengineered tissues and organs can be printed on demand.

PROJECT NAME HOT-BIOPRINTING

PROJECT SUMMARY

The innovation of HOT-BIOPRINTING lies in the development of a disruptive technology for single and automatised multiple-cell 3D bioprinting. HOT-BIOPRINTING capabilities for manipulating single cells for printing will drive a new paradigm shift: "BIOPRINTING resolution will be dictated by the cell size instead of by the mechanical component of the instrumentation". This new technological advancement for resolution enhancement but maintaining bioprinting speed using holographic automatised can open new opportunities for the tissue engineering and regenerative medicine community to respond to the demand for fast fabrication of complex microtissues and organ structures with unprecedented cell-driven resolution.

PROJECT LEAD PROFILE

Daniel Nieto García is a distinguished researcher (ERC Consolidator Fellow) at the University of La Coruña. Leader at Advanced Biofabrication Laboratory - Dnieto Lab. He has an MSc in Experimental Physics from the National University of Ireland and a PhD in Photonics and Laser Technologies (Extraordinary Award, 2012) from the University of Santiago de Compostela. He holds various professorships and postdoctoral positions at the MERLN Institute of the Faculty of Health Sciences, Maastricht University (The Netherlands), the University of Granada (Spain), the National University of Ireland (Ireland) and the International Nanotechnology Laboratory (Portugal). He has been a visiting professor in the Division of Health Sciences and Technology (HST) at Harvard Medical School-MIT, Brigham and Women's Hospital and the Institute of Biomedical Engineering at the University of Oxford (United Kingdom). In 2023, he obtained a Consolidator grant from the European Research Council (ERC) for the development of a new 3D biofabrication technology for multi-scale vascularised artificial tissues and organs.

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