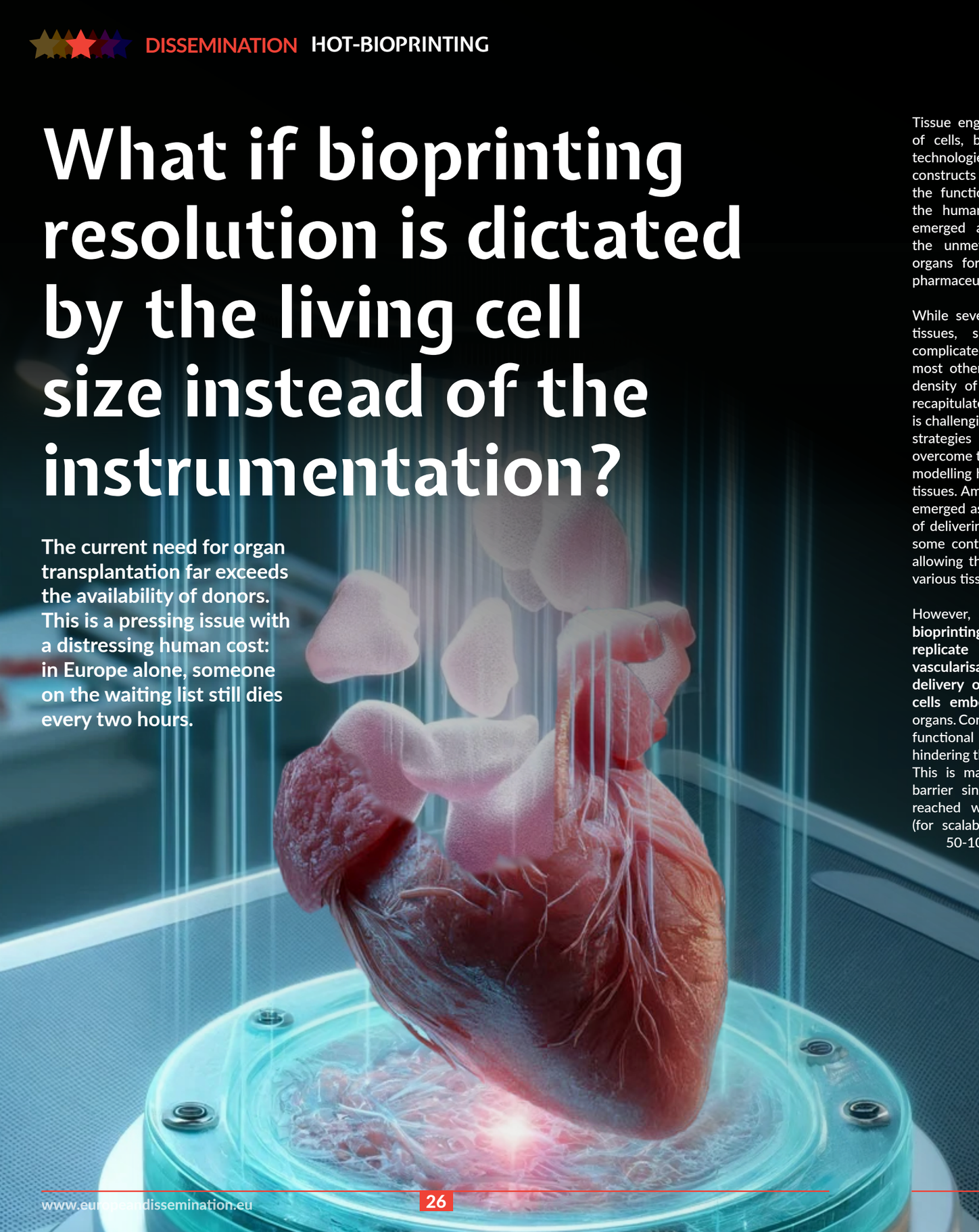
What if bioprinting resolution is dictated by the living cell size instead of the instrumentation?

The current need for organ transplantation far exceeds the availability of donors. This is a pressing issue with a distressing human cost: in Europe alone, someone on the waiting list still dies every two hours.



Tissue engineering uses a combination of cells, biomaterials and engineering technologies to fabricate biological constructs that mimic and improve the functions of their counterparts in the human body. This discipline has emerged as a promising solution to the unmet demand for tissues and organs for regenerative medicine and pharmaceutical research.

While several poorly microvascularised tissues, such as cornea, are less complicated to engineer, fabrication of most other tissues that rely on a high density of multiple cell types to fully recapitulate tissue/organ-level functions is challenging. Various tissue engineering strategies have been developed to overcome the barriers to regenerating or modelling highly complex and functional tissues. Among them, 3D bioprinting has emerged as the first technology capable of delivering cells and biomaterials with some control over spatial distributions, allowing the successful development of various tissue structures.

However, **state-of-the-art 3D bioprinting methodologies fail to replicate the intricate multi-scale vascularisation needed for the optimal delivery of nutrients and oxygen to cells embedded in** large tissues and organs. Consequently, they fail to develop functional and physiological organs, hindering the potential of 3D bioprinting. This is mainly due to a technological barrier since the maximum resolution reached with current instrumentation (for scalable printing frame time), i.e. 50-100 microns, allows neither the accurate printing of single cells nor precise control of how cells are positioned concerning each other, being these two aspects crucial for growing functional living organs, including their microvasculature.

A scientific breakthrough is needed for the successful engineering of functional tissues (i.e. with microvessel-like structures) suitable for microtissue regeneration/transplantation, as well as the construction of in vitro models to understand underlying disease causes and screen pharmaceutical compounds.

3D bioprinting is an emergent technique that allows the generation of tissues that incorporate various cell types in a complex and defined spatial architecture whose main aim is to better mimic human physiology and functions at multiple scales, from the molecular to the cellular, tissue and organ level (Kačarević *et al.*, 2018; Skardal, 2018; Dey and Ozbolat, 2020). 3D bioprinting can be used to reproduce the micro-physiological systems that reconstitute tissue–tissue interfaces and the tissue microenvironment, thus expanding the capabilities of organ-on-a-chip (OoC) models (Huh, Hamilton and Ingber, 2011; Datta *et al.*, 2020; Knowlton *et al.*, 2015; Miri *et al.*, 2018; Miri *et al.*, 2019).

The potential of generating transplantable tissue structures using bioprinting technologies has also grown with the development of new biomaterials and bioprinting processes demonstrated in various tissue structures, including vascular grafts (Hoch, Tovar and Borchers, 2014), nerve grafts (Owens *et al.*, 2015), trachea (Zopf *et al.*, 2013), muscles (Farina *et al.*, 2012), bone (Inzana *et al.*, 2014), cartilage (Gao *et al.*, 2015), heart tissue and thyroid.

Despite the significant advances in 3D bioprinting, no artificial tissue has been used to replace a part of an organ, except for simple or avascular tissues like skin or cartilage. The limitations of current 3D bioprinting technologies stem mainly from the inability to replicate the multi-scaled vasculature associated with human microtissues and organs. As a result, these technologies often fail to develop functional and physiologically realistic organs.

While much progress has been made with contact and non-contact bioprinting technologies, numerous bioprinting limitations (size, resolution and/or complexity) still hinder the real potential of 3D bioprinting to generate functional organs to cover the transplantation demand (Miri *et al.*, 2019). Almost every human body tissue requires oxygen and nutrients supplied by blood vessels. The inability of these technologies to bioprint structures of densely packed cells with controlled position and composition is thus a major barrier to bioprinting real tissues, including the multi-scale vasculature, which is essential for obtaining completely functional tissues and organs.

Most biological structures are composed of different components playing distinct roles, such as providing structural support or performing an essential function. If such structures are to be constructed by single-cell printing, it is thus necessary to print different cell types. A new approach for fast and precision single-cell bioprinting is needed to enable the manufacturing of new tissues with properties mimicking the complexity of biological systems.

Moreover, manipulating single cells is of paramount importance in other areas of biomedical research such as in vitro fertilisation, cell-cell interaction, cell adhesion, embryology, microbiology, stem cell, regenerative medicine and single-cell transfection. Examining how individual cells operate, function and interact with each other can reveal invisible processes, such as single-cell gene expression and chemical communication inside a single cell, and can elucidate connections between subcellular processes and population-level behaviour.

HOT-BIOPRINTING proposes a novel 3D bioprinting methodology able to deal with the high level of complexity in fabricating different microtissue structures with different sizes at a clinically relevant time scale (minutes). This ultra-precise bioprinting process introduces the possibility of individual cell manipulation for bioprinting multi-scaled vascularised tissues and organs, creating tissues and organs with vessels ranging from microcapillaries (3–20 microns), arterioles (40–100 microns) to the aorta (2–3 cm) with greater overall functionality. Furthermore, this free-form biofabrication with spatial organisation of cells and materials will be demonstrated to bioprint a multi-scale vascularised lymph-node tissue for this project, which has immediate implications for creating implantable tissue as a segue to whole organ printing.

Through single positioning of cells, materials and bio-inks in space and time, HOT-BIOPRINTING will also help to recapitulate cells' biophysical contexts, thus unveiling a mechanosensing mechanism at the cellular and tissue level.

Innovation

The innovation of HOT-BIOPRINTING lies in the development of a disruptive technology system named 'holographic optical tweezers bioprinting' (HOTB) for single and automatised multiple-cell 3D bioprinting. The non-contact nature of light will eliminate the failure of bioprinting associated with instrumentation compound, which, along with the HOTB 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 enhances resolution while maintaining bioprinting speed through holographic automation. It can open new opportunities for the tissue engineering and regenerative medicine community. This addresses the demand for rapid fabrication of complex microtissues and organ structures with unprecedented cell-driven resolution.

HOT-BIOPRINTING aims to surpass current bioprinting standards by addressing the following overarching objectives in the creation of human mimetic tissue models.

1. Generate the knowledge and develop a holographic optical tweezer bioprinter (HOTB) for high-definition single/multiple-cell bioprinting that can accurately print single cells from a mixture of multiple-cell sourcing candidates.
2. Demonstration and automatisation of multicellular printing for large-area tissue generation.
3. Overcome the challenges associated with existing biofabrication techniques (limited multi-scale vascularisation and oversimplified structures) that prevent the generation of functional tissues and organs.
4. Demonstrating lymph-node bioprinting with integrated multi-scale vascular network. HTBO will also be capable of recreating human cell-to-cell interactions and immune responses. This represents a big challenge that, if achieved, will revolutionise bioprinting technology by increasing the complexity of microtissue design, experimental conditions and data analysis.

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Scientific impact

HOT-BIOPRINTING will deepen into a new concept of bioprinting **resolution dictated by the cell size instead of by the mechanical component of the instrumentation**. Thus, HOTB will develop a novel methodology for single-cell bioprinting and full control of cell positioning when printing, allowing the development of fully vascularised functional multi-scaled bioprinted tissues and organs. Consequently, the project will *generate frontier knowledge* in three fields.

Bioprinting via the advancement of new concept methodology for high-definition single/multiple-cell bioprinting that can accurately print single cells from a mixture of multiple-cells- sourcing candidates.

Tissue engineering via overcoming the challenges associated with existing biofabrication techniques (limited multi-scaled vascularisation and oversimplified structures) that prevented the generation of functional tissue models, and via examination of how individual cells operate, function and interact with each other in 3D bioprinted tissue microenvironment which can reveal invisible processes such a single cell gene expression and chemical communications between subcellular processes and population-level behaviour.

Regenerative medicine bioprinted organs don't fit easily into our existing system of clinical trials, so scientists may need to develop new systems of preclinical and clinical trials to test the safety of bioprinted tissues in humans.

HOT-BIOPRINTING via precise positioning of cells, materials and bio-inks in space and time, will help to recapitulate cells' biophysical contexts, thus unveiling mechanosensing mechanism at the cellular and tissue level that can be used to elucidate the intrabody response of bioprinted tissues for organ regeneration and transplantation.

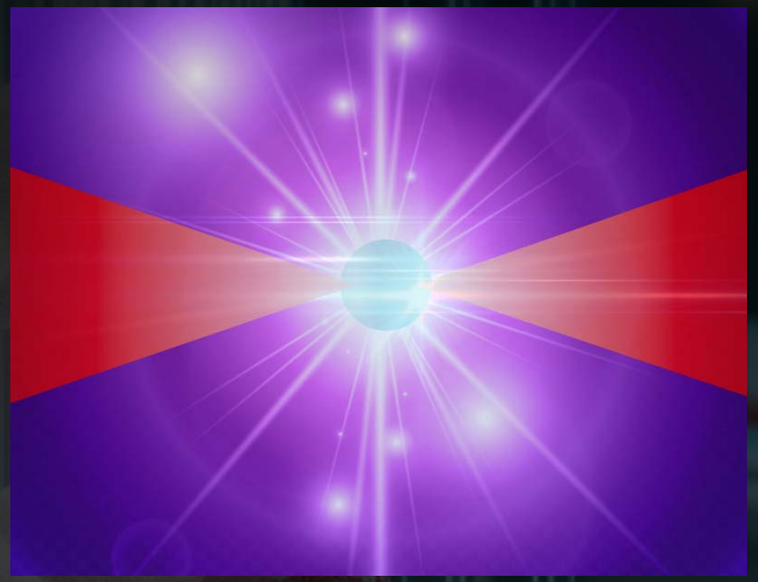

Figure 1: Image illustrating the optical tweezers concept.

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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 automatisation can open new opportunities to the tissue engineering and regenerative medicine community for responding to the demand for fast fabrication of complex microtissues and organ structures with un-precedent 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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