Cancer Letters 504 (2021) 116–124

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Emerging biofabrication approaches for gastrointestinal organoids towards patient specific cancer models

Fernando Soto [a,1], Carlos F. Guimarães [a,b,c,1], Rui L. Reis [b,c], Walfre Franco [d,e], Imran Rizvi [f,g], Utkan Demirci [a,*]

[a] Canary Center at Stanford for Cancer Early Detection, Bio-Acoustic MEMS in Medicine (BAMM) Laboratory, Department of Radiology, School of Medicine Stanford University, Palo Alto, California, 94304-5427, USA
[b] 3B's Research Group, Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal
[c] ICVS/3B's, PT Government Associate Laboratory, University of Minho, Braga/Guimarães, Portugal
[d] Department of Biomedical Engineering, University of Massachusetts, Lowell, 01854, MA, USA
[e] Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, 02114, MA, USA
[f] Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC and North Carolina State University, Raleigh, NC, 27599, USA
[g] Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC, 27599, USA

ARTICLE INFO

Keywords:
Bioengineering
Organ on a chip
Self-assembly
Externally driven assembly
Personalized medicine

ABSTRACT

Tissue engineered organoids are simple biomodels that can emulate the structural and functional complexity of specific organs. Here, we review developments in three-dimensional (3D) artificial cell constructs to model gastrointestinal dynamics towards cancer diagnosis. We describe bottom-up approaches to fabricate close-packed cell aggregates, from the use of biochemical and physical cues to guide the self-assembly of organoids, to the use of engineering approaches, including 3D printing/additive manufacturing and external field-driven protocols. Finally, we outline the main challenges and possible risks regarding the potential translation of gastrointestinal organoids from laboratory settings to patient-specific models in clinical applications.

1. Introduction

The diagnosis and treatment of gastrointestinal (GI) cancer remains a challenge for clinicians and researchers, as most cases are asymptomatic until they reach advanced stages due to a lack of sensitivity and specificity to detect premalignant lesions. The heterogeneous and stochastic distribution of gastric tumors highlights the unmet need to develop early cancer detection models that are patient-specific to provide better care. The development of GI cancer models that mimic native tissue could enable clinicians to accelerate discovery and testing of novel screening and disease treatments. Key advances have enabled GI models to capture important features of normal physiology and disease [1]. These advances include significant progress in culture methods for primary cells, improved understanding about the importance of biochemical cues, chemical gradients and physical forces in GI physiology. Simultaneously, development of engineering methods to capture these gradients and physical forces has led to increasingly sophisticated *in vitro* models as organ-on-a-chip devices that can simulate the functionality, mechanical factors and physiological response or an organ within a single microfluidic interface. When designing early cancer models, choosing the right platform becomes important. *In vitro* 2D monolayer cell cultures provides a simple and easy-to-use approach, while lacking of the shape and functionality of the corresponding tissue; thus, they are not considered a translatable model [2]. In contrast, animal models provide the complexity of GI fluid dynamics and interactions with the biome. The use of animal models, however, requires complex and expensive research protocols that reduce their potential throughput, as well as their natural deviation from human physiology. Recent developments in tissue engineering aim to bridge the gap between *in vitro* and *in vivo* models, and provide unique advantages by using direct self-assembly or bottom-up assembly approaches to create three-dimensional (3D) artificial cell constructs that mimic the structure and function of specific organs [3].

Organoids present important advantages that make them attractive

* Corresponding author.
E-mail address: utkan@stanford.edu (U. Demirci).
[1] F.S and C.F.G. contributed equally to this work.

https://doi.org/10.1016/j.canlet.2021.01.023
Received 11 November 2020; Received in revised form 14 January 2021; Accepted 23 January 2021
Available online 9 February 2021

models. These advantages include the fact that they are simple to work with, mimic the 3D structure of native human tissue closely, reduce experimental complexity, and are compatible with common bioanalysis methodologies, such as imaging. Thus, in general, organoids provide robust models of disease. An organoid is a multicellular unit derived from cells that form 3D structures to simulate a native organ/tissue development, functions and structure [4]. Many fabrication methods aim to create close-packed cell aggregates that undergo a fusion process to generate specific organoids. The development of engineered GI organoids presents unique fabrication challenges and requirements, as the GI tract ranges from esophagus to anus, and includes the various organs of the digestive system (Fig. 1) (see Fig. 2).

This review focuses on emerging trends in the biofabrication of GI organoids and their use as patient-specific cancer models. Recent reviews have covered different aspects of GI organoids while focusing mainly on biological aspects such as the source or differentiation of the organoid [5–7]. Herein, we aim to provide a multidisciplinary perspective to discuss the challenges and opportunities of various fabrication approaches, ranging from the application of biochemical and physical cues to guide the self-assembly of organoids, to advanced engineering protocols (additive manufacturing and external field-driven scaffoldless assembly) to enhance the throughput and design capabilities towards generating constructs that better mimic native tissues. Moreover, after providing a summary of different fabrication approaches, we outline main challenges and potential risks regarding the translation of gastrointestinal organoids from laboratory settings to the use of patient-specific models in the clinic.

2. Cell sources for GI organoid generation

There are two global parameters to consider when fabricating a 3D structure that recapitulates or aims to mimic the GI tissues generated through physiological development: First, the choice of cell(s) for the formation of the organoid; second, the cues that are to be provided for the selected cells to proliferate, differentiate, and organize into tissue-like architectures [8].

The available cell choice in organoid fabrication is relatively limited but can still vary, depending on the assembly mechanism. Adult differentiated cells can be used for direct assembly of organoid structures (e.g., by bioprinting) [9]. However, for cases where a more developmental route is envisioned, the cell source relies heavily on the need for proliferation and differentiation responses to derive multiple cellular subtypes with distinct functionality. For that, the choice usually involves stem cells [10,11].

Pluripotent stem cells can differentiate into any adult cell type and are extremely sought after for multiple purposes [12–14]. The naturally occurring pluripotent stem cells are Embryonic Stem Cells (ESCs), which exist only in the embryo, present significant ethical constraints and are very hard to translate into the clinical setting [15]. As an alternative, induced Pluripotent Stem Cells (iPSCs) have similar differentiation capacity and can be derived from adult and patient-specific cells [16,17]. GI organoids have also been extensively obtained with the use of iPSCs [18].

In adult organisms, stem cells naturally reside within specific tissues and can proliferate to generate different subtypes of cells and structures (multipotent). In the intestine, these are commonly called Intestinal Stem Cells (ISCs). ISCs are frequently identified by the Lgr5 (Leucine-rich repeat-containing G protein-coupled receptor 5) marker. This receptor is fundamental for embryonic development and is also expressed by various adult stem cells, highlighting the role of Lgr5 as a bona fide marker of stemness. Despite having varied biological functions, Lgr5 is often upregulated in cancers from various tissues [19]. In the normal intestine, Lgr5 is most notably expressed by the crypt cells that are capable of gradually differentiating and replace the tissue's lining over

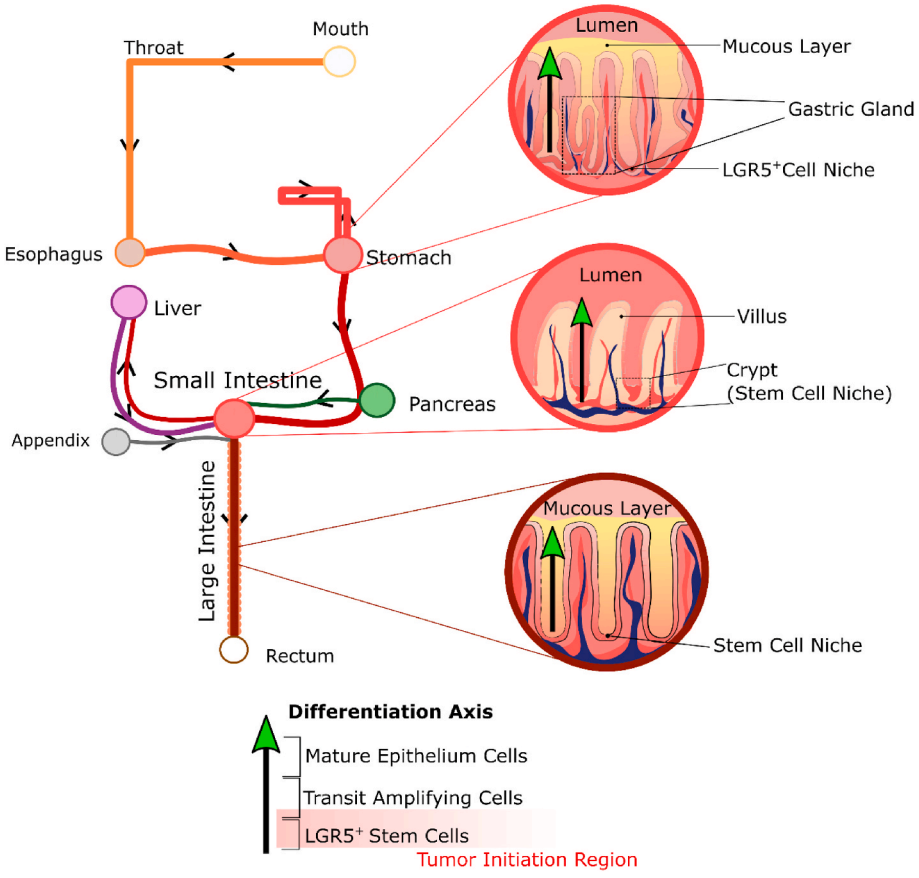

Fig. 1. The GUT Network: Schematic organization of the Gastrointestinal System and its main components connected in an anatomically relevant manner. The main tissues approached so far with organoids (stomach and intestine) are amplified to represent the details of the distinct epithelial structures, as well as common features such as the presence of crypts, where stem/progenitor cells are present. In all cases, the top-bottom axis represents the differentiation route cells from the crypt take until becoming mature epithelial cells, as represented in the differentiation axis schematics. The region of accelerating stem cell proliferation and differentiation is where recurring cell divisions occur to regenerate top layer's tissue loss, hence prone to the emergence of mutations that may lead into pre-neoplastic changes. As such, it is here identified as "tumor initiation region". It is in this region that $LGR5^{+}$ cells reside, which are adult stem cells that are capable of proliferating and differentiating along the epithelial axis, with higher potential for initiating tumors.

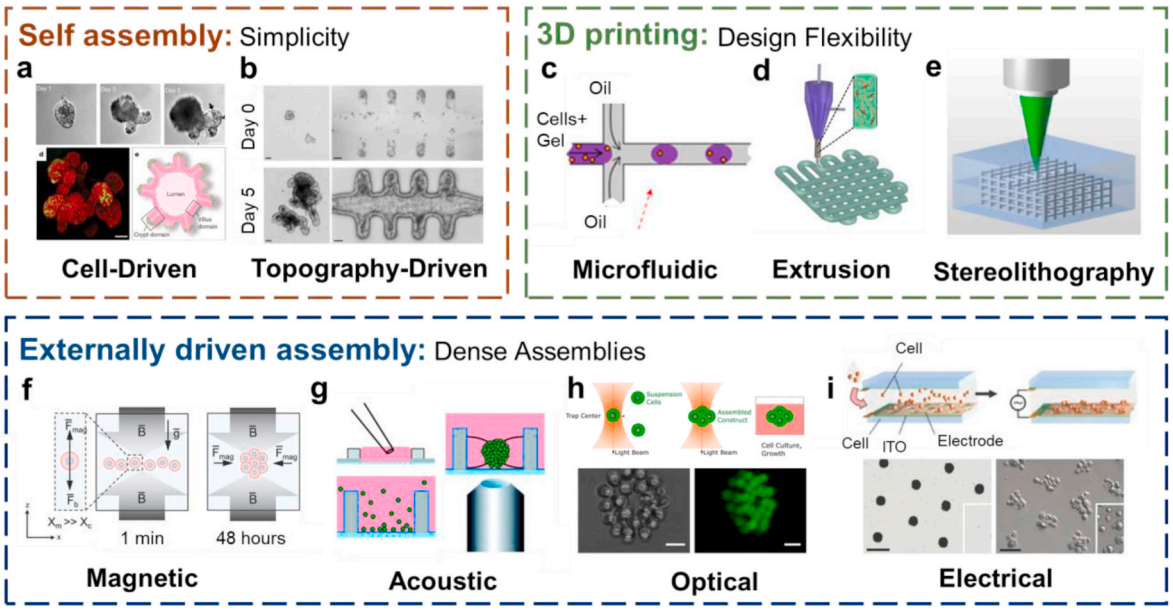

Fig. 2. Emerging 3D biofabrication approaches a) Biochemical cues to induce differentiation. Reprinted with permission of ref. [19] b) use of physical scaffolds to generate crypt structure. Reprinted with permission of ref. [54] c) Continuous flow based microdroplet filled with cells. Reprinted with permission of ref.[56] d) Extrusion based deposition of cell-loaded bioink. Reprinted with permission of ref. [58] e) Stereolitograpy based cell-loaded tissue construct. Reprinted with permission of ref. [62] f) Magnetic levitation-based assembly. Reprinted with permission of ref. [74] g) Acoustic node spheroid formation. Reprinted with permission of ref. [77] h) Optical tweezer-based cell assembly. Reprinted with permission of ref. [89] i) Electrically driven assembly of cell aggregates. Reprinted with permission of ref. [94].

time. Indeed, these cells can single-handedly create crypt-villus architectures, even in the absence of a mesenchymal niche [20]. Similarly, $Lgr5^{+}$ cells are also present in other GI tissues, such as those of the stomach. However, certain gastric stem cells were shown to be $Lgr5^{-}$, depending on their anatomic location [21–23]. This evidence demonstrates that there might be more complex molecular signatures of GI cell stemness other than the most widely studied Lgr5, and that different types of cells and molecular signatures might play an important role in the development of cancers. Overall, the diverse pool of gastrointestinal stem cells enables different approaches where specific subtypes might be selected to better recapitulate certain tissues of the GI tract, either healthy or cancerous.

Naturally, when stem cells are used, their differentiation must be properly directed towards the desired phenotypes avoiding unwanted behavior such as, tumor formation by uncontrolled pluripotent stem cell proliferation/differentiation [24]. Therefore, once the cellular entity is chosen, the next question must focus on the required stimuli for GI development. On one hand, biochemical cues are extremely important and different protocols have been studied and optimized to integrate the most essential soluble components for stem cell differentiation towards GI-like organoid formation. On the other hand, physical cues have gained increased importance as equivalent regulators of tissue development, with force and shape being primal parameters for cell differentiation, orientation and functional development [25,26].

3. Biochemical cues

The protocols that must be employed to differentiate stem cells might vary considerably depending on the potency of the initial cell source. While adult ISCs are already close to the GI phenotypes, iPSCs are able to differentiate into almost any type of adult cell regardless of origin or location and, as such, must go through more extensive and selective differentiation. Derivation of iPSCs is achieved through the transformative insertion of particular genes, the so-called Yamanaka factors. These factors are comprised of four genes: Oct4, Sox2, Klf4 and cMyc. By inducing their expression in adult cells, such as skin fibroblasts, these cells can revert to a pluripotent state (i.e. become iPSCs), as widely established elsewhere [16,17]. As such, this section focuses on how iPSCs can be used for GI organoid derivation. Initially, iPSCs must be directed towards endodermal tissue lineages in order to differentiate towards gut-like spheroids, eventually maturing into organoids [27]. The first steps rely heavily on developmental signals such as Wingless-related integration site (WNT) and Fibroblast Growth Factor (FGF), which can bring these stem cells closer to gut lineages, from where organoid differentiation can start [27–29]. This differentiation is also guided by specific biochemical factors, among which are Hepatocyte Growth Factors (HGFs) and Epidermal Growth Factors (EGFs) [27,30]. Of note, several molecular signaling pathways, including those related to WNT, are also intimately related with cancer and play a significant role in the genesis of cancer stem cells [31,32].

Naturally, these signals attempt to mimic the natural cell-cell signals during development and also converge with the process of ISC organoid formation [7], where EGF is also commonly employed [33]. Additionally, certain small molecules such as Noggin and R-Spondin are widely implicated in organoid creation. R-Spondin is the ligand of the previously discussed Lgr5 receptor, and is capable of stimulating stem cells for proliferation and organoid development, while Noggin antagonizes Bone Morphogenic Protein (BMP)-induced differentiation, facilitating the maintenance of stemness [33–35].

Although the fabrication of different types of intestinal and gastric organoids relies on variations in the biochemical factors and the timing of their addition as well as duration of exposure, these are mostly shared throughout the GI tract. WNT, FGF, EGF, retinoic acid and noggin are also involved in obtaining iPSC-derived structures [36], even though variations in the protocol timing might be needed [27,36]. Generally, organoid differentiation protocols can last well over 21 days into months [27]. As such, approaches that might accelerate this process, discussed later in this review, namely externally controlled assembly, can significantly impact organoid research by shortening their extensive differentiation times, increasing throughput.

The signaling pathways involved in these developmental processes are naturally complex and still under discovery. Among these pathways is signaling mediated by retinoic acid, which was recently reported to improve barrier function in epithelial monolayers [37] and has

significant impact on cell-fate transitions of the intestinal epithelia and *in vivo* regeneration [38]. The complexity behind intestinal organoid derivation leaves considerable room for further optimization, even though the main biochemical tools and protocols are already at our disposal. We must also consider physical signals, namely their spatial variation and their role in further governing GI organoid formation. A recent study demonstrated the formation of a physiologically-relevant model for intestinal epithelium using colonic crypts isolated from human biopsies taken during routine colonoscopies [39]. The crypts were cultured in arrays of microfabricated collagen scaffolds with invaginations created by polydimethylsiloxane (PDMS) stamps designed to support a biologically informed shaped epithelial monolayer. Gradients of cytokines, metabolites and growth factors were applied to these cultures to induce differentiation or to maintain the features of stem cells across relevant sections of the shaped monolayer. It was shown that the combination of the engineered invaginations to support a shaped monolayer along with chemical and molecular gradients resulted in an *in vitro* model for human colon crypts from patient-derived samples that recapitulated physiologically relevant tissue polarity, cell migration, architectural features, among other metrics. In fact, the role of purely physical parameters such as force and shape also play a defining role in organoid assembly, as discussed in subsequent sections.

4. Physical cues

For this discussion, we will divide organoid formation into two primary approaches. The first is self-assembly, i.e., the process that gives cells full autonomy to generate spheroid and organoid structures, most commonly accomplished with Matrigel either in 3D hydrogels or liquid hanging-drops [36,40]. The second is a topography-driven assembly, which is achieved by combining cells with pre-fabricated architectures that enforce a certain degree of cellular organization, potentially pushing the process to a more advanced start [41].

In self-assembly protocols, the goal is to provide the cells with an environment that fosters proliferation, differentiation, and organization. Such an environment has been described as biolabile [26], representing the ease with which cells can manipulate their surroundings to approximate natural development. Of note, biological tissue development occurs from a soft to stiff mechanical environment [26], and the formation of GI organoids is no exception [42,43]. As previously referred, Matrigel has been widely used as a soft, biologically derived 3D hydrogel for organoid generation over many decades [44]. Matrigel has also been used for hanging-drop intestinal organoid fabrication, a well-known method where cells aggregate by gravity within a suspended drop of medium, potentially supplemented with Matrigel to promote cell aggregation [40]. However, the difficulty in defining Matrigel composition and well-described batch-to-batch variation have continuously driven the community towards more reliable alternatives [45]. Overall, different hydrogels have been studied to obtain soft 3D environments for organoid development, most within the 200–1000 Pa stiffness range [33,34,46,47], using materials from both synthetic [33,34] and natural origins [48].

In fact, in the search for these “designer matrices” [33,34], scientists have been looking not only to 3D stiffness but also to the relevance of cell-adhesive sites. Commonly present in Matrigel and also in decellularized ECM-derived hydrogels [49], the ability of cells to adhere to the material is not a straightforward parameter in organoid formation. Even though most approaches integrate cell adhesive moieties within hydrogels [47], it has been demonstrated that non-adhesive environments, such as soft alginate hydrogels, could promote intestinal organoid maturation and functionality to an extent that is similar to that of Matrigel (adhesive) [48]. This suggests that cell-centered organoid formation is mostly dependent on adequate mechanical support and environmental softness, and adhesiveness might not be as critical as initially thought.

Naturally, most of these studies attempt to reconstruct the healthy GI environment, but the focus might be on recapitulating diseased tissues, namely GI cancers. Tumor tissue is often stiffer and more fibrotic than healthy tissue [26]. As such, when translating organoid formation to the study of cancer as well as to disease modeling, certain changes must be considered. First, the cells should be from cancerous origins, and represent similar genetic/phenotypic alterations [50]. Second, the cues which are provided to these cells might need to be rethought. For example, increasing matrix modulus to match increased tumor stiffness, as well as introducing hypoxia to the environment, have both been shown to positively favor GI cancer organoid formation [51], contrary to what happens in healthy scenarios.

While soft 3D gel environments are clearly important for self-assembly based organoid generation, using prefabricated GI-like surfaces as scaffolds for organoid formation might function in distinct mechanical stiffness ranges (e.g., soft, or rigid). To achieve well-defined crypt-villus architectures and cell-lineage compartmentalization, pre-shaped collagen scaffolds with stiffness around 9 kPa (9–10 times higher than 3D hydrogels) were employed [52,53] and PEG-based structures with similar architecture on the 40–90 kPa range also led to proper intestinal-like cell phenotypes and functionality [41]. Indeed, it was previously reported that matrix stiffness could have an important impact on ISC differentiation [33]. When deriving an organoid from single-cell approaches, a soft mechanical microenvironment (sub-KPa) appears to be fundamental for proliferation and maturation. However, when accelerating the process towards pre-engineered GI-like surfaces, stiffer environments might be considered as cells adhere to a 2D surface instead of expanding within a 3D restrictive environment. Researchers recently demonstrated that pre-shaped microfluidic chips within sub-kPa stiffness could also form highly complex mini-intestines on-a--chip [54]. In this work, the integration of crypt-villus architectures within a microfluidic chip added a new layer of complexity by creating an open-flow environment that mimics the natural GI cavities, unlike classical organoids that present closed cavities that hinder lifespan, experimental setups and homeostasis [54]. Together, these results demonstrate that classical organoid assembly methods might lack the required complexity to fully recapitulate living tissue dynamics, which allow proper modelling of healthy or diseased organs. As such, we next explore how distinct state-of-the-art techniques for 3D biofabrication might be employed to advance organoid assembly to higher levels of shape complexity, as a way to better model physiological events, and efficiency, as a way to accelerate organoid differentiation.

5. 3D printing/additive manufacturing

Biochemical and physical differentiation methods are employed in combination with microfabrication methodologies to design and fabricate increasingly complex geometries and designs, enabling rapid prototyping and the ability to explore multiple designs and variables. Although some of the fabrication methods that will be described in the following sections do not directly report the fabrication of gastrointestinal organoids, they serve as an introduction point towards future gastric organoid designs that employ programmable bioassembly based on the combination of diverse fabrication methodologies.

The fabrication of organoids using microfluidic devices has been widely reported. Continuous flow-based microfluidics relies on trapping liquid flow between another immiscible flow, allowing to encapsulate different components or cells into microdroplets [55]. The proliferation of stable cell assemblies relies on the chemical and physical environment and types of cells. The microdroplets can serve to encapsulate a few living cells within tunable physical and chemical parameters that enable the formation of organoids [56]. The main advantages of these methods are their inexpensive and high throughput mini reactors that use small liquid volumes where a single droplet can contain nutrients, support materials and biochemical signals introduced by multiple inlets of continuous flows [57].

3D printing of tissue commonly relies on extrusion or photo

crosslinking mediated bioprinting based on bioinks that serve as extracellular matrices for living cells to form simplified organ models [58]. The automated and continuous deposition of the mixture over a substrate is driven by extruding the material in the shape of a continuous filament. The deposition position is dictated by a predetermined pattern using computer-aided design (CAD). The bioinks employed in bioprinting consist of hydrogels that can be designed to promote cell differentiation. The bioinks can serve as a support for cells as well as sacrificial (removed from final design) and/or functional layers that contribute to the final 3D shape of the assembled organoid. As bioinks are optimized for commercial distribution, their reliability has increased, enabling homogeneous cell concentration in each print. Nevertheless, most of these inks can be expensive and proprietary, limiting further optimization to adapt to other uses and applications. Combining multiple printing nozzles has resulted in the promising capability to create vascularized tissue assemblies by the sequential printing of cells and vascular networks. We note that the resolution of this type of 3D-printer relies on the diameter of the printing nozzle and deposition pressure; thus, not all bioprinters that are commercially available are capable of direct printing of organoids.

Multiphoton laser lithography [59] and stereolithography [60] based biofabrication use rely on optical energy sources to photocrosslink a bioink or resin (commonly based on acrylate hydrogels) containing living cells over a substrate. This method offers high-resolution fabrication with micron size limit and could be considered a digital fabrication approach, as the layer-by-layer deposition of voxel-based patterns can be differentiated, minimizing material requirements when compared to extrusion methods. Two-photon lithography can generate micron-size structures that present topographical cues which are able to regulate cell differentiation depending on shape and material selection [61]. Stereolithography methods are commonly faster than laser-based bioprinting due to their large working area provided by digital light micromirror array projectors that direct the photo-crosslinking regions [62]. A layer-by-layer adhesion of different segments constructs the bioassembly, thus the fabrication time depends on the number of Z-layers. Stereolithography methods require planning and optimization as the UV light used in some of these approaches can damage cell DNA and the photocuring agents can produce toxic side effects. These toxicities have been overcome, in part, by fine-tuning different conditions, including concentration, pH, temperature and reaction time.

With such a diverse toolkit, bioassembly has permitted the development of customized organoid fabrication with complex geometries [63]. Micron-sized positioning and the placement of organoids has been reported. However, the size of organoid constructs depends on a variety of non-trivial parameters, such as nozzle diameter for extrusion methods and Z resolution for layer-by-layer methods. Moreover, there are certain limitations for using bioprinters, as these methods might entail long fabrication times, as these types of additive manufacturing methods require building the structure one layer at a time. Bioprinting methods can also induce stress to live cells due to exposure to heat, shear stress and chemicals used to stabilize the bioinks. The cell density of the organoid assembly can be reduced by diluting the cells into the support bioinks to enable the optimal viscosity and mechanical proprieties to print.

6. External field driven assembly

External field-driven assembly offers a promising scaffoldless fabrication approach to drive and direct the rapid assembly of tissue engineering, offering complimentary design opportunities [64–66]. External fields generate physical forces that push cells intro predetermined shapes, which can be stabilized using crosslinking hydrogels. In general, preconcentration of cells into confined spatiotemporal regions provides a higher packing density and dynamic reconfiguration of the cell assembly in real time compared to passive fabrication approaches. The main external fields used in microfabrication include magnetic, acoustic, optical, electrical and combinations of these fields [67,68].

Magnetic assembly can manipulate and assemble cells into organoids and tissues. The main methods rely on applying oscillating magnetic fields to push the assembly into predetermined designs. Individual cells must be labeled with magnetic materials in order to be manipulated by an external magnetic field, commonly generated by a Helmholtz coil. (an electromagnet device capable of generating localized magnetic fields) [69,70] These fields are ideal when high spatiotemporal control is desired, although the need for trained technicians and specialized equipment could limit the widespread use of this approach. Magnetic levitation utilizes a different approach by introducing individual cells within a magnetic gradient, which repel the cells from magnetic fields [71–73]. In order to achieve this, cells are submerged in a paramagnetic medium placed in between a permanent magnet. Depending on their magnetic susceptibility and other properties (e.g., density, size, compressibility), cells will levitate into different 3D-planes and form engineered tissues [74]. Magnetic levitation currently offers limited types of geometrical shape assemblies. The ease of use of magnetic levitation for organoid assembly could be utilized in a wide range of locations, as demonstrated by the use of this technique in the space station [75,76]. Acoustic assembly is another candidate to drive the assembly of cells, as it has been widely used in biomedical applications due to its safety and tissue penetration. The assembly of organoids driven by acoustic forces relies on the formation of pressure nodes that drive cell migration towards pre-established patterns [77]. The main parameters that govern organoid formation rely on the container shape, voltage, and frequency, although different acoustic transducers produce different types of assemblies. For instance, standing waves can generate large-scale 3D patterns that simulate complex tissue assemblies such as brain-like cortex [78], cardiac tissue [79] or ring-shape assemblies [80].

Surface acoustic waves can generate smaller organoids in a scalable manner. This method relies on the generation of traveling waves parallel to the substrate that create smaller “acoustic traps” or tweezers that can arrange small numbers of cells into organoids [81]. Acoustic levitation has also been employed for the scaffoldless assembly of cell constructs based on the projection of focus ultrasound patterns that generate 3D pressure nodes that trap cells into predesigned shapes overcoming gravity [82]. Although the generation of ultrasound holograms has typically relied on arrays of acoustic transducers, more recently, 3D printed masks have been used to create acoustic holograms using a single transducer [83,84]. Acoustic levitation systems can create more complex structures than other acoustic systems due to the ability to project and reprogram the shape. However, acoustic levitation systems are best-suited to generate millimeter size constructs [85,86].

Optical tweezers can manipulate and assemble living cells into organoid constructs [87]. These methods rely on focused or counter-propagating laser beams that trap and confine cells in optical traps [88,89]. Optical forces can be tuned by modulating intensity, medium and working area. Optical forces operate in a biocompatible range, enabling them to manipulate cells with a high degree of precision without damaging cell integrity, being even capable of subcellular manipulation [90]. Holographic optical tweezers have been used to fabricate sequential cell assemblies by positioning living cells in different positions [89,91]. Optical assembly methods rely on translucent media, limiting the application of this approach for *in vivo* assemblies. Electrically driven assembly of cellular constructs commonly relies on the use of dielectrophoretic force to manipulate and isolate cells towards electrodes [92,93]. The working mechanism relies on the polarization of cells subjected to an electric gradient, and the subsequent accumulation of polarized cells at the surface of microelectrodes [94,95]. The limit of this approach relies on the formation of 3D assemblies [96,97], potential damage to cell membrane under high electric field and medium constraints, as the presence of diverse analytes could directly affect the dielectrophoretic assembly mechanism.

Employing external fields to generate scaffoldless cellular assemblies has great potential for scaling the fabrication of gastrointestinal cancer

models with a high degree of flexibility in the design and preconcentration of cells in confined geometries. Most methods require chemical or optical cross-linking to make permanent tissue constructs. The parallel use of multiple external fields could overcome the limitations of each method and permit the manipulation and assembly of distinct cellular building blocks. As a potential research area, external fields could enable an on-site assembly of engineered tissue inside a living body. In this direction, the use of external fields could increase the reproducibility and throughput of organoids as the external fields can be continuously applied using a predetermined parameter to promote the formation of a GI model.

7. Outlook

In summary, GI cancer organoids can be designed and fabricated by various approaches. It is essential to consider the type of cells required to form the organoid (adult, differentiated cells, or stem cells), and the biochemical and physical environmental cues that will guide the proliferation and differentiation of such cells into gastrointestinal tissue-like architectures. The merging of biology and engineering has led to hybrid biofabrication protocols enabling the development of increasingly intricate designs that mimic the complexity of native tissue. For instance, microfluidic platforms offer large throughput and lower cost. They are better suited for the fabrication of mostly spherical constructs. Additive manufacturing consists of different methods based on layer-by-layer deposition; the most common methods rely on extrusion or photomasking. While these methods offer a high degree of design opportunities, they have less cellular density due to the need for a scaffold, required by the inherent transfer of materials for 3D printing methods. External field-driven assembly enables the rapid formation of organoids, although the diverse assembly protocols that depend on the cellular proprieties as well as the microenvironment and offer less design flexibility than 3D printers.

The successful translation of gastrointestinal cancer organoids for patient-specific cancer models requires the fabrication and assembly of millions of spheroids as biofabricated building blocks without compromising their differentiation capacity. Therefore, standardization is of key importance to ensure reproducibility and translation of these models. The combinatorial use of the diverse self-assembly and directed approaches for organoid differentiation could induce a programable organization that better mimics native tissues.

Engineered tissues could be a poor predictor for human models if the characteristic biochemical and mechanical parameters of different tissues are not adequately considered. Despite the recent progress in developing organoids as early gastrointestinal cancer models, key challenges include: **(i)** increasing the reproducibility of organoid fabrication methods and characterization protocols. As organoid fabrication is a developing field with a fast pace of growth, there is a lack of proper standardization from lab-to-lab. This is particularly challenging when trying to mimic native tumor heterogeneity. However, the development of more complex and tunable organoid models could integrate both healthy and cancer cells into a complete simulation model.**(ii)** The integration of 3D arrays of organoids with vascular inputs that mimics functional aspects of the GI tract (digestion, absorption of nutrients and excretion of digestion waste products). (iii) Mimicking he effect an organ physical behavior such as interstitial pressure flow conditions, dynamic tissue growth and mimicking gut motility (peristalsis) (**iv)** Finally, special attention should be placed on studying the microbiota's effect in organoid models.

Organoids could be the building blocks of organ-on-a-chip devices, taking advantage of the lower cost and potentially reducing the burden on animal models. Organ-on-chip models could introduce physical forces generated by fluid shear and geometrical confinement to mimic the tumor physiological microenvironment, while also simplifying bioanalysis by integrating different optical [98] or electrochemical sensors [99] into the device. Arrays of organoid circuits that combine cellular structures from different gastrointestinal tract sections allow for analysis of the synergistic toxicity and metabolite response of organoids to novel therapeutic models. The ability to generate lab on a chip organoid platform could enable early cancer models, potentially overcoming the lack of heterogeneity in mono cell cancer culture designs by integrating massively parallel models that incorporate both healthy and cancer cells. Moreover, once the gastric organoids are developed, they could be transplanted with the assistance of microrobotic technology to enable regenerative tissue engineering. To this purpose, organoids are ideal, since they could be delivered directly into target areas, thus increasing their ability to proliferate and survive. Perhaps more importantly, direct organoid delivery could assist in reducing cost, risk and discomfort associated with major surgery for organ transplantation [100].

With continuing advances in science, technology, and engineering, today's healthcare problems are open to innovative solutions than ever before. There are many competing technologies focused on enhancing throughput, improving translational relevance and developing platforms for potential commercialization of gastric models [101]. Commercialization poses significant hurdles to medical technology innovators/entrepreneurs and requires skillful management of patents, regulatory approval, market dynamics, business models, competition, financing, and technical feasibility among other potential challenges. Thus, emphasis should be placed on identifying market and clinical needs, embracing the engineering aspects, and focusing on value generation. The field could benefit from developing robust intellectual propriety portfolios and engaging private enterprises. As the use of gastrointestinal organoids matures into a fully reproducible and viable tool in addition to other existing alternatives, the advances in field and successful translational examples could fuel the next round of innovation, enabling the standardized organoid growth and assembly methodologies as ubiquitous tools or protocols. When integrated with novel analysis and downstream omic characterization tools for either using their cellular, extracellular or secretome such as extracellular vesicles, they present a promising vision into the future of prevision medicine [102]. In conclusion, we envision that organoids as patient-specific models has great potential to revolutionize tissue engineering and drug testing, enabling personalized and precision medicine approaches, improved understanding and therapy design for patients suffering from gastrointestinal cancers.

CRediT authorship contribution statement

Fernando Soto: All the authors have contributed to the, Conceptualization, writing, and editing, of the final document. **Carlos F. Guimarães:** All the authors have contributed to the, Conceptualization, writing, and editing, of the final document. **Rui L. Reis:** All the authors have contributed to the, Conceptualization, writing, and editing, of the final document. **Walfre Franco:** All the authors have contributed to the, Conceptualization, writing, and editing, of the final document. **Imran Rizvi:** All the authors have contributed to the, Conceptualization, writing, and editing, of the final document. **Utkan Demirci:** All the authors have contributed to the, Conceptualization, writing, and editing, of the final document.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Prof. Utkan Demirci (UD) is a founder of and has an equity interest in: (i) DxNow Inc., a company that is developing microfluidic IVF tools and imaging technologies, (ii) Koek Biotech, a company that is developing microfluidic technologies for clinical solutions, (iii) Levitas Inc., a company focusing on developing microfluidic sorters using magnetic levitation, and (iv) Hillel Inc., a company bringing microfluidic cell phone tools to home settings. UD's interests were viewed and managed in accordance with the conflict of interest policies.

Acknowledgements

This work was supported by research funding provided by DoD grants (LC150650, W81XWH-16-1-0200) and (LC19029, W81XWH-20-1-0746), NCI Center for Cancer Nano-technology Excellence for Translational Diagnostics (NCI– U54CA199075), NIH grant (R01 EB029805), the International Alliance for Cancer Early Detection (ACED) pilot award, and the Canary Center at Stanford for Cancer Early Detection Seed Award. F.S was supported by Stanford Molecular Imaging Scholars program, 5R25CA118681. (NIH T32 postdoctoral fellowship) and Schmidt Science Fellows. C.F.G acknowledges support from Fundação para a Ciência e Tecnologia (Grant no. PD/BD/135253/2017), Fundação Luso-Americana para o Desenvolvimento (FLAD) and Stanford RISE COVID-19 Crisis Response Faculty Seed Grant Program. I.R. acknowledges funding provided by the NC Translational and Clinical Sciences Institute (NCTraCS), supported by the National Center for Advancing Translational Sciences (NCATS, National Institutes of Healthh (NIH), through Grant Award Number UL1TR002489, and UNC-NC State Joint BME Department Start-up Fund. F.S. and C.F.G. contributed equally to this work. The authors thank D. Akin for his helpful discussions and assistance.

References

[1] J.S. Dutton, S.S. Hinman, R. Kim, Y. Wang, N.L. Allbritton, Primary cell-derived intestinal models: recapitulating physiology, Trends Biotechnol. 37 (2019) 744–760, https://doi.org/10.1016/j.tibtech.2018.12.001.

[2] Y. Wang, R. Kim, S.H.J. Hwang, J. Dutton, C.E. Sims, N.L. Allbritton, Analysis of interleukin 8 secretion by a stem-cell-derived human-intestinal-epithelial-monolayer platform, Anal. Chem. 90 (2018) 11523–11530, https://doi.org/10.1021/acs.analchem.8b02835.

[3] F. Soto, J. Wang, S. Deshmukh, U. Demirci, Reversible design of dynamic assemblies at small scales, Adv. Intell. Syst. (2020), 2000193, https://doi.org/10.1002/aisy.202000193.

[4] L. Yang, S. Yang, X. Li, B. Li, Y. Li, X. Zhang, Y. Ma, X. Peng, H. Jin, Q. Fan, S. Wei, J. Liu, H. Li, Tumor organoids: from inception to future in cancer research, Canc. Lett. 454 (2019) 120–133, https://doi.org/10.1016/j.canlet.2019.04.005.

[5] M. Li, J.C. Izpisua Belmonte, Organoids - preclinical models of human disease, N. Engl. J. Med. 380 (2019) 569–579, https://doi.org/10.1056/NEJMra1806175.

[6] M.J. Kratochvil, A.J. Seymour, T.L. Li, S.P. Paşca, C.J. Kuo, S.C. Heilshorn, Engineered materials for organoid systems, Nat. Rev. Mater. 4 (2019) 606–622, https://doi.org/10.1038/s41578-019-0129-9.

[7] S. Date, T. Sato, Mini-gut organoids: reconstitution of the stem cell niche, Annu. Rev. Cell Dev. Biol. 31 (2015) 269–289, https://doi.org/10.1146/annurev-cellbio-100814-125218.

[8] S.R. Merker, J. Weitz, D.E. Stange, Gastrointestinal organoids: how they gut it out, Dev. Biol. 420 (2016) 239–250, https://doi.org/10.1016/j.ydbio.2016.08.010.

[9] L.R. Madden, T.V. Nguyen, S. Garcia-Mojica, V. Shah, A.V. Le, A. Peier, R. Visconti, E.M. Parker, S.C. Presnell, D.G. Nguyen, K.N. Retting, Bioprinted 3D primary human intestinal tissues model aspects of native physiology and ADME/tox functions, IScience 2 (2018) 156–167, https://doi.org/10.1016/j.isci.2018.03.015.

[10] V. Prisk, J. Huard, Stem cells in tissue engineering, Nature 414 (2001) 531–544, https://doi.org/10.5772/54371.

[11] X. Yin, B.E. Mead, H. Safaee, R. Langer, J.M. Karp, O. Levy, Engineering stem cell organoids, Cell Stem Cell 18 (2016) 25–38, https://doi.org/10.1016/j.stem.2015.12.005.

[12] R. Morizane, A.Q. Lam, B.S. Freedman, S. Kishi, M.T. Valerius, J.V. Bonventre, Nephron organoids derived from human pluripotent stem cells model kidney development and injury, Nat. Biotechnol. 33 (2015) 1193–1200, https://doi.org/10.1038/nbt.3392.

[13] L. Yang, Y. Han, B.E. Nilsson-Payant, V. Gupta, P. Wang, X. Duan, X. Tang, J. Zhu, Z. Zhao, F. Jaffré, T. Zhang, T.W. Kim, O. Harschnitz, D. Redmond, S. Houghton, C. Liu, A. Naji, G. Ciceri, S. Guttikonda, Y. Bram, D.H.T. Nguyen, M. Cioffi, V. Chandar, D.A. Hoagland, Y. Huang, J. Xiang, H. Wang, D. Lyden, A. Borczuk, H.J. Chen, L. Studer, F.C. Pan, D.D. Ho, B.R. tenOever, T. Evans, R.E. Schwartz, S. Chen, A human pluripotent stem cell-based platform to study SARS-CoV-2 tropism and model virus infection in human cells and organoids, Cell Stem Cell 27 (2020) 125–136, https://doi.org/10.1016/j.stem.2020.06.015, e7.

[14] M.A. Lancaster, J.A. Knoblich, Generation of cerebral organoids from human pluripotent stem cells, Nat. Protoc. 9 (2014) 2329–2340, https://doi.org/10.1038/nprot.2014.158.

[15] M.J. Sandel, Embryo ethics - the moral logic of stem-cell research, N. Engl. J. Med. 351 (2004) 207–209, https://doi.org/10.1056/NEJMp048145.

[16] I.H. Park, P.H. Lerou, R. Zhao, H. Huo, G.Q. Daley, Generation of human-induced pluripotent stem cells, Nat. Protoc. 3 (2008) 1180–1186, https://doi.org/10.1038/nprot.2008.92.

[17] S. Yamanaka, K. Takahashi, K. Okita, M. Nakagawa, Induction of pluripotent stem cells from fibroblast cultures, Nat. Protoc. 2 (2007) 3081–3089, https://doi.org/10.1038/nprot.2007.418.

[18] J.O. Múnera, J.M. Wells, Generation of gastrointestinal organoids derived from human pluripotent stem cells, Organ Regen. Based Dev. Biol. 1597 (2017) 179–192, https://doi.org/10.1007/978-981-10-3768-9_10.

[19] L. Xu, W. Lin, L. Wen, G. Li, Lgr5 in cancer biology: functional identification of Lgr5 in cancer progression and potential opportunities for novel therapy, Stem Cell Res. Ther. 10 (2019) 1–9, https://doi.org/10.1186/s13287-019-1288-8.

[20] T. Sato, R.G. Vries, H.J. Snippert, M. Van De Wetering, N. Barker, D.E. Stange, J. H. Van Es, A. Abo, P. Kujala, P.J. Peters, H. Clevers, Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche, Nature 459 (2009) 262–265, https://doi.org/10.1038/nature07935.

[21] J.C. Mills, R.A. Shivdasani, Gastric epithelial stem cells, Gastroenterology 140 (2011) 412–424, https://doi.org/10.1053/j.gastro.2010.12.001.

[22] M. Hata, Y. Hayakawa, K. Koike, Gastric stem cell and cellular origin of cancer, Biomedicines 6 (2018) 1–9, https://doi.org/10.3390/biomedicines6040100.

[23] S. Bartfeld, T. Bayram, M. Van De Wetering, M. Huch, H. Begthel, P. Kujala, R. Vries, P.J. Peters, H. Clevers, In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection, Gastroenterology 148 (2015) 126–136, https://doi.org/10.1053/j.gastro.2014.09.042, e6.

[24] I. Gutierrez-Aranda, V. Ramos-Mejia, C. Bueno, M. Munoz-Lopez, P.J. Real, A. Mácia, L. Sanchez, G. Ligero, J.L. Garcia-Parez, P. Menendez, Human induced pluripotent stem cells develop teratoma more efficiently and faster than human embryonic stem cells regardless the site of injection, Stem Cell. 28 (2010) 1568–1570, https://doi.org/10.1002/stem.471.

[25] T. Iskratsch, H. Wolfenson, M.P. Sheetz, Appreciating force and shape — the rise of mechanotransduction in cell biology, Nat. Rev. Mol. Cell Biol. 15 (2014) 825–833, https://doi.org/10.1038/nrm3903.

[26] C.F. Guimarães, L. Gasperini, A.P. Marques, R.L. Reis, The stiffness of living tissues and its implications for tissue engineering, Nat. Rev. Mater. 5 (2020) 351–370, https://doi.org/10.1038/s41578-019-0169-1.

[27] Y. Takahashi, S. Sato, Y. Kurashima, T. Yamamoto, S. Kurokawa, Y. Yuki, N. Takemura, S. Uematsu, C.Y. Lai, M. Otsu, H. Matsuno, H. Osawa, T. Mizushima, J. Nishimura, M. Hayashi, T. Yamaguchi, H. Kiyono, A refined culture system for human induced pluripotent stem cell-derived intestinal epithelial organoids, Stem Cell Reports 10 (2018) 314–328, https://doi.org/10.1016/j.stemcr.2017.11.004.

[28] J.R. Spence, C.N. Mayhew, S.A. Rankin, M.F. Kuhar, J.E. Vallance, K. Tolle, E. E. Hoskins, V.V. Kalinichenko, S.I. Wells, A.M. Zorn, N.F. Shroyer, J.M. Wells, Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro, Nature 470 (2011) 105–110, https://doi.org/10.1038/nature09691.

[29] P.J. Attayek, A.A. Ahmad, Y. Wang, I. Williamson, C.E. Sims, S.T. Magness, N. L. Allbritton, In vitro polarization of colonoids to create an intestinal stem cell compartment, PloS One 11 (2016) 1–23, https://doi.org/10.1371/journal.pone.0153795.

[30] R.R. Zhang, M. Koido, T. Tadokoro, R. Ouchi, T. Matsuno, Y. Ueno, K. Sekine, T. Takebe, H. Taniguchi, Human iPSC-derived posterior gut progenitors are expandable and capable of forming gut and liver organoids, Stem Cell Reports 10 (2018) 780–793, https://doi.org/10.1016/j.stemcr.2018.01.006.

[31] M. Cebrat, L. Strzadala, P. Kisielow, Wnt inhibitory factor-1: a candidate for a new player in tumorigenesis of intestinal epithelial cells, Canc. Lett. 206 (2004) 107–113, https://doi.org/10.1016/j.canlet.2003.10.024.

[32] N. Ashley, Regulation of intestinal cancer stem cells, Canc. Lett. 338 (2013) 120–126, https://doi.org/10.1016/j.canlet.2012.04.016.

[33] N. Gjorevski, N. Sachs, A. Manfrin, S. Giger, M.E. Bragina, P. Ordóñez-Morán, H. Clevers, M.P. Lutolf, Designer matrices for intestinal stem cell and organoid culture, Nature 539 (2016) 560–564, https://doi.org/10.1038/nature20168.

[34] N. Gjorevski, M.P. Lutolf, Synthesis and characterization of well-defined hydrogel matrices and their application to intestinal stem cell and organoid culture, Nat. Protoc. 12 (2017) 2263–2274, https://doi.org/10.1038/nprot.2017.095.

[35] M. Urbischek, H. Rannikmae, T. Foets, K. Ravn, M. Hyvönen, M. de la Roche, Organoid culture media formulated with growth factors of defined cellular activity, Sci. Rep. 9 (2019) 1–11, https://doi.org/10.1038/s41598-019-42604-0.

[36] K.W. McCracken, E.M. Catá, C.M. Crawford, K.L. Sinagoga, M. Schumacher, B. E. Rockich, Y.H. Tsai, C.N. Mayhew, J.R. Spence, Y. Zavros, J.M. Wells, Modelling human development and disease in pluripotent stem-cell-derived gastric organoids, Nature 516 (2014) 400–404, https://doi.org/10.1038/nature13863.

[37] S. Yamada, Y. Kanda, Retinoic acid promotes barrier functions in human iPSC-derived intestinal epithelial monolayers, J. Pharmacol. Sci. 140 (2019) 337–344, https://doi.org/10.1016/j.jphs.2019.06.012.

[38] I. Lukonin, D. Serra, L.C. Meylan, K. Volkmann, J. Baaten, R. Zhao, S. Meeusen, K. Colman, F. Maurer, M.B. Stadler, J. Jenkins, P. Liberali, Phenotypic landscape of intestinal organoid regeneration, Nature 586 (2020), https://doi.org/10.1038/s41586-020-2776-9.

[39] Y. Wang, M. DiSalvo, D.B. Gunasekara, J. Dutton, A. Proctor, M.S. Lebhar, I. A. Williamson, J. Speer, R.L. Howard, N.M. Smiddy, S.J. Bultman, C.E. Sims, S. T. Magness, N.L. Allbritton, Self-renewing monolayer of primary colonic or rectal epithelial cells, Cmgh 4 (2017) 165–182, https://doi.org/10.1016/j.jcmgh.2017.02.011, e7.

[40] M. Panek, M. Grabacka, M. Pierzchalska, The formation of intestinal organoids in a hanging drop culture, Cytotechnology 70 (2018) 1085–1095, https://doi.org/10.1007/s10616-018-0194-8.

[41] J. Creff, R. Courson, T. Mangeat, J. Foncy, S. Souleille, C. Thibault, A. Besson, L. Malaquin, Fabrication of 3D scaffolds reproducing intestinal epithelium

topography by high-resolution 3D stereolithography, Biomaterials (2019) 221, https://doi.org/10.1016/j.biomaterials.2019.119404.
[42] G.A. Kim, J.R. Spence, S. Takayama, Bioengineering for intestinal organoid cultures, Curr. Opin. Biotechnol. 47 (2017) 51–58, https://doi.org/10.1016/j.copbio.2017.05.006.
[43] H. Liu, Y. Wang, K. Cui, Y. Guo, X. Zhang, J. Qin, Advances in hydrogels in organoids and organs-on-a-chip, Adv. Mater. 31 (2019) 1–28, https://doi.org/10.1002/adma.201902042.
[44] N. Deuk, K. Paikb, K.H. Clifton, Inhibitory effects of retinoids on development of squamous metaplasia in rat mammary epithelial organoids cultured in Matrigel, Canc. Lett. 110 (1996) 217–223.
[45] E.A. Aisenbrey, W.L. Murphy, Synthetic alternatives to Matrigel, Nat. Rev. Mater. 5 (2020) 539–551, https://doi.org/10.1038/s41578-020-0199-8.
[46] N. Broguiere, L. Isenmann, C. Hirt, T. Ringel, S. Placzek, E. Cavalli, F. Ringnalda, L. Villiger, R. Züllig, R. Lehmann, G. Rogler, M.H. Heim, J. Schüler, M. Zenobi-Wong, G. Schwank, Growth of epithelial organoids in a defined hydrogel, Adv. Mater. 30 (2018), https://doi.org/10.1002/adma.201801621.
[47] S. Rezakhani, N. Gjorevski, M.P. Lutolf, Low-defect thiol-michael addition hydrogels as Matrigel substitutes for epithelial organoid derivation, Adv. Funct. Mater. 30 (2020) 2000761, https://doi.org/10.1002/adfm.202000761, 1–12, 2000761.
[48] M.M. Capeling, M. Czerwinski, S. Huang, Y.H. Tsai, A. Wu, M.S. Nagy, B. Juliar, N. Sundaram, Y. Song, W.M. Han, S. Takayama, E. Alsberg, A.J. Garcia, M. Helmrath, A.J. Putnam, J.R. Spence, Nonadhesive alginate hydrogels support growth of pluripotent stem cell-derived intestinal organoids, Stem Cell Reports 12 (2019) 381–394, https://doi.org/10.1016/j.stemcr.2018.12.001.
[49] G.G. Giobbe, C. Crowley, C. Luni, S. Campinoti, M. Khedr, K. Kretzschmar, M. M. De Santis, E. Zambaiti, F. Michielin, L. Meran, Q. Hu, G. van Son, L. Urbani, A. Manfredi, M. Giomo, S. Eaton, D. Cacchiarelli, V.S.W. Li, H. Clevers, P. Bonfanti, N. Elvassore, P. De Coppi, Extracellular matrix hydrogel derived from decellularized tissues enables endodermal organoid culture, Nat. Commun. 10 (2019), https://doi.org/10.1038/s41467-019-13605-4.
[50] T. Seidlitz, S.R. Merker, A. Rothe, F. Zakrzewski, C. Von Neubeck, K. Grützmann, U. Sommer, C. Schweitzer, S. Schölch, H. Uhlemann, A.M. Gaebler, K. Werner, M. Krause, G.B. Baretton, T. Welsch, B.K. Koo, D.E. Aust, B. Klink, J. Weitz, D. E. Stange, Human gastric cancer modelling using organoids, Gut 68 (2019) 207–217, https://doi.org/10.1136/gutjnl-2017-314549.
[51] S. Ng, W.J. Tan, M.M.X. Pek, M.H. Tan, M. Kurisawa, Mechanically and chemically defined hydrogel matrices for patient-derived colorectal tumor organoid culture, Biomaterials 219 (2019), 119400, https://doi.org/10.1016/j.biomaterials.2019.119400.
[52] Y. Wang, D.B. Gunasekara, M.I. Reed, M. DiSalvo, S.J. Bultman, C.E. Sims, S. T. Magness, N.L. Allbritton, A microengineered collagen scaffold for generating a polarized crypt-villus architecture of human small intestinal epithelium, Biomaterials 128 (2017) 44–55, https://doi.org/10.1016/j.biomaterials.2017.03.005.
[53] Y. Wang, D.B. Gunasekara, P.J. Attayek, M.I. Reed, M. Disalvo, D.L. Nguyen, J. S. Dutton, M.S. Lebhar, S.J. Bultman, C.E. Sims, S.T. Magness, N.L. Allbritton, In vitro generation of mouse colon crypts, ACS Biomater. Sci. Eng. 3 (2017) 2502–2513, https://doi.org/10.1021/acsbiomaterials.7b00368.
[54] M. Nikolaev, O. Mitrofanova, N. Broguiere, S. Geraldo, D. Dutta, Y. Tabata, B. Elci, N. Gjorevski, H. Clevers, M.P. Lutolf, Homeostatic mini-intestines through scaffold-guided organoid morphogenesis, Nature 585 (2020) 574–578, https://doi.org/10.1038/s41586-020-2724-8.
[55] L.Y. Wu, D. Di Carlo, L.P. Lee, Microfluidic self-assembly of tumor spheroids for anticancer drug discovery, Biomed, Microdevices 10 (2008) 197–202, https://doi.org/10.1007/s10544-007-9125-8.
[56] M.E. Dolega, F. Abeille, N. Picollet-D'hahan, X. Gidrol, Controlled 3D culture in Matrigel microbeads to analyze clonal acinar development, Biomaterials 52 (2015) 347–357, https://doi.org/10.1016/j.biomaterials.2015.02.042.
[57] R.K. Vadivelu, H. Kamble, M.J.A. Shiddiky, N.T. Nguyen, Microfluidic technology for the generation of cell spheroids and their applications, Micromachines 8 (2017), https://doi.org/10.3390/mi8040094.
[58] Y.S. Zhang, A. Arneri, S. Bersini, S.R. Shin, K. Zhu, Z. Goli-Malekabadi, J. Aleman, C. Colosi, F. Busignani, V. Dell'Erba, C. Bishop, T. Shupe, D. Demarchi, M. Moretti, M. Rasponi, M.R. Dokmeci, A. Atala, A. Khademhosseini, Bioprinting 3D microfibrous scaffolds for engineering endothelialized myocardium and heart-on-a-chip, Biomaterials 110 (2016) 45–59, https://doi.org/10.1016/j.biomaterials.2016.09.003.
[59] A. Ovsianikov, S. Mühleder, J. Torgersen, Z. Li, X.H. Qin, S. Van Vlierberghe, P. Dubruel, W. Holnthoner, H. Redl, R. Liska, J. Stampfl, Laser photofabrication of cell-containing hydrogel constructs, Langmuir 30 (2014) 3787–3794, https://doi.org/10.1021/la402346z.
[60] Z. Wang, H. Kumar, Z. Tian, X. Jin, J.F. Holzman, F. Menard, K. Kim, Visible light photoinitiation of cell-adhesive gelatin methacryloyl hydrogels for stereolithography 3D bioprinting, ACS Appl. Mater. Interfaces 10 (2018) 26859–26869, https://doi.org/10.1021/acsami.8b06607.
[61] I.C. Yasa, A.F. Tabak, O. Yasa, H. Ceylan, M. Sitti, 3D-Printed microrobotic transporters with recapitulated stem cell niche for programmable and active cell delivery, Adv. Funct. Mater. 29 (2019), 1808992, https://doi.org/10.1002/adfm.201808992.
[62] A. Ovsianikov, A. Deiwick, S. Van Vlierberghe, M. Pflaum, M. Wilhelmi, P. Dubruel, B. Chichkov, Laser fabrication of 3D gelatin scaffolds for the generation of bioartificial tissues, Materials 4 (2010) 288–299, https://doi.org/10.3390/ma4010288.
[63] J.A. Reid, X.L. Palmer, P.A. Mollica, N. Northam, P.C. Sachs, R.D. Bruno, A 3D bioprinter platform for mechanistic analysis of tumoroids and chimeric mammary organoids, Sci. Rep. 9 (2019) 1–10, https://doi.org/10.1038/s41598-019-43922-z.
[64] J. Laurent, G. Blin, F. Chatelain, V. Vanneaux, A. Fuchs, J. Larghero, M. Théry, Convergence of microengineering and cellular self-organization towards functional tissue manufacturing, Nat. Biomed. Eng. 1 (2017) 939–956, https://doi.org/10.1038/s41551-017-0166-x.
[65] Y. Zhu, V. Serpooshan, S. Wu, U. Demirci, P. Chen, S. Güven, Tissue engineering of 3D organotypic microtissues by acoustic assembly. Methods Mol. Biol., Humana Press Inc., 2019, pp. 301–312, https://doi.org/10.1007/7651_2017_68.
[66] W. Ye, C. Luo, C. Li, J. Huang, F. Liu, Organoids to study immune functions, immunological diseases and immunotherapy, Canc. Lett. 477 (2020) 31–40, https://doi.org/10.1016/j.canlet.2020.02.027.
[67] S. Tasoglu, U.A. Gurkan, S.Q. Wang, U. Demirci, Manipulating biological agents and cells in micro-scale volumes for applications in medicine, Chem. Soc. Rev. 42 (2013) 5788–5808, https://doi.org/10.1039/c3cs60042d.
[68] T. Ren, W. Steiger, P. Chen, A. Ovsianikov, U. Demirci, Enhancing cell packing in buckyballs by acoustofluidic activation, Biofabrication 12 (2020), 025033, https://doi.org/10.1088/1758-5090/ab76d9.
[69] S. Tasoglu, E. Diller, S. Guven, M. Sitti, U. Demirci, Untethered micro-robotic coding of three-dimensional material composition, Nat. Commun. 5 (2014) 1–9, https://doi.org/10.1038/ncomms4124.
[70] S. Tasoglu, C.H. Yu, H.I. Gungordu, S. Guven, T. Vural, U. Demirci, Guided and magnetic self-assembly of tunable magnetoceptive gels, Nat. Commun. 5 (2014) 1–11, https://doi.org/10.1038/ncomms5702.
[71] A. Winkleman, K.L. Gudiksen, D. Ryan, G.M. Whitesides, D. Greenfield, M. Prentiss, A magnetic trap for living cells suspended in a paramagnetic buffer, Appl. Phys. Lett. 85 (2004) 2411–2413, https://doi.org/10.1063/1.1794372.
[72] H. Tseng, J.A. Gage, T. Shen, W.L. Haisler, S.K. Neeley, S. Shiao, J. Chen, P. K. Desai, A. Liao, C. Hebel, R.M. Raphael, J.L. Becker, G.R. Souza, A spheroid toxicity assay using magnetic 3D bioprinting and real-time mobile device-based imaging, Sci. Rep. 5 (2015) 1–11, https://doi.org/10.1038/srep13987.
[73] G.R. Souza, J.R. Molina, R.M. Raphael, M.G. Ozawa, D.J. Stark, C.S. Levin, L. F. Bronk, J.S. Ananta, J. Mandelin, M.M. Georgescu, J.A. Bankson, J.G. Gelovani, T.C. Killian, W. Arap, R. Pasqualini, Three-dimensional tissue culture based on magnetic cell levitation, Nat. Nanotechnol. 5 (2010) 291–296, https://doi.org/10.1038/nnano.2010.23.
[74] A. Tocchio, N.G. Durmus, K. Sridhar, V. Mani, B. Coskun, R. El Assal, U. Demirci, Magnetically guided self-assembly and coding of 3D living architectures, Adv. Mater. 30 (2018), 1705034, https://doi.org/10.1002/adma.201705034.
[75] V.A. Parfenov, Y.D. Khesuani, S.V. Petrov, P.A. Karalkin, E.V. Koudan, E. K. Nezhurina, F. Das Pereira, A.A. Krokhmal, A.A. Gryadunova, E.A. Bulanova, I. V. Vakhrushev, I.I. Babichenko, V. Kasyanov, O.F. Petrov, M.M. Vasiliev, K. Brakke, S.I. Belousov, T.E. Grigoriev, E.O. Osidak, E.I. Rossiyskaya, L. B. Buravkova, O.D. Kononenko, U. Demirci, V.A. Mironov, Magnetic levitational bioassembly of 3D tissue construct in space, Sci. Adv. 6 (2020), https://doi.org/10.1126/sciadv.aba4174 eaba4174.
[76] P. Chen, S. Güven, O.B. Usta, M.L. Yarmush, U. Demirci, Biotunable acoustic node assembly of organoids, Adv. Healthc. Mater. 4 (2015) 1937–1943, https://doi.org/10.1002/adhm.201500279.
[77] K. Olofsson, V. Carannante, M. Ohlin, T. Frisk, K. Kushiro, M. Takai, A. Lundqvist, B. Önfelt, M. Wiklund, Acoustic formation of multicellular tumor spheroids enabling on-chip functional and structural imaging, Lab Chip 18 (2018) 2466–2476, https://doi.org/10.1039/c8lc00537k.
[78] C. Bouyer, P. Chen, S. Güven, T.T. Demirtaş, T.J.F. Nieland, F. Padilla, U. Demirci, A bio-acoustic levitational (BAL) assembly method for engineering of multilayered, 3D brain-like constructs, using human embryonic stem cell derived neuro-progenitors, Adv. Mater. 28 (2016) 161–167, https://doi.org/10.1002/adma.201503916.
[79] P. Chen, P. Chen, H. Wu, S. Lee, A. Sharma, D.A. Hu, S. Venkatraman, A. V. Ganesan, O.B. Usta, M. Yarmush, F. Yang, J.C. Wu, U. Demirci, S.M. Wu, Bioacoustic-enabled patterning of human iPSC-derived cardiomyocytes into 3D cardiac tissue, Biomaterials 131 (2017) 47–57, https://doi.org/10.1016/j.biomaterials.2017.03.037.
[80] T. Ren, P. Chen, L. Gu, M.G. Ogut, U. Demirci, Soft ring-shaped cellu-robots with simultaneous locomotion in batches, Adv. Mater. 32 (2020), 1905713, https://doi.org/10.1002/adma.201905713.
[81] H. Cai, Z. Wu, Z. Ao, A. Nunez, B. Chen, L. Jiang, M. Bondesson, F. Guo, Trapping cell spheroids and organoids using digital acoustofluidics, Biofabrication 12 (2020), 035025, https://doi.org/10.1088/1758-5090/ab9582.
[82] E.H. Brandt, Suspended by sound, Nature 413 (2001) 474–475, https://doi.org/10.1038/35097192.
[83] K. Melde, A.G. Mark, T. Qiu, P. Fischer, Holograms for acoustics, Nature 537 (2016) 518–522, https://doi.org/10.1038/nature19755.
[84] Z. Ma, A.W. Holle, K. Melde, T. Qiu, K. Poeppel, V.M. Kadiri, P. Fischer, Acoustic holographic cell patterning in a biocompatible hydrogel, Adv. Mater. 32 (2020), 1904181, https://doi.org/10.1002/adma.201904181.
[85] B. Kang, J. Shin, H.J. Park, C. Rhyou, D. Kang, S.J. Lee, Y. sup Yoon, S.W. Cho, H. Lee, High-resolution acoustophoretic 3D cell patterning to construct functional collateral cylindroids for ischemia therapy, Nat. Commun. 9 (2018) 1–13, https://doi.org/10.1038/s41467-018-07823-5.
[86] K. Chen, M. Wu, F. Guo, P. Li, C.Y. Chan, Z. Mao, S. Li, L. Ren, R. Zhang, T. J. Huang, Rapid formation of size-controllable multicellular spheroids: via 3D acoustic tweezers, Lab Chip 16 (2016) 2636–2643, https://doi.org/10.1039/c6lc00444j.

[87] K. Dholakia, B.W. Drinkwater, M. Ritsch-Marte, Comparing acoustic and optical forces for biomedical research, Nat. Rev. Phys. 2 (2020) 480–491, https://doi.org/10.1038/s42254-020-0215-3.
[88] P.Y. Chiou, A.T. Ohta, M.C. Wu, Massively parallel manipulation of single cells and microparticles using optical images, Nature 436 (2005) 370–372, https://doi.org/10.1038/nature03831.
[89] G.R. Kirkham, E. Britchford, T. Upton, J. Ware, G.M. Gibson, Y. Devaud, M. Ehrbar, M. Padgett, S. Allen, L.D. Buttery, K. Shakesheff, Precision assembly of complex cellular microenvironments using holographic optical tweezers, Sci. Rep. 5 (2015) 1–7, https://doi.org/10.1038/srep08577.
[90] Y.L. Han, A.F. Pegoraro, H. Li, K. Li, Y. Yuan, G. Xu, Z. Gu, J. Sun, Y. Hao, S. K. Gupta, Y. Li, W. Tang, H. Kang, L. Teng, J.J. Fredberg, M. Guo, Cell swelling, softening and invasion in a three-dimensional breast cancer model, Nat. Phys. 16 (2020) 101–108, https://doi.org/10.1038/s41567-019-0680-8.
[91] T. Yamazaki, H. Taniguchi, S. Tsuji, S. Sato, T. Kenmotsu, K. Yoshikawa, K. Sadakane, Manipulating living cells to construct stable 3D cellular assembly without artificial scaffold, JoVE 13 (2018) e0209162, https://doi.org/10.3791/57815.
[92] M. Hronik-Tupaj, D.L. Kaplan, A review of the responses of two-and three-dimensional engineered tissues to electric fields, Tissue Eng. B Rev. 18 (2012) 167–180, https://doi.org/10.1089/ten.teb.2011.0244.
[93] T. Matsue, N. Matsumoto, I. Uchida, Rapid micropatterning of living cells by repulsive dielectrophoretic force, Electrochim. Acta 42 (1997) 3251–3256, https://doi.org/10.1016/S0013-4686(97)00175-8.
[94] D.R. Albrecht, G.H. Underhill, T.B. Wassermann, R.L. Sah, S.N. Bhatia, Probing the role of multicellular organization in three-dimensional microenvironments, Nat. Methods 3 (2006) 369–375, https://doi.org/10.1038/nmeth873.
[95] A. Sebastian, A.M. Buckle, G.H. Markx, Tissue engineering with electric fields: immobilization of mammalian cells in multilayer aggregates using dielectrophoresis, Biotechnol. Bioeng. 98 (2007) 694–700, https://doi.org/10.1002/bit.21416.
[96] H. Tsutsui, E. Yu, S. Marquina, B. Valamehr, I. Wong, H. Wu, C.M. Ho, Efficient dielectrophoretic patterning of embryonic stem cells in energy landscapes defined by hydrogel geometries, Ann. Biomed. Eng. 38 (2010) 3777–3788, https://doi.org/10.1007/s10439-010-0108-1.
[97] B.A. Nestor, E. Samiei, R. Samanipour, A. Gupta, A. Van Den Berg, M. Diaz De Leon Derby, Z. Wang, H.R. Nejad, K. Kim, M. Hoorfar, Digital microfluidic platform for dielectrophoretic patterning of cells encapsulated in hydrogel droplets, RSC Adv. 6 (2016) 57409–57416, https://doi.org/10.1039/c6ra10412f.
[98] A. Miano, M.J. Liao, J. Hasty, Inducible cell-to-cell signaling for tunable dynamics in microbial communities, Nat. Commun. 11 (2020), https://doi.org/10.1038/s41467-020-15056-8.
[99] M.O. Din, A. Martin, I. Razinkov, N. Csicsery, J. Hasty, Interfacing gene circuits with microelectronics through engineered population dynamics, Sci. Adv. 6 (2020), https://doi.org/10.1126/sciadv.aaz8344 eaaz8344.
[100] F. Soto, J. Wang, R. Ahmed, U. Demirci, Medical micro/nanorobots in precision medicine, Adv. Sci. 7 (2020) e2002203, https://doi.org/10.1002/advs.202002203.
[101] I.A. Williamson, J.W. Arnold, L.A. Samsa, L. Gaynor, M. DiSalvo, J.L. Cocchiaro, I. Carroll, M.A. Azcarate-Peril, J.F. Rawls, N.L. Allbritton, S.T. Magness, A high-throughput organoid microinjection platform to study gastrointestinal microbiota and luminal physiology, CMGH 6 (2018) 301–319, https://doi.org/10.1016/j.jcmgh.2018.05.004.
[102] J. Wang, P. Ma, D. Kim, B. Liu, U. Demirci, Towards microfluidic-based exosome isolation and detection for tumor therapy, Nano Today 37 (2021) 4, https://doi.org/10.1016/j.nantod.2020.101066.