



Gut-on-a-chip: Mimicking and monitoring the human intestine

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ABSTRACT

Over the last few years, the intestine has been extensively studied using *in vitro* microfluidic systems, commonly known as gut-on-a-chip (GOC) devices. This interest has been due not only to the importance of the intestine's proper functions but also to the relationship that this organ and the microbiota that inhabits it has with the rest of the body's organs. The increased complexity of these *in vitro* systems, together with the need to improve our understanding of intestinal physiology interdependences, has led to greater focus on the integration of biosensors within these devices. However, the current number of GOC devices with integrated sensors for monitoring relevant physiological parameters are very limited and demand the use of external analytical techniques that delay the analysis and prevent real-time decision-making. This paper reviews the various materials, technologies, and structures that have been used both for mimicking the physiology of the intestine and monitoring relevant physiological parameters, such as permeability of the gut barrier, dissolved oxygen concentration, cytokines profile and the production of microbial short-chain fatty acids. We also propose alternative biosensing techniques demonstrated in other *in vitro* and lab-on-a-chip devices that could be translated to GOC models. A critical analysis of the requirements, limitations, and current challenges on the microenvironment replication and monitorization of GOC models is included, with a particular focus on the physiological parameters and biomarkers that should be detected simultaneously in real-time to get a proper framework of the gut function that until now, have not received the necessary attention.

1. Introduction

The intestine is a primary and intricate organ responsible for the uptake of nutrients and water and performs a critical immunological function. It is also the main route for drug absorption and houses a large number of symbiotic microorganisms (microbiota) that support digestion and absorption of nutrients. Intestine dysfunction may cause severe and chronic gut diseases including Inflammatory Bowel Disease (IBD) (e.g., ulcerative colitis and Crohn's disease), celiac disease or Irritable Bowel Syndrome. Although the incidence of intestinal disorders, especially in the case of IBDs, is increasing globally, their etiology is not fully understood (Molodecky et al., 2012). A complex interaction between the host immune system, genetics, microbiota and environmental factors is the most accepted causative agent, in which the imbalance between pro-inflammatory and anti-inflammatory cytokines and alterations of

the composition and function of the gut microbiota (dysbiosis) play a pivotal role (Neurath, 2014). Nowadays, Gut-microbiome research community commonly uses laboratory mice to study these diseases; however, animal models often fail when extrapolated to humans due to the differences in microbiota composition and immune system.

In recent years, organ-on-chip (OOC) technology has emerged as a very promising tool to overcome animal model limitations by recapitulating tissue- and organ-level physiology and function into biologically inspired microfluidic *in vitro* devices (Bhatia and Ingber, 2014; Zhang et al., 2018). OOC devices emulate relevant conditions of the microenvironment found *in vivo*, which is otherwise not possible with conventional cell cultures typically based on 2D monocultures plates. Due to the complex gut dynamics, host-microbiome interactions, and differences between species, gut-on-a-chip (GOC) systems are especially necessary models to advance the knowledge in the intestinal physiology and diseases etiology (Bein et al., 2018; Hewes et al., 2020; Maurer et al., 2019).

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List of abbreviations

COP	Cyclo olefin polymer
DO	Dissolved oxygen
ECM	Extracellular matrix
GC	Gas chromatography
GOC	Gut-on-a-chip
IrOx	Iridium oxide
ISFET	Ion-selective field effect transistors
LAPS	Light-addressable potentiometric sensors
LC	Liquid chromatography
LPS	Lipopolysaccharide
LSPR	Localized surface plasmon resonance
MS	Mass spectrometry
OOC	Organ-on-a-chip
PC	Polycarbonate

PDMS	Polydimethylsiloxane
PEG	Polyethylene glycol
PEGDA	Polyethylene (glycol) Diacrylate
PET	Polyethylene terephthalate
PEVA	Poly-ethylene-co-vinyl-acetate
PMMA	Poly(methyl methacrylate)
PP	Polypropylene
Pt-DENs	Platinum dendrimer-encapsulated nanoparticles
PTFE	Polytetrafluoroethylene
PU	Polyurethane
PVC	Polyvinyl chloride
RuOx	Ruthenium oxide
SCFA	Short-chain fatty acids
SEBS	Styrenic block copolymers
SCFA	Short chain fatty acids
TEER	Transepithelial electrical resistance

The interest in and advancement that OOC systems have made over the last decade, particularly in GOC systems, are indisputable, as shown in Fig. 1a. In addition to the increasing number of publications referring to GOC, in the last two years, several reviews dedicated to gut engineered *in vitro* models have been published, which highlights the importance and growing interest in this organ models (Ashammakhi et al., 2020; Fois et al., 2019; Hewes et al., 2020).

Recent advances in GOC systems attempt to replicate gut inflammation and host-microbiota interrelation to shed light on the pathological mechanisms of intestinal disorders at their early stage. Specifically, GOC models aim to recapitulate the main characteristics of intestinal physiology and include functional readouts for monitoring the biological responses, as schematized in Fig. 1b. Relevant mimicked gut features include barrier function, either by using 2D cell cultures and 3D microstructures (e.g., villi), and emulation of biomechanical cues (e.g., shear stress, oxygen gradient and mechanical deformations) by using perfusable chambers (Ashammakhi et al., 2020; Fois et al., 2019; Hewes et al., 2020). Functional readouts (e.g., integrated biosensors or ELISA test) to assess the barrier integrity, oxygen concentration and inflammation response (e.g., cytokines production) are also involved by using

sensors integrated into the system, or by post-analysis of manually extracted samples (Li and Tian, 2018; Soucy et al., 2019).

The increased complexity of GOC systems, together with the necessity of obtaining real-time information that may be used for *in-situ* decision making (Young et al., 2019), has led to increasing attention on the integration of biosensors within these devices. Fig. 1c shows a timeline of some representative GOC models, indicating the specific emulated feature and the monitorization performed by using sensors integrated into the system. Noteworthy progress has been made in mimicking the intestine environment, while the integration of biosensors still remains a challenge.

In this review, we give a complete overview of the current advances, limitations, and perspectives on the mimicking and monitoring of *in vitro* GOC models from a technological point of view, with particular attention paid to their capability for sensor integration, as this is currently one of the primary necessities and greatest challenge. Apart from revising sensors that have been integrated into GOC devices for monitoring relevant physiological parameters, this work also considers alternative biosensing techniques, especially for cytokines and short-chain fatty acids (SCFA) detection, which have been implemented in

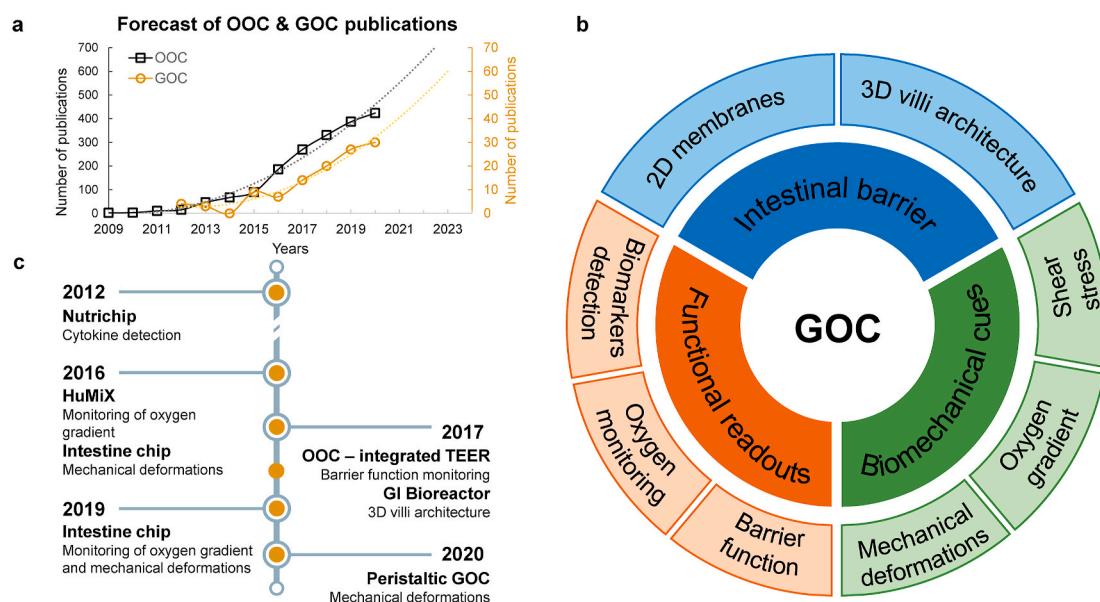


Fig. 1. Current GOC models. (a) OOC and GOC publication analysis carried out in December 2020 (Web of Science, database). (b) Important design considerations for GOC modeling. (c) Timeline of the most relevant GOC models: Nutrichip (Ramadan et al., 2012), Intestine Chip (Kim et al., 2016), HuMiX (Shah et al., 2016), OOC – integrated TEER (Henry et al., 2017), GI Bioreactor (Costello et al., 2017), Intestine chip (Jalili et al., 2019), Peristaltic GOC (Jing et al., 2020).

other *in vitro* and lab-on-a-chip devices and whose implementation could help to further evolve the GOC systems. In a final critical analysis, we propose a GOC system with integrated biosensors for monitoring relevant physiological parameters and biomarkers that if simultaneously detected in real-time, could provide a proper framework of the gut function, and that so far, have not received the necessary attention within the GOC field. We hope that our review of the current advances and sensing necessities will motivate researchers working in biosensing to help solve the current limitations that are preventing organ-on-a-chip technology in general and GOC in particular from reaching the clinic.

2. Physiology of the intestine

The gut (small and large intestine) is a long cylindrical tube with a large surface area featured by the epithelial layer's structural complexity, consisting of finger-like structures called villi, which selectively transport nutrients to the bloodstream (Peterson and Artis, 2014). The intestine also supports peristaltic movements that facilitate food and waste transit, altering the concentration of antimicrobial compounds. The epithelium and the mucus layer lining the gut represent an essential mechanical barrier that provides protection against a variety of infections by blocking the passage of antigens, toxins, and microbiota products. The integrity of this intestinal barrier (e.g., epithelium and mucosa) is vital in maintaining gut homeostasis. Barrier permeability is controlled by epithelium tight junctions, which contribute to the balance between tolerance and immune response to non-self-antigens, as they are responsible for the trafficking of macromolecules along the intestinal tract. Furthermore, the intestine performs a critical immunological function through lymphoid nodules (Peyer's patches) and immune cells such as macrophages, dendritic cells, and neutrophils. Cytokines and chemokines, produced by immune cells, infiltrating inflammatory cells, or from intestinal epithelial cells, support intestinal homeostasis and are key drivers of intestinal inflammation (Andrews et al., 2018).

The small and large intestine sustain a steep oxygen gradient from the epithelium (normoxic) to the lumen (oxygen-free), providing a suitable environment for the flourishing of anaerobic microbes. In some works, it is considered as a whole independent organ that encompasses 10^{14} commensal microorganisms, including bacteria, viruses, fungi, and protozoa (Gill et al., 2006). Among these, bacteria are the most well studied microorganisms. SCFA produced through dietary fiber fermentation by many intestinal bacteria have essential functions in lipid homeostasis and reducing inflammation. It is well known that different bacteria from the microbiota can selectively modulate immune pathways, including innate and acquired immunological responses (Neurath, 2020).

3. Engineering approaches of relevant features of the gut

GOC models represent just a small region of the intestine with the purpose of modeling functional units of this organ. To this end, multiple cell types including intestinal epithelial cells, vascular endothelial cells and immune cells are co-cultured within GOC devices. Established cell lines, including immortalized human colorectal carcinoma-derived cells (Caco-2), have been commonly employed since they are commercially available, robust, and easy to handle. For modeling the vasculature, human umbilical vascular endothelial cells (HUVECs) and human intestinal microvascular endothelial cells (HIMECs), among others, have been typically co-cultured with epithelial cells in separate chambers. In some works, peripheral blood mononuclear cells (PBMCs) isolated from human blood, which contain T-cells, have been added to the vascular compartment to achieve immune functions (Kim et al., 2016; Maurer et al., 2019). Biopsy-derived organoids have also been incorporated, increasing the physiological relevance of the models (Kasendra et al., 2018; Nikolaev et al., 2020). Finally, some representative bacterial strains have also been incorporated to simulate the human microbiome

on-chip, including strains of *Escherichia coli*, *Lactobacillus rhamnosus* GG and *Bacteroides caccae* (Kim et al., 2016, p. 201; Shah et al., 2016).

To recapitulate the gut physiology and functions *in vitro*, GOC systems must offer control over external and internal cell environments, reproducing the topography and the biomechanical cues of the intestine. The advances on material sciences and processing technologies have led to the development of engineering approaches able to reproduce the main relevant characteristics of the gut, as shown in Fig. 2: intestinal barrier, oxygen gradient, peristalsis, and shear stress and mass transport.

Basic engineering approaches of GOC models consist of an upper and bottom microfluidic channel interconnected by a suspended porous membrane that simulates the intestinal barrier. Perfusion through the channels creates the necessary shear stress experienced by the cells and transports the biochemical compounds. However, to faithfully emulate the human intestine, GOC models should: i) experience peristalsis-like motions, simulated by externally stretching the cell culture membrane, ii) recapitulate the complex 3D villi architecture of the intestine, reproduced by using hydrogels scaffolds, and iii) generate a physiological oxygen gradient, achieved by controlling the microfluidics design and material permeability.

Besides mimicking the relevant biological and mechanical cues to match gut-specific environments, microfluidic materials should comply with a specific set of requirements like biocompatibility and optical transparency. However, the integration of sensors within the microfluidic systems adds a degree of complexity to system manufacturing (Cacopardo, 2019; Lind et al., 2017; Perrier et al., 2018). Different techniques and materials have been used to reproduce and monitor these main relevant features, as discussed in the following section.

4. Fabrication techniques for GOC devices and sensor integration

Microfluidics for *in vitro* cell culture and microfluidic sensor integration have evolved following the advancements in lab-on-a-chip and point-of-care devices, as they have the same technological base.

Microfabrication processes inherited from the microelectronics industry (e.g., lithography, additive and subtractive processes) are commonly used to fabricate microfluidic structures down to the micrometer scale and integrate sensing elements. The materials typically employed are silicon, glass, and a few polymers. As example, photolithography is commonly used to fabricate molds for soft lithography using a chemically resistant photosensitive resist such as SU-8 (Huh et al., 2013). Combining photolithography and dry etching is possible to fabricate silicon-based molds for hot embossing to produce microfluidic structures (Illa et al., 2010) or molds containing circular pillars microarrays to manufacture porous membranes (Huh et al., 2013; Jing et al., 2020). Alternatively, e-beam lithography has been used to pattern pores on ultrathin membranes for OOC devices (Chung et al., 2018). Sensing elements integrated into OOC are mainly fabricated by the metal deposition on polymers by e-beam evaporation, due to low deposition temperatures, in a lift-off process (Illa et al., 2014; van der Helm et al., 2019). Another lithography technique adapted to sensor integration into OOC is stencil lithography, used to pattern metal millimeter scale structures on polymeric substrates (Henry et al., 2017).

Rapid prototyping techniques have received considerable attention in OOC manufacturing due to the wide range of workable materials, fast fabrication times, and reduced equipment investment. They encompass a wide range of methods including replication-based techniques, micromachining and printing techniques (Amin et al., 2016; Guckenberger et al., 2015a; Lerman et al., 2018). For OOC applications, the most widely used replication-based technique is soft lithography using PDMS as a substrate material due to the ease of microfabrication, high flexibility, and excellent biocompatibility (M. Whitesides, 2000). Micromachining techniques are very versatile for OOC applications since they can be used either for machining microfluidic features directly on the substrate material or fabricating master molds used in

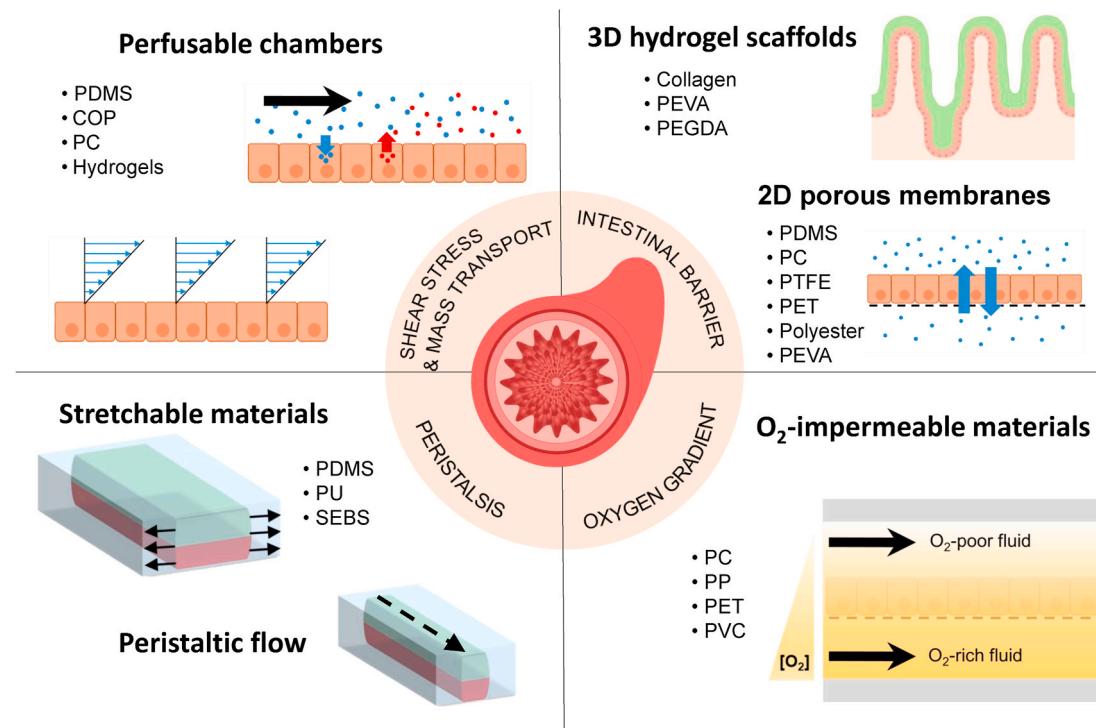


Fig. 2. Engineering approaches to recapitulate gut physiology and function. Main relevant reproduced features include shear stress and mass transport, peristalsis-like motion, intestinal barrier, and oxygen gradient.

replication-based techniques (Guckenberger et al., 2015b; Yen et al., 2016). Recently, three-dimensional (3D) printing has also emerged as a useful tool for fast, automated manufacturing of OOC devices (Knowlton et al., 2016; Lerman et al., 2018), due to its unprecedented ability to fabricate free-standing 3D structures and to manufacture devices in one step with micrometer resolution. Due to their low sintering temperatures and simpler manufacturing processes, printed electronics offers a great alternative to microelectronics-based sensor integration, enabling the patterning of sensors with feature sizes in the hundred micron range and on a wide range of substrates, as well as the embedding of sensors into OOC systems (Moya et al., 2017).

In the particular case of GOC devices, the integration of villi-like hydrogel scaffolds is playing a critical role to better represent the architecture of the gut. To that aim, soft lithography has been the conventional technique to obtain 3D hydrogel scaffolds for *in vitro* gut models (Shim et al., 2017; Wang et al., 2018). Recently, a novel moldless photolithography technique has been developed by Martinez's group and used to fabricate 3D villi microstructures on PEG-based hydrogels as a single-step process (Castaño et al., 2019). Laser-assisted stereolithography, in which a laser light raster scans the photosensitive hydrogel precursor layer by layer to induce the polymerization (Creff et al., 2019), and laser ablation (Nikolaev et al., 2020), have also been applied for 3D and in-plane villi structures generation, respectively. These techniques simplify the multi-step protocols of conventional micromolding techniques by reducing the fabrication time and increasing the resolution of the soft substrates.

Despite the wide range of current fabrication techniques and available materials, there are technological limitations in terms of material compatibility, sensor characteristics, and cytotoxicity, that limits the diversity of materials employed. A striking example is PDMS, which despite its wide use and outstanding features, is prone to leach uncured oligomers into the cell culture medium, absorb small hydrophobic molecules, and increase background fluorescence (Carter et al., 2020; Toepke and Beebe, 2006). Although polymers are preferred for microfluidics due to their low cost, high processability, good biocompatibility and optical transparency, most polymers are not compatible with

microfabrication-based processes. This is mainly due to exposure to high temperatures and chemical incompatibility with photoresists and polar solvents. Moreover, sensor fabrication approaches largely yield electrodes with low optical transmittance, which is counterproductive for the inspection of cells under the microscope. Therefore, micro-fabrication and sensor integration into polymeric devices remains technically challenging. Despite this, thermoplastic polymers like cyclic olefin copolymer (COP) have proven to be chemically compatible with conventional metal micropatterning procedures (Illa et al., 2010). Henry et al. have also integrated semitransparent electrodes on PC substrates by sequentially depositing thin layers of titanium (3 nm), gold (20 nm), and titanium (20 nm) by e-beam evaporation for electrochemical impedance measurements of cellular barriers (Henry et al., 2017).

Regarding 3D printing technology, there are concerns related to the cytotoxicity of printed materials. It has been demonstrated that materials used in 3D printing leach harmful chemicals such as monomers, photoinitiators, and plasticizers on cell cultures, which may result in poor cell adhesion or even cell death (Oskui et al., 2016). These issues of commercially available resins have driven research on novel printable substrates compatible with cell culture applications (Amin et al., 2016; Bhattacharjee et al., 2018; Zhu et al., 2018). In fact, Bhattacharjee et al. developed a 3D printing PDMS-based resin that displays similar properties to thermally cured PDMS in terms of biocompatibility, transparency, and elasticity.

5. Mimicking the human intestine microenvironment on a chip

As shown in Fig. 2, GOC models try to reproduce the intestinal barrier and incorporate biomechanical cues, including shear stress, hypoxia conditions, and cyclic strain, to emulate the intriguing complexity of the intestinal microenvironment. In this section, we discuss the impact that these system parameters have on chip design and provide examples of how these physiological factors have been translated into GOC modeling.

5.1. Intestinal barrier function

The major function of a cell barrier is to regulate and to separate two distinct physiological compartments. To simulate this separation, most GOC systems are based on the creation of independent compartments (e.g., two or more perfused microchambers) interconnected by physical interfaces (e.g., permeable membranes or gel-liquid interfaces) that serve as support for cell culturing (Yeste et al., 2018). The ability to generate an interface enables access to apical and basal domains, the assessment of intestinal barrier function, and analysis of drug metabolism and absorption.

2D barriers

Most GOC models are composed of two perfused microchambers interfaced by a semipermeable ECM-coated membrane in which intestinal cells are cultured on one of their sides forming a tight monolayer (Fig. 3a). Membrane properties including thickness, pore size and distribution are the most important parameters (Tan and Rodrigue, 2019). Pore size dictates whether cells are restricted to paracrine communication, granted cell-to-cell physical contact, or transmigrate from one compartment to another, whereas pore density and membrane thickness influence membrane permeability and transport mechanisms (Chung et al., 2018). Commercially available membranes that have been incorporated in intestinal tissue barrier studies are typically made of polycarbonate (PC), polyethylene terephthalate (PET), and polytetrafluoroethylene (PTFE) (Maurer et al., 2019; Shah et al., 2016; Tan et al., 2018). PC and PET membranes are generally fabricated by the track-etching technique in which pore size, shape, and density are well-controlled parameters. These membranes are available in pore sizes ranging from 0.1 μm to 12 μm. PTFE membranes are usually manufactured by electrospinning producing highly porous nonwoven semipermeable non-transparent membranes. Custom-made membranes are an alternative to commercially available ones as they can be easily adapted to the needs of a particular experiment. Polydimethylsiloxane

(PDMS) is the most employed material for fabricating custom membranes with typical pore size diameters between 2 μm and 10 μm (Fan et al., 2015; Quirós-Solano et al., 2018).

Villi-like intestinal architecture

Although spontaneous villi formation on planar substrates has been reported, it lacks control over villi dimensions and distribution and has poor reproducibility (Kim et al., 2012; Maurer et al., 2019). The integration of microfabricated 3D scaffolds has emerged as a solution to better recapitulate the human intestinal architecture (Sung et al., 2011; Yu et al., 2012). Furthermore, hydrogel-based scaffolds closely resemble the native ECM microenvironment providing a more biomimetic substrate and cell differentiation along with barrier function and integrity. In this regard, Shim et al. developed a perfusable platform with an integrated collagen-based villi-like structure via soft lithography using a SU-8 master mold. The height of the villi was 300 μm and intervilli spacing was 150 μm (Shim et al., 2017). In a similar approach, Costello et al. 3D printed a synthetic material called polyethylene-co-vinyl-acetate (PEVA) with the intestinal topography and integrated the scaffold in a bioreactor where cells were also exposed to cell media flow. This model permitted long-term culture up to 32 days and intestinal cells exhibited characteristics similar to those found *in vivo* in terms of nutrient absorption (Fig. 3b) (Costello et al., 2017). Recently, a hydrogel scaffold made of a mixture of collagen I and Matrigel and fabricated by laser photopatterning has been developed to reproduce the architecture of the gut crypts (Nikolaev et al., 2020). Seeded intestinal stem cells were able to self-organize within the device, forming perfusible organoid tubules with key physiological features such as intestinal tissue regeneration.

5.2. Biomechanical cues

Cells can sense a wide variety of biomechanical cues and respond to these stimuli by influencing essential aspects of cell behavior such as

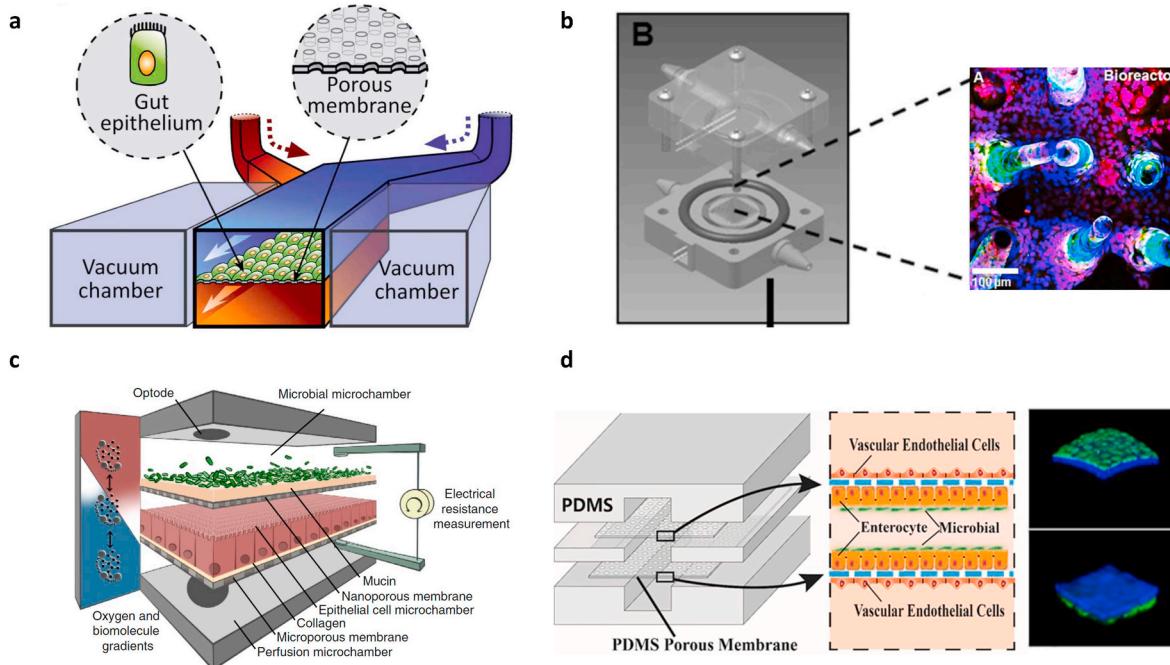


Fig. 3. Examples of GOC models that faithfully capture key aspects of the human intestine. (a) Basic design of a GOC model based on intestinal monolayers cultured on top of a flexible and porous membrane (Kim et al., 2012), with permission from the Royal Society of Chemistry. (b) A GOC model mimicking the 3D villus topography of the intestine by culturing cells on a villous-like scaffold (Costello et al., 2017), reproduced under the Creative Commons Attribution 4.0 International License. (c) The HuMiX model recreates a steep oxygen gradient across the device by using a gas impermeable material (Shah et al., 2016), reproduced under the Creative Commons Attribution 4.0 International License. (d) A peristalsis GOC model in which intestinal cells experience peristalsis-like motion by perfusing pulsatile flow into the middle chamber (Jing et al., 2020), reproduced under the Creative Commons Attribution 4.0 International License.

alignment, proliferation, and differentiation (Vogel and Sheetz, 2006). In the gut, shear stress, hypoxia conditions, and peristaltic muscular contractions can critically affect epithelial cell differentiation and bacterial overgrowth (Gayer and Basson, 2009).

Shear stress

In microfluidic devices, cell culture medium is perfused by pumping systems at desired flow rates through the microchannels resulting in associated shear stress experienced by the cells. Typically, shear stress can be tailored to the specific needs of the cultured cell type by adjusting fluid flow rate or channel dimensions. In the intestine, shear stress plays a fundamental role in cell differentiation including enhanced mucus production, increased mitochondrial activity, and higher drug absorption (Delon et al., 2019). Several studies have demonstrated that development of fully matured intestinal cells is achieved in 4 days under dynamic flow in contrast to the 21-day period for static conditions (Natoli et al., 2012; Trietsch et al., 2017).

The external pumping systems used to induce the fluid flow allow the fine-tuning of flow rates with high precision, but they imply bulky setups and low levels of parallelization. Despite this, Tan et al. increased its throughput by integrating two micropumps on a Thiolene Microchip to drive fluid flow through 16 microchannels (Tan et al., 2018). Common values of shear stress of GOC devices are usually in the range of 0.01–0.06 dyne/cm⁻² (Jalili et al., 2019; Jing et al., 2020; Maurer et al., 2019). Alternative pumping techniques include the pump-free perfusion using gravity-induced flow exploited by the OrganoPlate (OrganoPlate®, Mimetas, Netherlands) platform. Although this technique is very simple and has a higher degree of parallelization, control over shear stress is limited and the bidirectionality of the flow is not physiological (Trietsch et al., 2017).

Oxygen gradient generation

Oxygen levels in standard cell culture conditions are maintained at around 20%, which differs greatly from the intestinal physiological values. It is well known that intestinal-level oxygen concentrations encompass a steep gradient along the intestinal tract with oxygen levels below 0.5% (Zheng et al., 2015). Most materials used for static cell culture are gas permeable to avoid oxygen depletion and, therefore, cell death. However, to model the complex and rich intestinal milieu, a hypoxic microenvironment is required to co-culture eukaryotic cells with anaerobic bacteria. To achieve anaerobic conditions on GOC devices over extended periods of time, the material's gas permeability plays a fundamental role. In this regard, Shah et al. developed the HuMiX device using two PC enclosures acting as a barrier to gas passage into the culture area (Fig. 3c). By continuously perfusing anoxic medium (0.1% O₂), oxygen profiles across the microbial chamber were maintained at 0.8% (Shah et al., 2016). Other GOC systems, such as the Intestine Chip have been entirely made of PDMS, a well-known gas permeable material (Jalili et al., 2019). To provide adequate oxygen levels, the Intestine Chip is placed inside an anaerobic chamber made of poly(methyl methacrylate) (PMMA) that is continuously flushed with humidified 5% CO₂ in nitrogen gas. Although this method is costlier, as additional space and a continuous nitrogen supply are needed, oxygen levels of 0.3% have been attained with this system, which are much closer to the physiological values found in the intestine *in vivo*. Therefore, for the study of crosstalk between the human intestinal mucosa and microbiota under a hypoxia microenvironment, gas-impermeable materials, such as many thermoplastics, are the best option.

Cyclical mechanical deformations (peristalsis)

Cyclical mechanical contractions can be accurately reproduced on GOC platforms by applying an external loading in which muscle contractions or peristaltic-like flow are simulated. In this regard, the Intestine Chip developed by Kim et al. recreates intestinal mechanophysiology *in vitro* by applying computer-controlled vacuum suction to its lateral hollow chambers. This causes the PDMS-based

membrane to unidirectionally stretch outwardly. The model mimics the mechanically active intestine microenvironment by exerting a strain value of 10% at a frequency of 0.15 Hz (Jalili et al., 2019; Kim et al., 2012, 2016). Another system developed by Jing et al. simulates peristaltic-like motion by perfusing culture medium at fluctuating flow rates using a pneumatic pump (Jing et al., 2020). With this method, the PDMS-based membrane bends periodically in the z-direction, reaching 15% of membrane deformation (Fig. 3d).

The main factor in simulating peristalsis-like cues on-a-chip is that the membrane on which cells are cultured must sustain cyclical mechanical deformations for prolonged periods of time. Therefore, the material must be flexible enough to undergo such mechanical loading within the material's elastic region without fatiguing. Elastomeric materials are best suited for this application because they show wide elastic deformation regions. For example, PDMS-based membranes display a wide linear elastic region up to strain values of 40% (Mata et al., 2005). Other less well-known polymers that exhibit similar elastomeric properties and could be employed for OOC devices are polyurethane (PU) (Domansky et al., 2013) and styrenic block copolymers (SEBS) (Domansky et al., 2017). Although they display a narrower linear region up to 25%, these materials can be used for cyclic mechanical loading since the *in vivo* values lie within 10–15% of intestinal mechanical strain.

6. Monitoring the human intestine on-a-chip

GOC technologies seek to provide relevant physiological data about key biological processes in real-time and in a non-invasive fashion. To this end, high-resolution monitoring of the GOC microenvironment is of paramount importance. As GOC models have improved in biomimetic performance, the necessity to integrate accurate and high-throughput monitoring tools into the microfluidic devices has increased in parallel. Sensing systems including physical, chemical, and biochemical approaches have been pursued to capture the intestinal physiology dynamics including the complex host-microbiota interplay (immune responses, epithelial barrier monitoring, mucosa, viruses affecting the gut, etc.). Furthermore, multiplexed readout technologies have been shown as crucial for upgrading the parallelization capacity allowing high data throughput (Suresh et al., 2009). These analytical tools can be categorized according to their position in the operation workflow of the GOC. In-line and on-line monitoring provides real-time data by means of fully integrated sensors (in-line) or by analysis of automatically collected samples (on-line). On the contrary, at-line and off-line schemes require manual sample collection and subsequent analysis in the laboratory or off-site analysis, respectively; thus not allowing real-time data acquisition and delaying decision-making actions (Chu et al., 2011).

Various parameters can be monitored in GOC systems owing to the complex intestinal physiology and pathophysiology. Relevant factors of the cell microenvironment include physicochemical properties (e.g., oxygen, pH, temperature, CO₂, and osmolarity), physical cues (e.g., mechanical interactions from cell-cell and cell-ECM contact), and biochemical cues (e.g., hormones, cytokines, and growth factors). So far, the parameters monitored in GOC devices have been limited to barrier permeability, dissolved oxygen (DO) levels, and cytokines production. More recently, SCFA production has also been off-line monitored. In this section, the most relevant sensing technologies used in GOC models for in-line and off-line monitoring of these key parameters are reviewed in detail.

6.1. Intestinal barrier function assessment

Quantifying the permeability of barrier tissues is necessary to assess the state of the barriers and identify the factors contributing to barrier dysfunction. For example, in toxicology, monitoring of the barrier integrity permits evaluation of the effects of toxic compounds; in disease modeling, examining the barrier breakdown during a disease progression; and in drug development, testing the ability of new drugs to cross

the barriers. The most common laboratory techniques are based on permeability assays and transepithelial electrical measurements. The former can be used to determine apparent permeability coefficients of test compounds (e.g., to predict drug absorption), whereas the latter provides information on transepithelial ion transport (e.g., to assess barrier integrity). Permeability assays involve the addition of tracer molecules in a donor compartment (either apical or basolateral) and diffusion measurements in the opposite side of the barrier (Huxley et al., 1987). The most commonly used tracer compounds are fluorescent dyes, e.g., fluorescein isothiocyanate or Rhodamine, while molecular weight is an important factor in studying the size-selectivity of biological barriers (Linnankoski et al., 2010; Mari Hämäläinen et al., 1997). These assays permit quantifying transepithelial transport in both directions and can discriminate between active and passive transport mechanisms (Artursson et al., 2001; Hubatsch et al., 2007). Thus, permeability tests are a valuable tool in barrier assessment and drug permeability prediction, but they entail time-consuming and invasive protocols.

On the other hand, transepithelial electrical measurements enable real-time monitoring and are label-free and non-invasive. In particular, electrical impedance spectroscopy can determine: i) transepithelial electrical resistance (TEER), which evaluates the barrier integrity (closely related to the tightness of the intercellular space); ii) the cell layer capacitance, which can yield information about the cell membrane surface area; and iii) the contribution of the medium solution to the impedance (Benson et al., 2013). While these measurements are easy to carry out only by means of external electrodes, it is challenging to achieve uniform current distribution required for accurate transepithelial electrical measurements in the miniaturized cell culture channels of OOC, which may account for the large discrepancies between TEER values reported in the literature (Odijk et al., 2015). Several researchers have proposed particular strategies and electrode configurations to address this issue in microfluidic cell cultures and have integrated TEER measurement electrodes to allow monitoring of the barrier state in real-time (Henry et al., 2017; Yeste et al., 2016).

In the case of microfluidic intestinal barrier models, TEER measurements have been performed by means of external electrodes such as

Ag/AgCl electrode wires or chopstick-like electrodes inserted into the microchambers (Shah et al., 2016; Shin et al., 2019). Odijk et al. compared direct current TEER values obtained from a GOC model with a simple Transwell culture system, demonstrating that differences mainly arise from the device geometry rather than biological factors. Remarkably, small changes in the cell layer coverage (e.g., 0.4%) could dramatically decrease TEER (80%) (Odijk et al., 2015). Owing to this, other researchers have employed integrated microelectrodes to improve accuracy and parallelization capacity. For instance, integrated TEER gold electrodes were used for real-time epithelial barrier monitoring into a PC chip, illustrated in Fig. 4a (Henry et al., 2017). In a different approach, indium alloy bottom electrodes and platinum wire top electrodes were embedded directly on both sides of the membrane in a thiol-ene GOC (Tan et al., 2018). The device, which contained eight parallel chambers, enabled monitoring intestinal drug transport in real-time with increased throughput. Following this trend, Trietsch and co-workers developed a high-throughput platform containing 40-microfluidic cell culture structures embedded in a 384-well glass plate format (OrganoPlate®, Mimetas, Netherlands). The system was composed of a polystyrene top plate and a bottom plate made of glass (with optical quality) containing microfluidic elements made of glass and proprietary polymers (low compound-absorbing). Thanks to its high-throughput capability, the device enabled the assessment of barrier permeability in several intestinal tubules in parallel by perfusion of fluorescent dyes. Barrier response to different drugs was studied by following the dye leakage with automated imaging techniques, making it a user-friendly technology compatible with standard laboratory equipment (Trietsch et al., 2017). Later, the device was coupled to TEER electrode-pair arrays for simultaneous assessment of intestinal barrier integrity (OrganoTEER®, Mimetas, Netherlands). By monitoring barrier function and cytokines production, the platform was successfully validated for large scale disease modeling and drug discovery (Beaurivage et al., 2019).

6.2. Dissolved oxygen monitoring

Precise oxygen supply is a critical issue owing to the essential role of

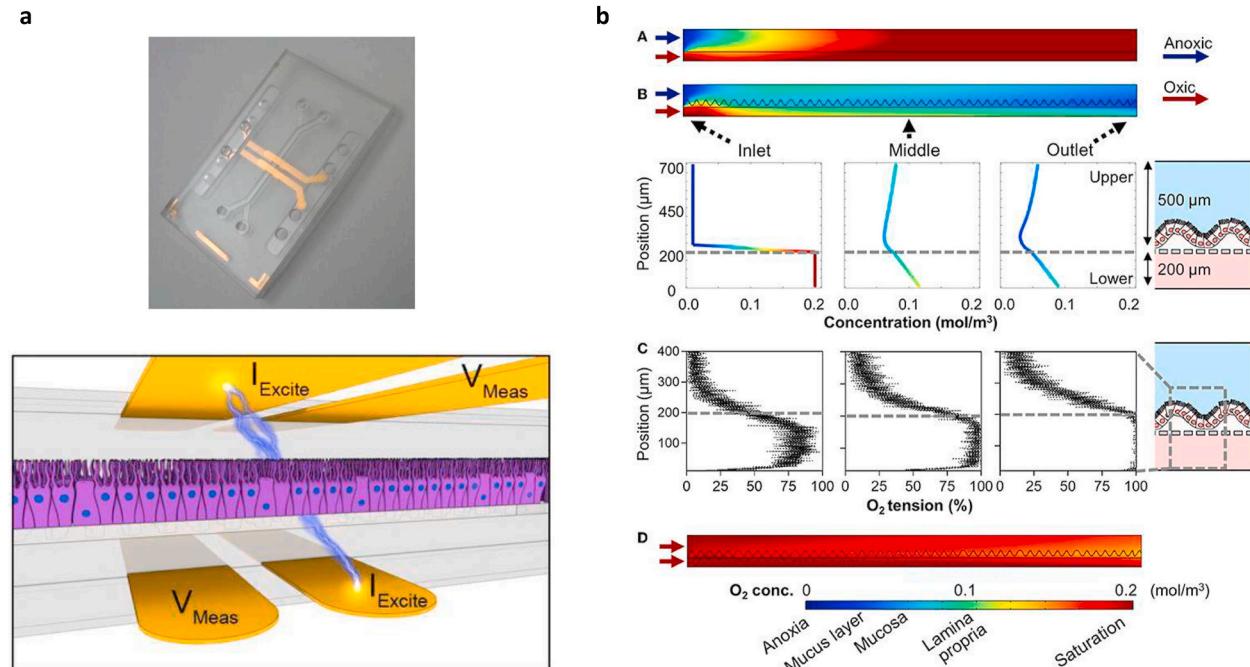


Fig. 4. GOC monitoring. (a) Chip with integrated TEER gold electrodes patterned onto polycarbonate substrates and scheme depicting the 4-point impedance measurements. Reproduced from Henry et al. with permission from the Royal Society of Chemistry (Henry et al., 2017). (b) Profiles of oxygen concentration at the inlet, middle and outlet regions of an anoxic-oxic GOC. Reproduced from Shin et al. under the Creative Commons Attribution 4.0 International License (Shin et al., 2019). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

DO gradients in shaping the gut-microbiome ecosystem. Luminal oxygen levels below 0.5% are required and should be kept constant over time since most commensal bacteria are anaerobic (Zheng et al., 2015). There are several approaches to measure oxygen concentration based in optical, electrochemical, infrared, ultrasonic, paramagnetic, and very recently, laser methods (Pereira et al., 2017; Suresh et al., 2009; Wei et al., 2019). Most extended probes for measuring DO in liquid are electrochemical (Clark-type microsensors) and optical (Chu et al., 2011; Moya et al., 2016, 2018).

DO monitoring in GOC has been performed in-line using non-invasive and biocompatible integrated optical sensors (optodes). Regarding the low intestinal oxygen levels, optical sensors are more advantageous since an oxygen depletion region is not created and direct physical contact with the solution can be avoided. The first GOC model to include oxygen sensors was the HuMiX device, which incorporated four sensors made of commercial oxygen-sensitive material (pst3) bonded to deep-machined pockets of the PC enclosure by applying silicone-based adhesive. The constructed 5 mm diameter disk optodes were affixed close to the inlets and outlets of the microbial and perfusion microchambers to achieve spatially-resolved fluorescence readouts using a commercial recorder (Shah et al., 2016). As a step forward, an Intestine Chip developed by Jalili et al. allowed high-resolution DO monitoring thanks to the integration of 6 sensor disks composed of oxygen-quenched fluorescent microparticles within the microfluidic structure (Jalili et al., 2019). In this study, oxygen sensors were fabricated by mixing oxygen-sensitive and optical particles with PDMS prepolymer and curing agent. After spin-coating and curing the mixture, 1 mm oxygen sensor disks were obtained using a biopsy punch and embedded in the PDMS microfluidics channels. These sensors could be interrogated in a non-invasive way by fluorescence readout and displayed in pseudocolors for further quantification.

Shin and co-workers used an interesting approach to validate the oxygen gradient generated in an anoxic-oxic interface-on-a-chip containing a human intestinal epithelium, shown in Fig. 4b. In this work, cell culture medium was mixed with platinum dendrimer-encapsulated nanoparticles (Pt-DENs) and a fluorescent reagent. Pt-DENs acted as peroxidase-like nanozymes converting molecular oxygen into reactive oxygen species, which induced the oxidation of the reagent by the Pt nanoparticles producing a fluorescent compound. The oxygen gradient was visualized in-situ by confocal microscopy obtaining accurate spatiotemporal concentration profiles allowing validation of the platform for the robust co-culture of anaerobic microbiome with intestinal epithelium. However, this strategy was not found suitable for monitoring DO profiles under real operating conditions since the employed nanomaterials and reagents could interfere with the microphysiological conditions (Shin et al., 2019).

6.3. Biomarkers detection

For many years, host immune factors such as cytokines, antibodies, and C-reactive protein (CRP), as well as cellular (regulatory T cells) and genetic biomarkers have been considered useful indicators of inflammatory processes in general and of IBDs in particular (Norouzinia et al., 2017). More recently, it has been suggested that several microbiome-derived and microbiome-modified metabolites act as signaling molecules in the host-microbiota interplay, encompassing carbohydrate and protein fermentation products (e.g., SCFAs), amino acid derivatives (e.g., indol) and others (Krautkramer et al., 2020). Due to their important physiological functions, cytokines and SCFAs have attracted much attention in the last years as biomarkers in GOC modeling and, therefore, are revised in detail in this section.

Cytokines

Cytokines are low-weight soluble signaling proteins that act as mediators and modulators of the complex functional interactions and responses of the immune system. Given their central role in host immune

response to infection, inflammation, cancer and many other processes, cytokines are among the most informative biomarkers in GOC models. The conventional standard methods for cytokine quantification are immunoassay-based techniques including ELISA test and bead-based immunoassay, which currently require a minimum assay time of 3–8 h in a centralized clinical laboratory setting. These at-line techniques have been widely employed to monitor cellular responses to toxins, drugs, or other test compounds in GOC models (Beaurivage et al., 2019; Kim et al., 2012, 2016; Maurer et al., 2019; Shah et al., 2016). For example, the immunological response of epithelial cells to co-cultured bacteria was assessed by cytokine analysis in the HuMiX model by determining the concentration of eight different pro-inflammatory cytokines (GM-CSF, IL-1, IL-6, IL-10, IL-12 and TNF- α) in eluate samples from the perfusion microchamber (Shah et al., 2016). In a similar way, analysis of the epithelial response through cytokines detection (IL-8, IL-6, IL-1 and TNF- α), in both luminal and capillary chambers of a GOC containing intestinal cells and microbiota, revealed pro-inflammatory effects induced by immune cells and bacterial lipopolysaccharide (LPS) endotoxin (Kim et al., 2016). Similarly, in an on-chip model of gut inflammation harboring immune cells, intestinal epithelium and microbiome, dextran sodium sulphate-sensitized epithelium produced inflammatory cytokines stimulated by LPS, which were detected in the apical chamber of the microdevice (Shin and Kim, 2018). Interestingly, an analysis of apical and basal cytokines secretion in a high-throughput GOC allowed the formulation of a cytokine cocktail with pro-inflammatory capacity comparable to *E. coli*-activated dendritic cells (Beaurivage et al., 2019). The complex crosstalk between epithelial, immune and microbial components has also been studied in terms of cytokines secretion. In particular, the effect of microbial interactions was studied by detecting IL-6 and TNF- α secreted by epithelial and endothelial cells in an immunocompetent GOC (Maurer et al., 2019). In the Nutrichip, a detection chamber was created downstream of the culture chambers, which were separated by a valve (Ramadan et al., 2012). Dedicated to cytokines detection, the chamber contained magnetic beads functionalized with antibodies which allowed in-situ capture of the biomarkers and simultaneous washing prior to fluorescent detection (Lehmann et al., 2001).

Short chain fatty acids (SCFA)

The human gut is a dynamic environment in which microorganisms constantly interact with the host via their metabolic products. Some of the most important microbial metabolic products are fermentation products such as SCFAs (Levy et al., 2016). SCFA (specifically, acetic, butyric, propionic, valeric, isobutyric, and isovaleric acids) are the primary end-products of fermentation of non-digestible carbohydrates, which serve as energy sources for gut epithelial cells, modulate cytokines production, and induce expansion of regulatory T cells (Schirmer et al., 2016). Numerous efforts are focused on providing evidence for the role of SCFA as key signaling molecules between the gut microbiome and host health and their role in human metabolic health (den Besten et al., 2013).

Despite the high interest, we have only found one work using GOC models to study the production and relation of SCFA on gut inflammation. In this work, Trapecar et al. used a microphysiological system that modelled the gut-liver-immune axis, and showed that microbiome-derived SCFA may either improve or worsen inflammatory bowel disease and liver disease severity, depending on the activation state of CD4 T cells (Trapecar et al., 2020). In this work, the SCFA were measured by liquid chromatography and mass spectrometry (LC-MS) by collecting samples from the GOC system.

7. Emerging biosensing technologies for GOC monitoring

The field of biosensing technology is continuously evolving towards the development of analytical devices with improved performance for label-free and real-time detection of clinically relevant biomarkers

(Vigneshvar et al., 2016). In our opinion, GOC modeling could take advantage of recent advances in biosensing (especially for cytokines and SCFA) to get an accurate perspective of the intestine function as a whole and, in turn, enable rapid decision-making.

The cytokine biosensor (Stenken and Poschenrieder, 2015) has positioned itself as a promising alternative to standard methods in cell culture for automated and real-time monitoring of cytokine profiles, and its integration in GOC models could be enormously beneficial. On-line cytokine monitoring in *in vitro* microfluidic systems mostly involve electrochemical detection since microelectrode arrays can be easily microfabricated and integrated into microfluidic platforms. Electrochemical sandwich immunosensors either direct or magnetic bead-based have been coupled to automated microfluidic platforms for multiplexed monitoring of cell secretomes on-line. Due to their easy functionalization, microfabricated gold electrodes (Riahi et al., 2016) or gold screen-printed electrodes have been used (Ortega et al., 2019). For example, the use of magnetic beads with microfluidics allowed on-chip capture of IL-6 and TNF- α with detection performed off-chip (Hernández-Albors et al., 2019). These systems yield high sensitivity measurements but are laborious, time-consuming, and thus unable to provide real-time data. For this reason, label-free sensing strategies have been developed taking advantage of the sensitive nature of impedimetric methods. Microfluidic impedimetric immunosensors demonstrated capacity for continuous monitoring of cell-secreted biomarkers for up to 7 days in a liver-on-a-chip platform (Shin et al., 2017). In addition, the antibody-functionalized gold microelectrodes could be regenerated (and reused) with a cleaning solution and application of electrical sweep potential. This was followed by implementation of a label-free biosensor in a fully integrated modular heart-liver-on-chip multisensor platform for automated in-line monitoring, as schematized in Fig. 5a (Zhang et al., 2017).

As a step forward, Liu et al. developed an on-chip microfluidic platform containing micropatterned gold electrodes for detecting

multiple cell-secreted cytokines (Liu et al., 2015). Label-free, sensitive, and specific detection of the pro-inflammatory cytokines IFN- γ and TNF- α was demonstrated using aptamers labelled with redox reporters, based on the fact that binding of target cytokines induced conformational changes in the bioreceptor molecules. Furthermore, the sensors could be regenerated on-chip with urea buffer (Zhou et al., 2014). The device was also employed to study TGF- β secretion induced by alcohol in a liver-on-a-chip model (Zhou et al., 2015).

Alternatively, localized surface plasmon resonance (SPR) technology has evolved as a sensitive and versatile tool for detecting cytokines in microfluidic cell culture systems. An example is the development of a SPR imaging biochip mounted on an optical inverted microscope for detecting the cytokine secretions of T lymphocytes (Baganizi et al., 2015). Oh et al. developed a nanoplasmonic biosensing approach for the detection of a single cytokine inside a cell culture chamber (Oh et al., 2014). They then went on to develop a different localized SPR approach to quantitatively characterize cytokine secretion behaviors of T cells with a fine time-resolution that were altered by immunosuppressive drugs (Oh et al., 2016). This technique enabled simultaneous multi-time-point measurements of pro-inflammatory (IL-2, IFN- γ , and TNF- α) and anti-inflammatory (IL-10) cytokines secreted by T cells, as illustrated in Fig. 5b. However, the detection system was based on bulky dark-field microscopy with a motorized stage, and the sample had to be extracted from the cell culture and injected into the biosensing chip for the analysis.

The identification and quantification of SCFA in biological samples, usually fecal samples, is quite extensive and commonly performed by so called metabolomics techniques (Primec et al., 2017). Although these methods could be implemented for SCFAs analysis in GOC devices, it would require manual sample collection from the outlet port of the microfluidic system, preventing the continuous monitoring information. As stated in numerous papers, the rate of production of each SCFA in the gut lumen is an important factor to measure as this parameter regulates

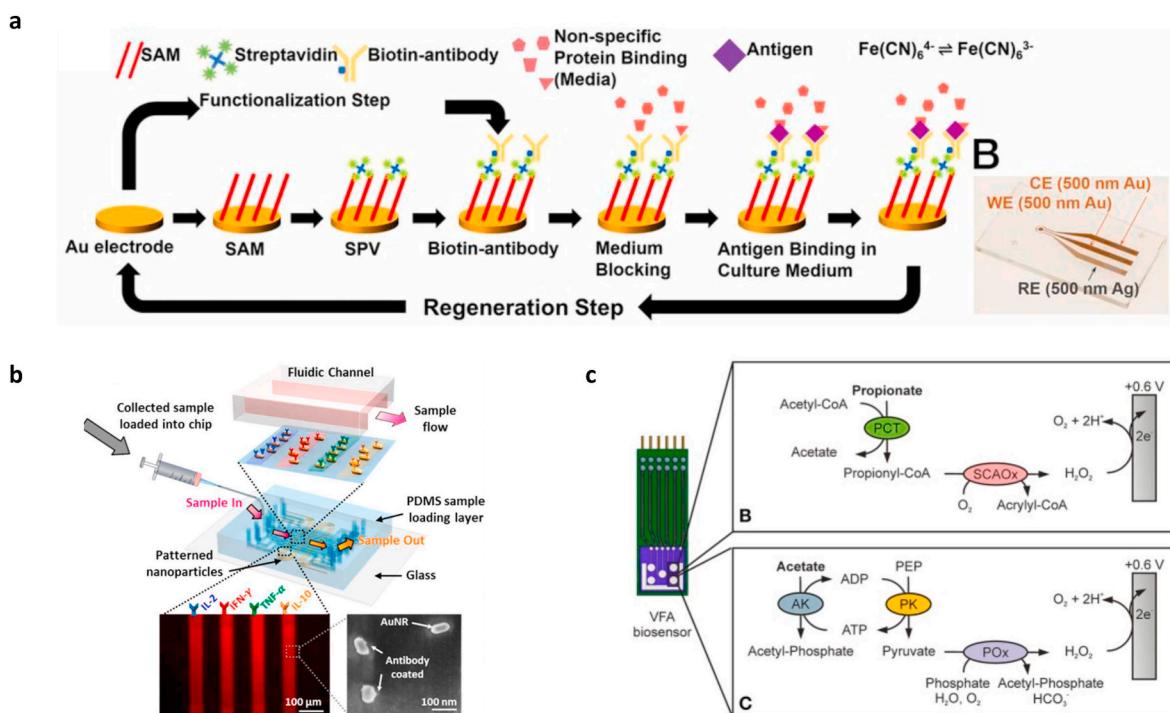


Fig. 5. Emerging biosensing approaches for GOC monitoring. (a) Schematic showing the functionalization and regeneration process of the electrodes and image of the electrochemical sensor for biomarkers detection in an OOC platform (Zhang et al., 2017). Reproduced from Zhang et al. with permission from the National Academy of Sciences. (b) Multiplexed cytokine detection using LSPR nanoplasmonic biosensor microarray chip (Oh et al., 2016). Reproduced from Oh et al. with permission from the American Chemical Society (c) Amperometric detection principles of acetate and propionate using enzyme-modified electrodes. (Röhlen et al., 2018). Reproduced from Röhlen et al. under the Creative Commons Attribution 4.0 International License.

bacterial fermentation in the intestine (Sakata, 2019), and is only possible by the integration of SCFA biosensors into or in-line within GOC devices.

Microbially produced SCFAs have been the focus of attention in many different areas, including the food industry and biofuel cells, where the advance in sensor integration in microfluidics for on-line and in-line monitoring has been the greatest. For example, acetate determination is very important for food industries. Mieliauskiene et al. proposed in 2006 an amperometric biosensor for acetate monitoring in vinegar and wine (Mieliauskiene et al., 2006). This was a sensitive, selective, and stable tri-enzyme electrode developed by immobilization of the enzymes on the surface of the graphite electrode. In this case, miniaturization of the sensor was feasible, although it was conceptually very complex. More recently, microfabricated platinum electrodes enzymatically modified have been used for the amperometric quantification of acetate and propionate, mediated by oxidation of hydrogen peroxide, as shown in Fig. 5c (Röhlen et al., 2018). Alternative approaches have demonstrated the use of Fourier transform infrared spectroscopy for the analysis and prediction of fatty acid profiles (Shapaval et al., 2014, 2019), and also the detection of volatile SCFA by electronic nose (eNose) (Wilson, 2018).

Other types of SCFA biosensors are based on living microorganisms. The first demonstration of SCFA quantification using microbial fuel cells was presented by Kaur et al. (Kaur et al., 2013, 2014). However, the detection range (less than 80 mg/L) was quite limited for real applications although it functioned as a sensor of total organic matter with real wastewater. An innovative biosensor based on a microbial electrolysis cell was developed by Jin et al., in 2017 to monitor SCFA concentrations resulting from anaerobic digestion processes in wastewater over a 5 month period (Jin et al., 2017). However, this bio-electrolytic sensor is a long way from being miniaturized to fit onsite clinical devices. Atci et al. presented two novel prototypes of punctual amperometric microbial

biosensors with immobilized specific microorganisms over the working electrode that were able to oxidize SCFAs, specifically acetate (Atci et al., 2016) and lactate (Atci et al., 2017). The authors demonstrated the feasibility of monitoring these analytes in depth profile inside a biofilm, as the microelectrodes were all enclosed in a glass outer case with a 30-μm tip diameter. Although these sensors were miniaturized, their fabrication presented several technical difficulties. However, as these microbial biosensors could be connected in-line to GOC devices to perform measurements in the outlet ports of the system, they are a promising tool for SCFAs monitoring.

Finally, apart from acting as biocompatible 3D scaffolds for cell culture, hydrogels can also be exploited as biosensing elements in GOC monitoring. For many years, stimuli-responsive hydrogels have been recognized as able to respond to many different cytocompatible factors including biomolecules, live cells, and pH, by changing their water content and/or physicochemical properties (Mohamed et al., 2019). Hydrogel response may be monitored by optical, electrochemical and micromechanical methods (Tavakoli and Tang, 2017). For example, biorecognition elements such as antibodies and cells have been encapsulated within hydrogel matrices for detection of biomarkers and bio-active compounds, as demonstrated for cytokines, phenolic compounds and toxics (Sanahuja et al., 2015; Shin et al., 2016; Vigués et al., 2018). Remarkably, sensing hydrogels have proved useful for assessing cell activity in-vivo and even in-situ manipulation and self-reporting on cell scaffold stiffness (Choi et al., 2013; Li et al., 2020). Hydrogel photonic crystals, in which the refractive index has a periodic variation, have attracted much attention in recent years for enabling facile colorimetric detection of biomolecules, e.g., volatile organic compounds (Qin et al., 2018).

Table 1
Technological overview of representative GOC models.

In vitro platforms	Fabrication technique	Material	Chip configuration	Membrane properties	Mechanical & biochemical cues				Integrated readouts			Throughput
					Shear stress	Cyclic strain	Oxygen gradient	3D	TEER	Oxygen	Cytokines	
HuMiX Shah et al. (2016)	Micromachining	PC	3-layered channels	PC Øpore = 50 nm	Yes	No	Yes	No	No	Yes	No	Low
OOC – integrated TEER (Henry et al., 2017)	Soft lithography	PDMS	2-layered channels	PDMS Øpore = 10 μm	Yes	No	No	No	Yes	No	No	Low
Intestine Chip (Jalili et al., 2019)	Soft lithography	PDMS	2-layered channels	PDMS Øpore = 10 μm	Yes	Yes	Yes	Yes	No	Yes	No	Low
OrganoPlate (Trietsch et al., 2017)	Photolithography	Glass	3-side by side channels	Membrane-free	Yes	No	No	Yes	No	No	No	Medium
Thiol-ene Microchip (Tan et al., 2018)	Soft lithography	PDMS	2-layered channels	PTFE Øpore = 0.4 μm	Yes	No	No	No	Yes	No	No	Medium
Anoxic-oxic interface chip (Shin et al., 2019)	Soft lithography	PDMS	2-layered channels	PDMS Øpore = 10 μm	Yes	No	Yes	Yes	No	No	No	Low
Immuno Biochip (Maurer et al., 2019)	Injection molding	Polystyrol	2-layered channels	PET Øpore = 8 μm	Yes	No	No	Yes	No	No	No	Low
NutriChip (Ramadan et al., 2012)	Soft lithography	PDMS	2-layered channels	Polyester Øpore = 0.4 μm	Yes	No	No	No	No	No	Yes	Low
GI Bioreactor (Costello et al., 2017)	3D printing	VeroClear-RGD810	1 chamber	PEVA scaffold	Yes	No	No	Yes	No	No	No	Low
Peristaltic GOC (Jing et al., 2020)	Soft lithography	PDMS	3-layered channels	PDMS Øpore = 10 μm	Yes	Yes	No	No	No	No	No	Low

8. Critical discussion

Table 1 summarizes the characteristics that have been discussed in the previous sections for different GOC devices that have been reported in the literature since 2016. The table reveals great variability between devices, making it difficult to compare or standardize the models, even though some of them are commercially available. The most common chip configuration comprises two perfused compartments separated by a semipermeable membrane. Furthermore, PDMS is the preferred material for developing GOCs, although it is not suitable for certain applications due to its gas-permeability and capacity to absorb small hydrophobic molecules (Toepke and Beebe, 2006). However, as PDMS is not suited for mass production, commercial GOC devices are made of materials compatible with conventional microfabrication processes such as glass, polystyrol and PC. Almost all models incorporate fluid flow through the perfusion channels generating a shear stress mimicking the intestinal fluid transport. In most cases, shear stress is induced by peristaltic pumps, which greatly increase the bulkiness of the setup and limit its parallelization capacity (low throughput capability).

To the best of our knowledge, there are only five GOC models that incorporate built-in sensors for non-invasive and real-time monitoring. The two intestinal physiological parameters measured are intestinal barrier integrity and DO concentration. None of them (except the Nutrichip, and only as a proof of concept) integrates any type of biomarker sensor.

Therefore, an ideal GOC device for *in situ* decision-making and future personalized medicine should include a series of sensors and biosensors for measuring key biochemical interdependencies in real-time, as it is shown in **Fig. 6**. The multiparametric monitoring GOC device should include: i) physical parameter sensors to control the microenvironment (DO and pH), ii) TEER sensors to measure barrier permeability, iii) biosensors to detect protein biomarkers (cytokines) in real time and, iv) biosensors to monitor the generation of microbial metabolites and signaling molecules (Kim et al., 2017; Yu et al., 2018). While the type of biosensor currently available is quite large, recent advances in technologies offer new possibilities for its integration into the GOC models.

From the proposed key parameters, only sensors for measuring DO and barrier function have been successfully integrated into GOC devices. For the proper culture of the gut microbiota, beside oxygen levels, environmental pH has demonstrated to be among the strongest drivers of microbial community structure and function (Duncan et al., 2009). Thus, in-line pH monitoring of the GOC microenvironment may allow controlling the acidity of the media as well as following acidification rates with precision. Alternative detection techniques include metal oxide-based potentiometric sensors, ion-selective field effect transistors (ISFET), light-addressable potentiometric sensors (LAPS) or optical sensors (Duroux et al., 1991; Hafeman et al., 1988; Manjakkal et al., 2020). In recent years, IrOx-based sensors have emerged as a suitable technology for monitoring *in vitro* systems since they can be fabricated by low-cost methods (e.g., inkjet-printing) on a wide variety of substrates (Prats-Alfonso et al., 2013; Zea et al., 2019).

The interrelation between microbiota fermentation products, e.g., SCFA, barrier permeability (microbial translocation), and cytokines production is currently one of the issues currently attracting considerable attention, not only because of its effect on the intestine homeostasis but also its influence on other organs such as the brain (gut-brain axis). However, the multidetection capability of these interrelated parameters in real-time is still at its infancy. As explained in Section 7, only a few examples can be found of integrated sensors for detecting specific SCFA in real-time. Electrochemical, microbial biosensors, and infrared sensors are promising candidates. Although the real-time monitoring of SCFA represents a challenge in itself, developments in this area would greatly contribute to our understanding of the function of SCFA and permit a further study on the link between gut microbiota and host physiology.

However, monitoring the produced cytokine profile by using in-line biosensors also has its limitations and challenges. Overall, significant advances towards real-time and continuous monitoring of biomarkers in microfluidic cell cultures have been made, especially in the field of electrochemical and localized SPR biosensors. Nevertheless, their integration into *in vitro* microfluidic systems is still at an early stage. Most of the cases are only based on an on-line connection between the microfluidic device and the biosensor to deliver the secreted cytokines into the sensing surface or in a post-analysis after manually extracting the

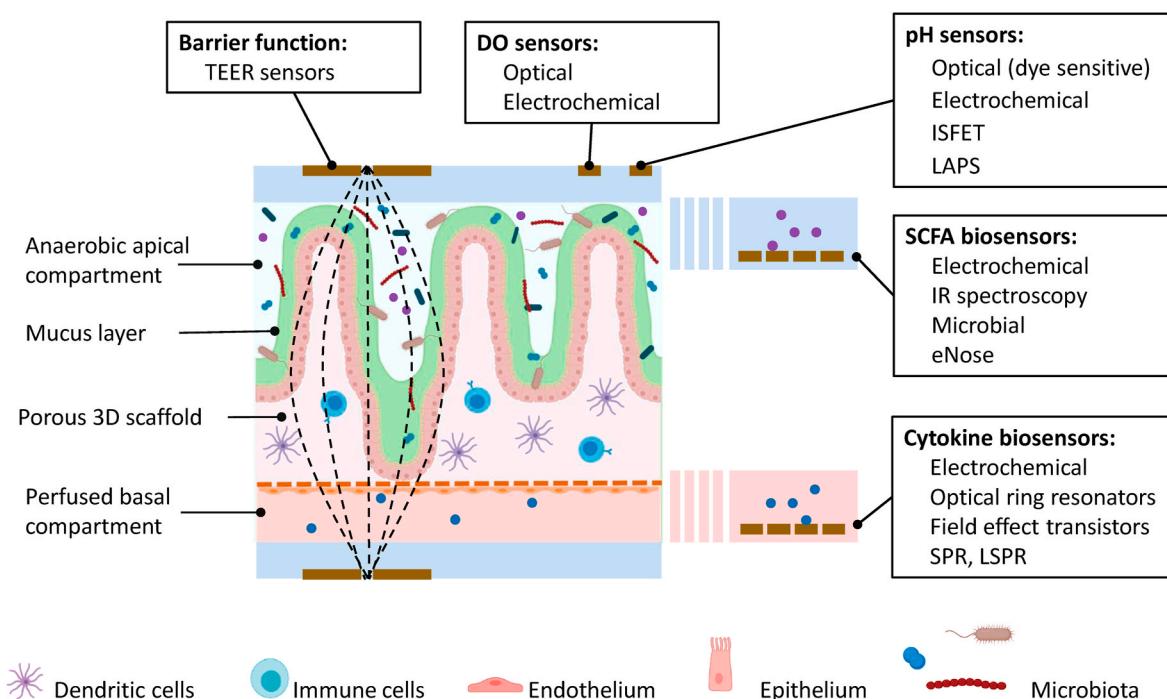


Fig. 6. Multiparametric monitoring GOC device. Schematic representation of a biologically relevant GOC model with integrated biosensors for DO, pH and TEER measurements and for quantification of cytokines and SCFA biomarkers (created with BioRender.com).

sample. Further efforts must be made to achieve biosensors capable of determining the cytokines profile with enough sensitivity, in real-time and automatically. Despite advances in the biosensing field, there are still challenges related to the multiplexing capability of label-free biosensors without increasing complexity in their operations or using bulky instrumentation. An extra challenge in the development of biosensors for long continuous monitoring is sensor saturation and surface regeneration. Moreover, on-chip regeneration represents a critical issue in the development of reusable biosensors for affordable monitoring of time-dependent cytokine profiles (Goode et al., 2015).

From the clinical point of view, a GOC device that yields a particular pattern of cytokines, in a short period of time, according to the immunological response derived from the exposition to different microbiota configurations, would be an extremely useful tool. Such a tool, for example, could be used to follow-up patients who recently received a fecal microbiota transplant (e.g., refractory pseudomembranous colitis) in order to confirm the correct implantation of the normal colonic flora with no inflammatory responses; deepen our understanding of the impact on the microbiota of both antibiotic treatment and changes in diet; and investigate the role of drugs in tight junction regulation (e.g., Zoulin, Larazotide acetate) under different modulated conditions (inflammatory or pathological environment). In this context, a novel strategy is being developed in the microbiome research field to precisely modulate the gut microbiota to improve vaccination outcomes in humans (de Jong et al., 2020).

9. Conclusion

Over the last decade, GOC systems have undergone a pronounced evolution towards developing complex systems to emulate intestinal physiology. However, despite the rapid progress made, many technical challenges remain. On the technical side these include the need to develop more biocompatible materials; optimization of microfluidic designs to tailor mechanical and biochemical cues; sensor integration for multiparametric monitoring in real-time, and ways to improve standardization and throughput of GOC platforms. A second issue is that as GOC combines diverse technologies, these challenges can only be overcome by adopting an interdisciplinary approach.

We hope that this review of the advances and potential of GOC technologies will motivate researchers working in biosensing and related fields to help solve the current limitations that are preventing organ-on-a-chip technology in general, and GOC in particular, from reaching the clinic. In so doing, GOC technology will be able to make a significant contribution to human health, reducing the number of animals used in experiments, facilitating major improvements in disease modeling and drug development, and paving the way towards precision medicine.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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