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REVIEW

In vitro micro-physiological models for translational immunology

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The immune system is a source of regulation of the human body and is key for its stable functioning. Animal models have been successfully used for many years to study human immunity and diseases and provided significant contributions to the development of powerful new therapies. However, such models inevitably display differences from the human metabolism anddisease state and therefore may correlate poorly with the human conditions. This explains the interest for the use of *in vitro* models of human cells, which have better potential to assist in understanding the physiological events that characterize the immune response in humans. Microfluidictechnologies offer great capabilities to create miniaturized*in vivo*-like physiological models that mimic tissue-tissue interactions and simulate the body metabolism in both the healthy and diseased states. The micro-scale features of these microfluidic systems, allow positioning heterogeneous cellular cultures in close proximity to each other in a dynamic fluidic environment, thereby allowing efficient cell-cell interactions and effectively narrowing the gap between *in vivo* and *in vitro* conditions. Due to the relative simplicity of these systems, compared toanimal models, it becomes possible to investigate cell signaling by monitoring the metabolites transported from one tissue to another in real time. This allows studying detailed physiological events and in consequence understanding the influence of metabolites on a specific tissue/organ function as well as on the healthy/diseased state modulation.Numerous *in vitro* models of human organs have been developed during the last few years aiming to mimic as closely as possible the *in vivo* characteristics of such organs. This technology is still in its infancy, but ispromised a bright future in industrial and medical applications. Here we review recent literature, in which functional microphysiological models have been developed to mimic tissues and to explore multi-tissue interactions, focusing in particular on the study of immune reactions, inflammation and the development of diseases. Also an outlook on the opportunities and issues for further translational development of functional *in vitro* models inimmunologywill be presented.

List of abbreviations

PAMPs	:	Pathogen-associated molecular patterns
TLRs	:	Toll-like receptors
NLDs	:	Nucleotide binding receptors
NF-κB	:	Nuclear factor kappa-light-chain-enhancer of activated B cells
IL-1β	:	Interleukin-1-beta
IL-6	:	Interleukin-6
TNF-α	:	Tumor necrosis factor-alpha
IL-8	:	Interleukin-8
PDMS	:	Polydimethylsiloxane
PBPK	:	Physiologically based pharmacokinetic
iPSC	:	Induced pluripotent stem cells
VEGF	:	Vascular endothelial growth factor
BTHS	:	Barth syndrome
ECM	:	extracellular matrix
TC	:	Tumor cell
EC	:	Endothelial
IBD	:	Inflammatory bowel disease
GIT	:	Gastrointestinal tract
BBB	:	blood-brain barrier
NK	:	Natural killer
TJ	:	Tight junction
GALT	:	Gut-associated lymphoid tissue
FAE	:	Follicle-associated epithelium
LPS	:	Lipopolysaccharide
TEER	:	Transepithelial Electrical Resistance
EHEC	:	Enterohemorrhagic*E. coli*
BECs	:	Bladder epithelial cells
ACD	:	Allergic contact dermatitis
ICD	:	Irritant contact dermatitis
LLNA	:	Local lymph node assay

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ARTICLE TYPE

GPMT	:	Guinea pig maximization test (GPMT)
KCs	:	Keratinocytes
DPRA	:	Direct peptide reactivity assay
h-CLAT	:	human cell line activation test
MoDCs	:	Monocyte-derived dendritic cells
ECVAM	:	European Centre for the Validation of Alternative Methods
CNS	:	Central nervous system
hCMEC	:	human brain endothelial cell
ADME	:	Absorption, Distribution, Metabolism and Exertion
hPSCs	:	human pluripotent stem cells

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REVIEW

1. Introduction

1.1 The immune system and inflammation

The human immune system has recently gained a significant interest from a wide scientific community, including clinicians, biologists, engineers, computer scientists, mathematicians and others, who are particularly interested in the capabilities and complexity of this system. The immune system is a complex system which employs specialized cells, molecules, tissues and organs to combat endogenous dysfunction and the action of exogenous infectious microorganisms. The interaction between the immune system and several other systems and organs allows the regulation of the body, guaranteeing its stable functioning [1, 2]. In his topical review, Tomio Tada (1997) described the immune system as a "supersystem" which can engender itself by generation of its diverse components from a single progenitor, creating a dynamic self-regulating system. The generation of its components is mostly by a stochastic process followed by selection and adaptation, the consequence of its self-organization [3]. After a few decades of studying the IS, immunologists have become convinced that there are no such simple rules that govern the immune system and there are no mathematically linear cause-effect relationships in many of the important immune phenomena, e.g. in the responses of T and B cells to exposure to an antigen. A single cause, such as peptide recognition, can result in multiple outcomes, such as activation, apoptosis, etc.,depending on co-stimulatory signals and other environmental factors. Also multiple causes can induce a single uniform process, leading to similar or dissimilar ends; for example, different cytokines can activate the same signaling pathway in different cells, producing the same or entirely different effects at the end [3].

There are two inter-related systems, by which the body identifies foreign substances: the *innate immune system* and the *adaptive immune system* [2-6]. The innate immunesystem consists of cells and proteins that are always present and ready to mobilize and fight microbes at the site of infection. The main components of the innate immune system are: physical epithelial barriers, phagocytic leukocytes, dendritic cells, a special type of lymphocytes, called natural killer cells, and circulating plasma proteins [7-9].Some innate immune responses are temporarily up-regulated as a result of exposure to microbes, but the components of the innate immune system do not change permanently during an individual's lifetime. The most important aspect of innate immune recognition is the fact that it induces the expression of co-stimulatory signals in antigen-presenting cells that will lead to T cell activation, promoting the start of the adaptive immune response. The latter is called upon against pathogens that are able to overcome innate immune defenses. The adaptive responses are highly specific to the particular pathogen that induced them and can provide long-lasting protection. The function of adaptive immune responses is to destroy invading pathogens and any toxic molecules they produce. Because these responses are destructive, it is crucial that they aremade only in response to molecules that are foreign to the host and not to the molecules of the host itself. The ability to distinguish what is foreign from what is self is a fundamental feature of the adaptive immune system, while the innate immune system lacks this ability [10-11].

Inflammation is an immunological mechanism that assists in the removal of infectious and other damaging foreign materials, as well as damaged native tissue materials, from the body. Inflammation consists of a tightly regulated cascade of immunological, physiological and behavioral processes, which are orchestrated by soluble immune signaling molecules, called cytokines [12]. This also leads to a variety of cellular responses in tissues, and ultimately to leukocyte activation and phagocytosis. The cascade also includes elevated permeability in microvessels, attachment of circulating cells to the vessels in the vicinity of the injury site, migration of several cell types, cell apoptosis, and growth of new tissue and blood vessels [13].The first step of inflammatory cascade involves the recognition of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) [12]by specific receptors, such as transmembrane Toll-like receptors (TLRs) and intracellular nucleotide binding receptors (NOD-like receptors or NLRs) [14,15].Once recognition of ligands occurs, TLRs activate common signaling pathways that culminate in the activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells). NF-κB translocates to the nucleus, where transcription is up-regulated through binding to target genes. Transcription and translation of genes lead to the next stage of the inflammatory cascade, which is the expression of pro-inflammatory cytokines, such as interleukin-1-beta (IL-1β), IL-6, tumor necrosis factor-alpha (TNF-α), and others. These cytokines facilitate the recruitment of effector cells, such as monocytes and neutrophils, to the site of disturbance. The resulting effect of these cellular signaling processes appears as the stereotypical signs of inflammation, including heat, swelling, redness, pain and loss of function. Inflammation varies spatially, temporally and in magnitude. It begins in a localized area but spreads rapidly as a response to the release of several secreted cytokines. Acute inflammation is an immediate reaction to infection and injuries and is quickly resolved once the disturbance is removed; however, chronic inflammation results when the disturbance persists [12,16]. The magnitude also varies from a low to a high grade inflammatory response. Chronic and high-grade inflammatory responses are associated with a variety of cardiovascular [17] and metabolic [18,19] diseases. For more details about the mechanism of inflammation, readers may refer to [9,12].

1.2 From animal testing to organs-on-a-chip

Many aspects of the human immune response may be modeled in animals and/or *in vitro*, which allows investigating the various physiological events that are crucial to system biology and allow the development of predictive models that can be used to design rational interventions to modulate the immune response. Animals

have been often used as models to study human immunity for many years and have been successfully used to enhance the understanding of the human disease and have made significant contributions to the development of powerful new therapies. Although the use of such models is an important task of understanding human disease, they inevitably display significant differences from the human disease state and correlate poorly with the human conditions [20]. For example, the study of sepsis in animal models have failed in identifying targets for drug discovery and have led to repeated clinical failures [21]. Recent studies indicated that translational medical research should be focused more on complex human factors and conditions, rather than relying purely on mouse models [20], and reconstituted "humanized" mice were used to analyze human immune responses [22]. It became clear that the significant differences between human and animal metabolism necessitate the use of human cells. *In vitro* models of human cell culture have the potential to assist in understanding the physiological events that characterize the immune response in human. Such models would increase the predictability of human response to drugs and significantly reduce expenses associated with the clinical studies.

The simplicity of traditional *in vitro* models, usually consisting of a single cell type, makes them robust and suitable for high throughput research, but unfortunately provides only little biological relevance to the complex biological tissues of the human body. Furthermore, the culture conditions do not closely mimic the *in vivo* microenvironment due to the absence of the fluid flow and shear stresses [23]. Also, these cultures are less suited to provide a dynamically controlled flow of cell nutrients and stimuli and, additionally, the accumulation of waste leads to a pH drift in a static culture. Other drawbacks of the classical cell culture systems are the long growth times needed for cells to differentiate into functional cells and, and the dependence on external signal detection systems that require the manual withdrawing and manipulation of samples.

Recent developments in microfabrication and microfluidics technologies provided the tools to create advanced cell culture systems, aiming to provide *in vivo* -like cellular microenvironments [24, 25]. The small culture volumes within these devices consume much less cells as well as culture and analytical reagents. They allow control of microenvironmental parameters, such as cell-cell and cell-matrix interactions. Additionally, the tissue-to-fluid ratio within these devices can be adjusted to mimic the physiological ratios. Perfusion-based media supply allows delivery and removal of soluble molecules into the cell microenvironment and controlled application of shear stresses by the fluid flow. These tools are therefore well suitable for studying the biological interactions down to the cell and molecular levels and have tremendous potential to be applied to study human physiology and pathogenesis, by effectively creating microphysiological models of human organs. These "organs-on-a-chip" can be designed to process extremely small volumes of complex fluids with high efficiency and speed, with a high degree of accuracy and without the need for an expert operator. A clear advantage of using organ-on-a-chip systems is the control of the fluid flow in physiologically-relevant dimensions, which makes it possible to regulate nutrient and drug concentrations at the levels of single cells or small cell clusters. This gives the microphysiological models strong potential forcreating dedicated platforms for compact immune system analysis.

1.3 Scope of this review

The number of publications on organ-on-a-chip technology is on the rise in the academic community. Several excellent reviews on the field have been published during the year 2014, which cover different aspects of this technology such as physiological relevancy [26], therapy development [27] and drug development [28]. Bhatia and Ingber have recently published a comprehensive review on the role of microfluidics in realizing organ-on-a-chip devices and discussed in details the challenges and opportunities of the technology [29]. Another excellent review with wider scope was published by Giese and Marx[30]. The authors discussed in detail the status of various approaches to emulate innate immunity in non-lymphoid organs and adaptive immune response in human professional lymphoid immune organs in vitro with emphasis on the tight relationship between the necessarily changing architecture of lymphoid organs at rest and when activated by pathogens. Junkin and Tay [31] reviewed single-cell-based microfluidic investigations of immunity.

This review provides an up-to-date sketch of recent developments of microengineered physiological systems that reconstitute physiologically features of the human immune system. We first review microfabrication approaches to construct cellular architectures that provide key elements of the physiological microenvironments of human organs, emphasizing the relationship between tissue architecture, the cellular microenvironment and the cellular response to external stimuli. We also provide a survey of the various microfluidic systems that have been recently reported to emulate specific human tissues and immune reactions, and which allowed the study of immune reactions, with a focus on aspects of inflammation and disease development.Finally, further expected translational developments and outstanding issues of functional *in vitro* models in the different organ-on-a-chip application fields will be presented.

1.4 Synopsis of the Reviewed Work

Microfluidics has the potential to overcome many of the practical limitations that have impeded progress in the *in vitro* modeling of human organs. Among the major advantages of microfluidic systems is that they have the potential to mimic the true three-dimensional (3D) nature of the *in vivo* situation, as characterized by an enhanced cell exposure to the extracellular environment. Cells in microfluidic devices can be fluidically accessed with spatio-temporal gradients of media that provide minimal disturbance to the cells. This mild flow of media can be adjusted to impose shear stresses on the cell surface, analogous to the *in vivo* conditions. Additionally, multiple cell types can be grown into a multi-compartment device [32], so that they can interact over physiological length scales. Microfabrication also allows creating arrays of cell hosting chambers, which provide an opportunity of high-throughput testing. Furthermore, microfluidic systems not only can host heterotypic cell co-cultures, but can be used to accommodate living tissue or biopsy samples, within which multi-cell types co-exist. This raises the potential of these systems to screen for the efficiency of patient-specific therapies. Organ-on-a-chip technology has therefore high ambitions and, while still being in its infancy, several promising studies have demonstrated *in vitro* models of the human gut [33-36], lung [37-42], blood vessel [43-51], liver [52-58], kidney and renal tubule [59-61], pancreatic islets [62], blood-brain barrier [63-65]and skin [66]. To give the reader a quick overview of the literature, Table 1 summarizes recent developments in miniaturized *in vitro*

models of human organs with their structures and functions. Many of these models represented a first step towards developing more complex modular systems with multi-organs-on-a-chip or a complete "human-on-a-chip". Such *in vitro* human model finally would allow for completely animal-free screening.

2. Microfluidics-based physiological models

2.1 Physiologically based pharmacokinetic (PBPK) models

Of the reported model systems, those that were aimed for drug screening and the study of cell/tissue-drug interactions were developed first, and this research provided the major driving force for the development of *in vitro* models of human organs. Traditionally, drug discovery techniques are time-consuming and expensive and still pose significant challenges to pharmaceutical industry. In pharmaceutical research, cell culture-based assays are exploited to bridge the gap between molecular level assays and animal testing, because they can evaluate drug effects on cell proliferation, apoptosis and migration, and provide higher throughput than time-consuming animal experiments [67, 68]. However, these methods encounter a number of limitations, including the consumption of large amounts of cells, and reagents, and the requirement of costly equipment. Most importantly, and as discussed above, these traditional models provide only little biological relevance. Therefore, these challenges necessitated the exploration of new strategies for drug development. Miniaturization of the traditional cell culture tools using microfluidics is a promising approach to overcome these limitations. Miniaturized bioreactors can provide a dynamic, tunable environment emulating *in vivo* conditions and hence providing more physiologically relevant *in vitro* models.

The liver is the major organ integrating metabolic and immunologic homeostasis in the human body, and is the target of most molecular therapeutics. A wide spectrum of therapeutic and technological needs drives efforts to capture liver physiology and pathophysiology *in vitro* to predict the metabolism and toxicity of drugs molecules [69]. Feasibility studies to demonstrate long-term cell survival have been exploited using simple polydimethylsiloxane (PDMS) devices [52, 54, 56, 70]. The feasibility of using microfluidics-based *in vitro* models for physiologically based pharmacokinetic (PBPK) measurements and toxicity analysis has been pioneered by the Shuler group at Cornell University [53, 71, 72].

Cancer and its pharmaceutical treatment is also a focus of microfluidic studies, that particularly try to develop *in vitro* models of tumors within an "*in vivo*-like" microenvironment that enables studying the cellular interactions within and with the matrix surrounding the tumor [73-76].Tumor growth involves both cell-cell communication and cell-matrix communication and the changes that occur inthese relationships may contribute to abnormal proliferation and metastasis. Also, control of the physio-mechanical properties to study the fundamental biological processes associated to primary tumors and metastasis and the exploration of new therapeutic strategies were reported [72, 77].

2.2 Disease models

The questionable scientific value and relevance of animal models triggered the exploration of corresponding human-based *in vitro* model systems to study human pathogenesis at the cellular level. It is difficult to identify critical cellular and molecular contributors to the development of a disease or to vary them independently in whole animal models. Ideally, an *in vitro* physiologically relevant human disease model would therefore be established from human tissue/cells or induced pluripotent stem cells (iPSC).Several examples of the study of human organs via simple models have already shown promising results of cell-cell interactions, cell stimulation and tracking, as well as detection/quantification of cellular response. Therefore, the predictive capabilities of these models would prove to be extremely useful for understanding the patho-physiological mechanisms of diseases too. A few studies have reported miniaturized *in vitro* models for studying the diseased state, such as lung injury [78], endothelial cells-circulating tumor cell interaction [79], as well as tumors [80-84].

Huh et al., 2007 [79]described an *in vitro* model of the human small airway to reproduce under flow conditions cellular level lung injury, which causes symptoms that are characteristic of some pulmonary diseases [Fig. 1a]. Specifically, propagation and rupture of liquid plugs that simulate surfactant-deficient reopening of closed airways lead to significant injury of small airway epithelial cells by generating deleterious fluid mechanical stresses. Bischel et al, 2013[47] developed 3D lumens with circular cross-sectional geometries through extracellular matrix (ECM) hydrogels that are lined with endothelial monolayers to mimic the structure of blood vessels *in vitro*. This setting was used to investigate the biological response to vascular endothelial growth factor (VEGF) gradients in the form of angiogenic sprouting and the ability to generate vessel networks on demand. Recently, Wang et al, 2014 [85] demonstrated a "heart-on-a-chip" tissue system to model the mitochondrial cardiomyopathy of Barth syndrome (BTHS) using iPSCs[Fig. 1b]. Using their system, the authors defined the metabolic, structural and functional abnormalities associated with a mutation in the gene encoding tafazzin(TAZ).

In oncology research, reconstituting the tumor microenvironment *in vitro* is important, in particular for the understanding of the influence of microenvironmental parameters, such as the ECM, hypoxia, biochemical gradients and angiogenesis, on tumor proliferation. Also metastasis and tumor metabolism can be studied, which may facilitate the discovery of potential drug candidates and improve early diagnosis. Some progress has been made using microfluidics to answer questions related to different aspects of the tumor microenvironment, yet much still need to be explored. Wlodkowic and Cooper , 2010 [76] published an excellent review on the application of microfluidic technologies in cancer biology and experimental oncology and the reader is recommended to refer to this review for related development in this area and we will only summarize some recent related efforts. Tumor growth involves both cell-cell communication as well as cell-matrix communication and the changes that occur by these relationships may contribute to abnormal proliferation and metastasis [86]. In this respect, microfluidics is superior for allowing understanding of the cellular processes and physio-mechanical properties of the tumor microenvironment. A critical step in cancer metastasis is the entry of tumor cells into the blood stream, but the underlying mechanism of cancer cell intravasation remains largely unknown. Zervantonakis et al., 2012 [82] investigated tumor cell-endothelial cell (TC-EC) interaction and used a microfluidics-based assay to recreate the tumor-vascular interface in 3D, which allowed quantification of the endothelial barrier function and

enabled testing the hypothesis that carcinoma cell intravasation is regulated by biochemical factors from the interacting cells and cellular interactions with macrophages (Fig. 1c). This study found that endothelial barrier impairment was associated with a higher number and faster dynamics of TC-EC interactions. The authors concluded that the endothelium poses a barrier to tumor cell intravasation that can be regulated by factors present in the tumor microenvironment.

During cancer development, the expanded tumor actively promotes the growth of new blood vessels (angiogenesis) due to itsdemand for oxygen and nutrients for cell survival. It has been found that tumor cells can create their own blood vessels through a process, in which tumor-associated stem cells differentiate into endothelium [87, 88]. Microfluidic-based tumor angiogenesis models would therefore enable a parametric *in vitro* study with tumor cells in close proximity to and actively signaling with endothelial cells.

2.3 Inflammation models

Traditionally, inflammation has been associated with bacterial, viral, fungal or parasitic infections and with the induced response of our immune system. But it becomes increasingly evident that inflammation is associated with a variety of diseases, including chronic vascular diseases [89], myocardial ischemia [90], acute cerebral stroke Alzheimer's [91-93], type 2 diabetes [18, 19, 94, 95], hypertension [96] and cancer [97-99]. A severe form of inflammation also is observed in multi-organ failure [100]. Therefore, inflammation analysis becomes central in clinical research. As said before, inflammation consists of a tightly regulated cascade of immunological, physiological, and behavioral processes that are orchestrated by soluble immune signaling molecules called cytokines [101, 102].Most aspects of the inflammatory cascade are still at the beginning of being quantitatively analysed and significant effort has been made in identification of the tissues, cells, proteins, and genes that participate in the inflammation cascade. So far, no quantitative prediction model of the inflammatory cascade is available, but only a limited number of studies which considered the cascade as a whole [102].The various forms of inflammation differ in large part by their location in the tissue and timing. The magnitude of inflammation spans from low grade to high grade and extends from acute to chronic effects. Acute inflammation is characterized by the immediate and early responses to a disturbance agent and is quickly resolved. However, chronic inflammation results when the disturbance persists.

Epithelial tissues, such as those in the intestine, lung and skin, form protective barriers that physically separate an organism from the outside world, yet form life-sustaining immunological barriers at the interface between the body and the environment. Immune homeostasis in these epithelial tissues depends on tightly regulated interactions between epithelial, stromal and immune cells and commensal and environmental microorganisms. Failure to properly control epithelial immune responses causes severe diseases, such as inflammatory bowel disease (IBD), psoriasis and asthma [103, 104].The role of epithelial cells in the regulation of immune homeostasis and inflammation in barrier surfaces has gained increased attention. Particularly, signaling pathways controlling epithelial cell responses to microbial, immunological, physical and chemical assaults have central functions in the regulation of immune homeostasis at barrier surfaces. In the following sections, we will discuss recent research on tissue inflammation by considering specific organs, including the gastrointestinal tract (GIT) or gut, skin, lung and blood-brain barrier (BBB).

2.3.1 Gut inflammation

Intestinal inflammation is widely recognized as a pivotal player in health and disease. It is defined as the infiltration of leukocytes in the *lamina propria* layer of the intestine and, in consequence, leads to damage of the epithelium and, on a chronic basis, can induce inflammatory bowel disease and potentially cancer [105].The intestinal epithelium acts as a selectively permeable barrier, permitting the absorption of nutrients, electrolytes and water, while maintaining an effective defense against toxins, antigens, and enteric flora. The permeability of the intestinal epithelium depends on the regulation of the intercellular tight junctions, which are involved in the pathological process [106]. Therefore the role of the intestinal barrier in the pathogenesis of gastro-intestinal diseases is receiving significant attention. The intestinal epithelium, which is a single-cell layer, is the largest mucosal surface in the human body, and provides an interface between the external environment and the host. Within this mucosal surface, the GIT microbiota and the GIT barrier are the two key functional entities formaintaining gut health [106, 107]. The GIT microbiota which consists of about 10^{14} bacteria [108] regulate the epithelial functions such as mucus production by goblet cells, prevent colonization of pathogens in the gut and regulate the mucosal immune system [109]. Any imbalance in microbiota, for example due to exposure to antibiotics [110] or by ingestion of a carbohydrate-rich diet [111] would influence the host local defense system. On the other hand, any malfunction of the epithelium would influence the microbiota diversity and functionality [109]. Alterations of the GIT barrier have shown to be crucial events in progression of diseases such as infectious diarrhea [112], IBD[113] and coeliac disease [114].

The transport of molecules between the apical and basolateral side of the epithelium occurs through two major routes: trans-cellular and paracellular pathways (Fig. 2a). The presence of the tight junctions reduces the para-cellular spacing to 0.8 nm in the human jejunm and 0.3 nm in the human colon [115]. This allows only small hydrophilic and polar molecules to cross the epithelial monolayer through the paracellular route [116]. The rate of transport through the epithelium depends on the concentration gradient and the permeation characteristics of the epithelium [117]. The tight junction defines the overall barrier function of the intestinal epithelium. This barrier function is severely compromised when epithelial cells are lost, for example, after exposure to microorganisms and their products. Therefore, intestinal permeability to macromolecules increases [118, 119].Consequently, this gives rise to a rapid immune response. The specific cells involved in this immune response include antigen-presenting cells, T and natural T killer lymphocytes (NK), B lymphocytes, and plasma cells [120]. These cells lie in close proximity to the intestinal epithelial barrier and facilitate immune responsiveness, especially in the presence of elevated intestinal epithelial permeability [121]. However, intestinal epithelia are programmed to rapidly heal and reseal the barrier within minutes of injury [122].*In vitro* and *in vivo* animal studies have demonstrated that intestinal permeability is regulated by multiple factors, including exogenous factors, epithelial apoptosis, cytokines, and immune cells [107]. For example, IFN-γ and TNF-α, which are central mediators of intestinal

inflammatory diseases induce intestinal epithelial barrier function [123] and incubation of intestinal epithelial cell monolayers (Caco2 and T84) with IFN-γ and TNF-α promoted the reorganization of several tight junction (TJ) proteins and decreased epithelial barrier function [124].

A quantitative analysis of the postprandial kinetics of inflammatory biomarkers provides information on the quality of the interaction between specific foods and the organism. Drugs and food screening using microfluidic devices that incorporate a human intestinal cell model can potentially provide better, faster and more efficient prediction of *in vivo* drug/nutrients fate and their functions in the body. The intestine is a fluidic tract where the cells are exposed to continuous in-and outflow. Microfluidic culture systems can provide steady state culture conditions by mimicking the *in vivo* fluid flow and shear stress in a controllable manner, thus bringing the GIT *in vitro* model closer to the physiological microenvironment. The mass transfer characteristics in the *in vivo* tissue environment, which is mainly determined by the transport of nutrients in capillary vessels that are in close proximity with the cell, can be maintained by a microfluidic perfusion system.

Epithelial cell cultures are currently prominent tools to study the absorption mechanisms and drug and nutrients transport. However, the intestinal epithelial cells are difficult to culture and have limited viability [125, 126]. Therefore attention has been turned to the use of human adenocarcinoma cell lines, such as HT-29 and Caco2 that show a number of characteristics of differentiated intestinal cells. The Caco2 cell line is widely used and characterized system for modeling the human small intestine. The cells exhibit many properties of the small intestinal epithelium, as they form a polarized monolayer of well differentiated columnar absorptive cells expressing a brush border on their apical surface [127]. The cells form dense intercellular junctional complexes, resulting in a tight epithelium and thus presenting a unique paracellular as well as transcellular barrier [128]. When cultured on microporous membranes, Caco2 cells expose their apical side to the upper compartment and the basolateral side to the lower one [129]. The transport studies require placement of a donor or stimuli solution on the upper side of the barrier and monitor its arrival into the receiver compartment as an indicator of diffusion rate. Many attempts to model the human stomach and small intestine have been made in the last two decades. These *in vitro* tools are static, include a limited number of simulated parameters and are dedicated to a particular application [130, 131].

The validity of an *in vitro* model is dependent on how well it reproduces the key physiological and biological characteristics of its *in vivo* analogue. The key characteristics of the inner intestinal lumen include: the primary structure, consisting of strongly expressed intercellular TJs between epithelial cells, which directly control compound permeability and the presence of a mucus layer, which covers the epithelial cells. The mucus-covered epithelial monolayer forms a selective interface and plays an important role in modulating the transport of nutrients and drugs from the digestive system to the circulation system and to other organs in the body. A major limitation of current intestine models is the fact that they are solely based on epithelial cell (e.g. Caco2 cell line) culture, whereas the intestinal epithelium is a conglomerate of absorptive enterocytes and other cells, such as mucus-secreting goblet cells, which represent a second barrier beside the enterocytes [132]. The HT 29 cell line can be used as a model of mucus-secreting goblet cells [133]. A co-culture of intestinal cells and goblet cells would provide a mucus-covered intestinal epithelium, which would transform the model system into an efficient testing barrier and closely mimic the human small intestine absorption model.An intact epithelial barrier is crucial for the physiological activities of the gut. However, this barrier is not static but can be modulated by specific stimuli. The increased permeability of intestinal epithelium has been linked to pathogenesis of inflammatory bowel disease, ulcerative colitis, Crohn's disease and food allergies. On the other hand, transient increases in paracellular transport could improve bioavailability of desirable bioactive compounds, which normally are poorly absorbed [134].

The intestinal epithelium monolayer is predominantly composed of enterocytes mixed with mucus-secreting goblet cells. The latter provide an effective barrier that prevents the entrance of microorganisms and other substances into the gut organ [135]. Gut-associated lymphoid tissue (GALT) is also scattered throughout the gastrointestinal mucosa [136] and consists of lymphoid follicles arranged either individually or in clusters to form distinct structures, such as Peyer's patches, situated immediately beneath the epithelial cell layer [135]. In the intestine, these lymphoid structures are separated from the lumen by the follicle-associated epithelium (FAE), which contains, in addition to enterocytes and some goblet cells, specialized epithelial M cells which perform a key role of luminal sampling and transport of antigens and pathogens invasion to lymphoid tissues cells beneath (FAE), initiating the mucosal immune response [135, 137, 138]. M cells are particularly common in the ileum and appendix but are also found in the colorectum [139]. Due to their ability to transport a broad range of materials, these cells have become a promising target of research, offering a putative way for oral delivery of nano-encapsulated therapeutic peptides and vaccines [135-142]. However, much remains to be learnt about the role of human M cells in physiological and pathological states. In particular the process of how particles are taken up and transported by M cells remains poorly understood, due to the small number of M cells in the human gastrointestinal tract (less than 1% of the total intestinal surface [143]. *In vitro* models of human FAE have been developed employing a co-culture of Caco-2 cells and isolated lymphocytes from mouse Peyer's patches [144] or of Caco-2/human Raji B lymphocytes [145, 146].

A biologically active *in vitro* co-culture cellular system has been proposed by Ramadan et al., 2013 [35]to measure the regulation of inflammatory processes by nutrients.The model comprises a confluent layer of Caco2 cells, which allows application of *in vitro*-digested food on its apical side, and a basolateralco-culture of a monocytic cell line (U937 cells) differentiated into macrophages. The two cell types are separated by a porous membrane (Fig. 2a). The system has been used to model the response of immune cells to pro-inflammatory stimuli applied on the epithelial layer such as lipopolysaccharide (LPS). The epithelial monolayer integrity was monitored by measuring the Transepithelial Electrical Resistance (TEER) using a set of Ag/AgCl electrodes. The tendency of TEER to increase to a maximumwas associated with formation ofa confluent layer of Caco2 cells, reached after one week of culture. Preliminary results showed a significant increase of the IL-6 concentration after treating the macrophages with LPS (Fig. 2b and c) and demonstrated the possibility of quantifying the induced cytokines

using an on-chip immunomagnetic assay. Kim et al, 2012 [34] demonstrated a co-culture of *Lactobacillus rhamnosus* GG (LGG) bacteria on the luminal surface of an epithelial cell monolayer, without compromising epithelial cell viability for extended periods (>1 week) using a "gut-on-a-chip" device enhanced with a peristaltic wall motion mechanism (Fig. 2d). This co-culture showed to improve the barrier function, as previously observed in humans [147]. The study also investigated the epithelial barrier integrity when LGG bacteria are grown on the apical surface of the epithelial cells. The results showed that LGG bacteria co-culture enhancedthe intestinal barrier integrity, as indicated by TEER measurements (Fig.2e). The co-existence of intestinal cells and the non-pathogenic (commensal) bacteria in close proximity within the GIT and the interaction between the intestinal epithelium and this residence microflora is central to maintain a healthy and functional GIT. Kim et al, 2009 [148] described an innovative system to investigate the pathogenic infection of the intestine (Fig. 3). The developed system enabled independent culture of eukaryotic cells and bacteria, and testing the effect of the commensal microenvironment on pathogen colonization. Bacterial biofilms were created in reversible islands along with culture of an epithelial cell monolayer.This setting was used to develop a commensal *Escherichia coli* biofilm among HeLa cells, followed by introduction of enterohemorrhagic*E. coli* (EHEC) into the commensal island, in a sequence that mimics the sequence of a GIT infectionevent. This co-culture model provided a useful tool for investigating the effect of GI tract signals on EHEC virulence as well as for applications, such as screening potential probiotic strains. Another infection model was reported by Andersen et al., 2012 [149], which emulated the uropathogenesis in the human bladder. Short-term bacterial colonization on the bladder epithelial cells (BECs) within a fluidic chamber layer led to intracellular colonization. Exposing invaded BECs to a flow of urine led to outgrowth of filamentous bacteria. These filaments were capable of reverting to rods that could invade other BECs. The authors concluded that the elements of the uropathogenic cascade were inducible in a human BEC *in vitro* model system.

2.3.2 Lung inflammation

Pulmonary allergy andassociated diseases are among the biggest health problems impairing the quality of life of a large population and remaining a major cause of mortality and morbidity of a significant part of world population with asthma beingthe primary cause of hospitalization in pediatric populations [150]. Primary risk factors for lung health include exposure to chemical vapors, smoke and fine particles, which often lead to respiration restriction and complications. Despite the significant development of asthma treatment medication, there is still an urgent need to accelerate the discovery and validation of new airways drugs and to find new clinically relevant tools to test these new drugs.

Lung and airways are characterized by two key physiological characteristics, these are: the air-liquid interface of the alveolar epithelium and the dynamic mechanical forces imposed on the tissue due to the breathing movements. An *in vitro* model for an alveolar cell monolayer at an air interface was reported by Nalayanda et al, 2009 [37]. The system showed structural and functional improvement, as compared to existing Transwellculture systems, due to the ability to expose cells to varying mechanical stimuli and the higher degree of monolayer integrity and a decrease in surface tension. Huh et al, 2010 [38] demonstrated a mechanically active lung-on-a-chip device which mimickedthe stretching mechanism of the alveolar epithelium by microfabricating a microfluidic system containing two closely superposed microchannels separated by a thin porous and flexible membrane made of PDMS. The mechanical stretching was achieved by incorporating two lateral microchambers into the device (Fig. 4a). When vacuum was applied to these chambers, it producedelastic deformation of the thin wall that separates the cell-containing microchannels from the side chambers; this causedstretching of the attached PDMS membrane and the adherent tissue layers and, when the vacuum was released, this causedthe membrane and adherent cells to relax to their original size. To demonstrate the immune-responsive lung-on-a-chip system, the authors incorporated blood-borne immune cells in the fluid flowing through the lower channel under the epithelial layer. Pulmonary inflammation was then induced by introducing a medium containing the pro-inflammatory mediator TNF-α into the alveolar microchannel in the presence of physiological mechanical strain and examined activation of the underlying microvascular endothelium by measuring ICAM-1 expression. This showed that TNF-α stimulation of the epithelium substantially increased endothelial expression of ICAM-1within 5 hours after the stimulation. Additionally, the epithelial layer was also challenged by adding living *E.coli* bacteria to the alveolar microchannel. The presence of these pathogens on the apical surface of the alveolar epithelium for 5 hours was sufficient to activate the underlying endothelium, as indicated by capture of circulating neutrophils and their transmigration into the alveolar microchannel.A recent publication by Nesmith et al., 2014 [39] reported an *in vitro* model of asthmatic inflammation using an engineered laminar bronchial smooth muscle tissue on elastomeric thin film (Fig. 4b). When challenged with a cholinergic agonist, the artificial muscle layer contracted and induced thin film bending, which served as an *in vitro* analogue for bronchoconstriction. Exposing the engineered tissues to interleukin-13 resulted in hypercontractility and altered the relaxation in response to cholinergic challenge. More interestingly, the asthmatic hypercontraction was reversed using a muscarinic antagonist and a β-agonist which are used clinically to relax constricted airways. These conceptual devices could pave the way to develop new enabling tools for accelerating drug discovery.

2.3.3 Skin allergy

Skin allergy is one of the major health problems affecting the quality of life of a significant proportion of the world population. In particular, allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD) resulting from skin sensitization are common occupational and environmental health problems and have major economic burden [151]. Five to ten percent of all occupational skin diseases are estimated to result from skin allergy [152]. The inflammatory process that results from an allergic substance is mediated through immunologic mechanisms, whereas irritant reactions result from direct tissue damage, which initiate alternative inflammatory reactions [153]. The mechanism of ACD is different from ICD, because it is an immunologic, type IV hypersensitivity response to skin contact with an allergen (e.g., poison ivy, nickel, fragrance, etc.). ACD tends to be more severe with repeated exposures to the allergen and can become systematized. In ACD, the body produces inflammatory

substances, such as cytokines, sending immune cells to the skin to kill "foreign" substances [153]. Visibly, it is difficult to distinguish between ICD and ACD [154]. ACD is an immunologic reaction, while ICD results from direct injury to the skin cells and is non-immunological and tends to be short-lasting [154]. Substances in products that come into contact with the skin, such as cosmetics, fragrances and clothing textiles play an important role as exogenous factors in the triggering of ACD. Since all new ingredients to be incorporated into a product are potential sensitizers, it is necessary to conduct a thorough skin sensitization risk assessment prior to the market introduction of new products or product ingredients that contact the skin to assure the product/ingredient will not induce ACD or ICD.

To date, the identification and evaluation of potentially sensitizing chemicals completely relies on animal testing, such as the local lymph node assay (LLNA) in mice and the Guinea pig maximization test (GPMT), as no validated alternative exists. Several legislations call also for significant reductions or even a complete ban on animal testing. In Europe, animal testing for chemical allergenicity used in cosmetic products is prohibited since2013. Therefore, there is an urgent need for some reliable alternative methods, which will be able to study the allergenic potential of chemicals. It is estimated that the number of chemicals to be submitted for registration will be68,000 to 101,000 chemicals between 2010 and 2022. This suggests a demand of 54 million animals and testing costs of 9.5 billion euro [155]. This clearly challenges the feasibility of implementing this type of testing and major investments into alternative and high-throughput test methodologies will be required.

Currently, several companies provide reconstituted human epidermal *in vitro* skin equivalents such as EpiDerm (MatTek, Ashland, MA, USA), Episkin (Episkin, Chaponost, France), Apligraf (Organogenesis Inc., MA, USA) and Skinethic (Skinethic, Nice, France) for *in vitro* toxicology studies. All models consist of keratinocytes (KCs), seeded on matrices of either dermal components or materials of non-biological origin [156]. The most significant difference between *in vitro* skin equivalents and normal *in vivo* skin is the cellular structure. For the evaluation of inflammation effect of chemicals, the interaction of KCs and the immune system, nerves, etc. is essential. Skin equivalents vary in the penetration rate of substances through the *stratum corneum* (the most outer layer of the skin). Compared to the normal skin, theseskin constructs haveapproximately 10-30-fold higher permeability [157, 158]. This probably results in an over-prediction of skin irritancy due to the higher penetration rate of applied substances. Ongoing research continues to provide new insights into the biological processes driving skin irritancy and sensitization. Cosmetics Europe (The European Cosmetics Industry Trade Association) member companies have developed three *in vitro* tests: 1)the direct peptide reactivity assay (DPRA), which is based on the evidence that there is a clear association between the degree of chemical reactivity and the sensitization potency of a chemical sensitizer [159]; 2)the myeloid U937 skin sensitization test (MUSST), which is developed by Cosmital SA, uses the human myeloid cell line U937 (a dendritic cell surrogate). Cells are treated with the test chemicals for 24 h, 48 h and 72 h and CD86 is measured by flow cytometry together with monitoring the cell viability [160]; 3)the human cell line activation test (h-CLAT) uses the human monocytic leukemia cell line THP-1, a dendritic cell line. The tests measure the up-regulation of the co-stimulatory surface molecules CD54 and CD863 as markers of dendritic cell activation [161].The Procter & Gamble, Wella-Cosmital laboratories have also developed an *in vitro* test that is based on human peripheral blood monocyte-derived dendritic cells (MoDCs). These cells are exposed for 24 or 30 hours to the test chemicals and dendritic cell maturation is measured by flow cytometry and mRNA expression of IL-1β, interleukin-8 (IL-8) and aquaporin-3 using real-time PCR [162]. Recently, the European consortium, SENS-IT-IV, has developed an *in vitro* test, which uses a KC cell line (NCTC 2544) and is based on the selective induction of IL-18 in the KCs by contact allergens. Cell-associated IL-18 was evaluated after 24 hours by ELISA [163].

The most commonly used parameters for skin sensitization are measurement of cell viability [164]. However, the European Centre for the Validation of Alternative Methods (ECVAM) agreed that measuring cytotoxicity alone does not always reveal the right prediction between irritants and non-irritants [165]. For that reason, additional, more specific, biomarkers should be incorporated. In this respect, measuring the release of cytokines or other molecules might be a promising approach. For example, IL-1α is believed to be the main switch in the initiation of inflammation [166]and induces the expression of itself, and other pro-inflammatory cytokines like IL-6 and IL-8. TNF-αalso can be produced by KCs following stimulation [167]. A quantitative analysis of the inflammatory biomarkers may provide information on the interactions between irritants/allergens and the immune system. Recapitulating the skin epidermal layer with immune system co-culture including T cells will be a powerful tool to assess the irritant and allergenic potential of chemicals used in industry and provide valuable insight into the molecular basis of skin irritation and sensitization. Research in this area is still in the very early stages, but the microsystem technologies used in the above-cited experimental reports on different human organs can also be applied to create an *in vitro* model of human skin, which, if combined with system biology and computational toxicity, could find a great and immediate industrial application. Ataç et al, 2013 [66] cultured *ex vivo* prepuce in a dynamically perfused chip-based bioreactor aiming to maintain the native state of the tissue for long periods (Fig. 5). The study showed a distinct difference between the static and perfusion chip cultures, and that tissue disintegration was prevented by dynamic perfusion. Additionally, hair follicle cultures in the chip showed a prominent hair-fiber elongation from the epidermis while tripling the culture period compared to the conventional culture.

2.3.4 Neuro-inflammation (disruption of the blood-brain barrier)

The blood-brain barrier (BBB) is a selective tissue structure formed by endothelial cells (EC) that line cerebral capillaries and separates the peripheral blood from the central nervous system, thereby maintaining brain homeostasis [53, 168, 169]. Due to the tight character of the BBB, nearly all polar or large compounds are blocked and the majority of the drugs targeting the central nervous system (CNS) fail to enter through the BBB. This endothelial lining is crucial in preventing the entrance of toxic substances to the brain [169]. On the other hand, many neuro-immune disorders are characterized by trans-endothelial leukocyte migration across the brain microvascular bed [170]. Increasing evidence indicates that disruption of the BBB, such as endothelial dysfunction, is implicated in many neuro-degenerative diseases, including Alzheimer's disease,

Parkinson's disease and multiple sclerosis [171, 172].Therefore, understanding the mechanism of leukocytes migration into the brain would provide useful insight into the pathogenesis of the neuro-immune disorders and how to modulate the host immune response. A growing number of studies have been reported aiming at developing an immune-competent micro-physiological model of the BBB barrier and enable the study of the physiology, pharmacology and pathology of this barrier.

A dynamic *in vitro* model of the BBB was constructed using modified hollow fibers that featured trans capillary pores with diameter of 2-4 μm, which were positioned inside a sealed chamber (the extraluminal space) (Fig. 6a) [170].The cell culture was provided with a pulsatile flow to mimic the intravascular perfusion pattern of the *in vivo* physiological blood flow. The porosity of the hollow fibers allowed gas and nutrient exchange between the luminal and the abluminal compartments, but did not permit cells to cross. This structure allowed measurements of the trans-endothelial electrical resistance, sucrose permeability, and BBB integrity during reversible osmotic disruption with mannitol; the study showed that the microholes did not hamper the formation of a tight functional barrier. This innovative design provided useful insight into how to modulate pathologic immune responses. For example, flow cessation followed by reperfusion in the presence of circulating monocytes caused a biphasic BBB opening paralleled by a significant increase of pro-inflammatory cytokines and activated matrix metalloproteinases. Another micro-BBB model was reported by Booth and Kim, 2012 [173], which comprised a Bend.3 endothelial cell culture, both with and without co-cultured C8-D1A astrocytes (Fig. 6b).The micro-BBB showed significantly higher TEER levels than that obtained in static Transwell-based models. Furthermore, instantaneous transient drop in TEER in response to histamine exposure was observed in real-time, followed by recovery. Another BBB-on-a-chip device that used the immortalized human brain endothelial cell line hCMEC/D3, was reported by Griep etal., 2012 [63]. The barrier function was modulated both mechanically, by exposure to fluid shear stress, and biochemically, by stimulation with TNF-α. The device had integrated electrodes to analyze barrier tightness by measuring the TEER. Shear stress positively influenced barrier tightness and increased TEER values with a factor 3, up to120 $\Omega.cm^2$, and subsequent addition of TNF-α decreased the TEER with a factor of 10, down to 12 $\Omega.cm^2$.

3. Translational developments of *in vitro* models

Organ-on-a-chip technology is certainly ambitious and could revolutionize many bio/medical/consumercare-related industrial practices. Despite the long way ahead towards the development of robust, reliable, physiologically relevant and immuno-competent models, ultimately, many industrial sectors could benefit from this technology. Here, we list examples of such possible translational research outcomes for different industrial sectors.

(i) Immuno-toxicology testing in chemical and skin care industry

Safety assessment of chemicals is one of the most important issues in regulatory science, as human beings become exposed to many chemicals in their daily life and only a small percentage of these have been assessed for safety. The ToxCastprogramme (http://epa.gov/ncct/toxcast/) has identified a risk assessment strategy, which is reviewed by [174], and the approach is broken into the following tasks: (i) identifying biological pathways that, when perturbed, can lead to toxicity; (ii) developing high throughput *in vitro* assays to test chemical perturbations of these pathways; (iii) identifying the universe of chemicals with likely human or ecological exposure; (iv) testing as many of these chemicals as possible in the relevant *in vitro* assays; (v) developing hazard models that take the results of these tests and identify chemicals as being potential toxicants; (vi) generating toxicokinetics data on these chemicals to predict the doses at which these hazard pathways would be activated; and (vii) developing exposure models to identify chemicals for which these hazardous dose levels could be achieved.

So far, the toxicity of chemicals is being evaluated by using rodents and the research results are then extrapolatedto humans. The major challenge in toxicology is in the large number of chemicals that need to be tested. Because of animal welfare concerns, as well as the requirement for high-throughput, *in vitro*, -omics, and *in silico* systems have been proposed or are being developed. Currently, toxicologists do not have the acceptable alternative tools to meet the requirements imposed by regulations. Among these approaches, the *in silico*method, i.e. the computational modeling of human organs, shows accelerating progress due to the promise of low-cost and obvious capability to achieve high-throughput screening. However, *in silico*methods may not be the only solution, but PBPK modeling, system biology,-omics techniques and the combination of these would be the key for shaping future toxicology. ACD accounts for 10% to 15% of all occupational diseases [175], has a major socio-medical impact and poses a huge challenge for clinicians and scientists. Understanding the mechanisms, through which chemical allergens induce ADC in humans, is essential to ensure that chemical ingredients can be assessed for their potential to induce skin sensitisation without the need for animal testing. However, at present, there are gaps in our fundamental understanding of how the adaptive immune responses to chemical sensitizers differ from those induced by pathogen antigens? Another open question is which sensitiser-induced innate immune pathways in the skin are predictive of the subsequent adaptive immune response? A "skin-on-a-chip" *in vitro* model will therefore be a powerful tool to assess the irritant and allergenic potential of any chemicals used in industry.

(ii) Pharmaceutical industry

Drugs may impose the risk of unexpected immuno-toxicological side effects, including the suppression or enhancement of the immune response. Suppression of the immune response can lead to decreased host resistance to infectious agents, while enhancing the immune response can exaggerate autoimmune diseases or hypersensitivity [176]. Therefore, the assessment of immunotoxicity of pharmaceuticals is an essential component of their safety evaluation. The International Immunotoxicity guidelines [S8 Immunotoxicity Studies for Human Pharmaceuticals] have been in effect since May 2006. These guidelines emphasize the need to investigate immunotoxic effects during the preclinical phase of drug development. It becomes accepted that animal models and humanized animal models can generate misleading extrapolations. Drug development requires extensive, costly and time-consuming pre-clinical testing and validation experiments before potential therapeutic compounds are approved to progress to the clinical evaluation. For every ten

drugs entering clinical trials, only one or two will typically be licensed for eventual use in humans [28,177]. Microphysiological*in vitro* models can be used in the early stages of drug development and significantly accelerate the testing process of potential compounds by screening of large numbers of compounds and compound mixtures on the organ models in healthy and diseased states with better authenticity comparedto animal models.

(iii) Food industry

There is an increased interest in understanding the complex relationship between food and health. It is now generally accepted that food modulates the physiological functions of the body, depending on the type of diet and it is believed that a meal can provoke an inflammatory response. The cellular metabolism of nutrients, culminating in the synthesis of biochemical energy, produces side effects that include oxidative stress. These effects accumulate at the level of the organism to produce a phenomenon, called postprandial stress [178]. Under normal conditions, postprandial stress is of low magnitude and disappears within a few hours post-ingestion. An unhealthy diet may, however, increase the magnitude of postprandial stress and/or delay its recovery [179]. A repetition of postprandial inflammatory stress over prolonged periods of time may significantly contribute to the development of chronic inflammation. Thereby the inflammatory stress imposed by inadequate or inappropriate diets may contribute negatively to the maintenance of health by reducing the metabolic plasticity of the organism.

A changing diet has been suggested to prevent or delay type 2 diabetes mellitus onset [180]. In particular, there is a growing number of examples, where nutrients or "nutraceuticals" possess modulatory roles in immunity, adipocyte functions and in promoting anti-inflammation [181, 182]. For example, omega-3 fatty acids, palmitate, quercetin and probiotics were shown to impact monocyte/macrophage polarization, adipose tissue inflammation and insulin resistance [183-186]. Therefore, using nutraceuticals to "re-polarize" immune cells and their interacting adipocytes to a favorable phenotype that blocks chronic inflammation and insulin resistance presents an attractive therapeutic strategy [182].Systematic *in vitro* screening of biologically active compounds and nutraceuticals for immune-metabolic modulatory functions in conjunction with well-structured randomised control trials would be necessary to assess the translational viability of this idea.

A gut-on-a-chip system which comprises the intestinal barrier, essential immune cells with the presence of intestinal bacteria (microbiome) can be a powerful tool for translational development of food processing (such as fermentation) to be used to generate "functional food" products. Healthy properties of food are currently evaluated by conducting human studies that analyze metabolites and biomarkers in the patients'blood after food intake. Using an *in vitro* model of the gut will help in correlating results from such human study with the direct analytical results obtained by analyzing the food compounds on the gut-on-a-chip. Food allergy is another potential application of such technology, which can be utilized to screen the allergenic properties of food components for personalized nutrition.

4. Challenges of organ-on-a-chip systems for translational immunology

Figure 7 shows a depicted view of a wishful microfluidic system, which contains the tissues/organs of interest that are connected via a common microfluidic circuit. This system would emulate the absorption, distribution, metabolism and exertion (ADME) process. In this example, the gastrointestinal tract, lung, and skin are listed as the major organs that function as the barriers, metabolizers or distributors of the compounds that are tested, while blood vessels transport their reaction products to the other major organs, including the heart, liver, kidney and urinary tract.

The ultimate goal of the organ-on-chip technology is to provide analytical platforms mimicking human normal and pathological physiology *in vitro*. These systems could integrate physiologically relevant models within a dynamic environment with sensing elements for monitoring the *in vitro* model integrity and quantification of the cellular system response to external stimuli, as well as tracing the cell-cell and cell-environment signaling. Basic immunology science and translational immunology will greatly benefit from human functional organ-on-chips in terms of reliability and costs. Current developments of organ-on-a-chip technology show a promising alternative to the use of animal models and, owing to their small size and the amenability to integration and automation, these systems will enable high-throughput experimentation that could be adopted for clinical and industrial usage. However, there are many challenges that must yet be overcome to enable transferring this technology from the laboratory to the industry, including:

(i) Manufacturability and scalability

The vast majority of tissue engineering devices use PDMS as a fabrication material due to the ease of fabrication, optical properties, gas permeability and mechanical properties (elasticity). Soft lithography became the most widely used fabrication method of microfluidic devices for rapid prototyping among the academic research communities after it was introduced by the George Whitesides group in 1998 [187]. Despite the success of using PDMS as a prototyping material, it cannot be adopted as a manufacturing material due to many limitations. For example, PDMS absorbs small molecules [188, 189], which can affect cell signaling [190]. PDMS is not easily amenable to integration, such as incorporating metallic microelectrodes and most importantly, PDMS-based devices are not scalable, because PDMS is not compatible with the standard manufacturing, technologies such as injection molding, embossing and rolling. For more details about the implication of using PDMS in cell culture, readers are advised to Regehr et al, 2009 [189]. Due to these limitations, the translational research community is continuously searching for a better material that can overcome these challenges. Polystyrene is a classical bio-ware material with long history of cell culture and has the potential to be employed for manufacturing of micro-scale systems, which could eliminate the limitations associated with PDMS [191]. However, if a thermoplastic material is adopted for microfluidic manufacturing, an urgent requirement is to develop an alternative pumping/valving mechanism than the one which currently relies on the PDMS elasticity.

(ii) Reliability and robustness

Organ-on-a-chip technology inherits all the challenges that has been facing microfluidic technology, including the lack of a standard fabrication method, bubble formation during reagent injection and experimentation, lack of standard "micro-macro"

interfacing tools, which makes handling the microfluidic devices by a researcher/operator in a standard biological lab or clinic a difficult task. Compatibility of an organ-on-a-chip system with existing biological protocols is another hurdle. Biological assay developers mostly design their protocols with improved performance in terms of sensitivity and/or the use of amount of sample and reagents. However, such protocol might not be straightforwardly transferrable to a microsystem from a conventional macro-system. Another requirement for robustness is the ability of the microsystems to maintain the cell/tissue culture environment in optimum conditions to keep the organ models intact for a long time (e.g. one month). This requires incorporating automated sample injection, processing and collection, real-time monitoring and feedback systems, particularly when the biological model comprises more than one organ/tissue type, which requires analytes or biological substances (e.g. metabolites) to pass through different tissues in a way that is mimicking the *in vivo* conditions.

(iii) Type of cells

A major advantage of the organ-on-a-chip technology is the employment of human cells to overcome the difference of metabolism between humans and animals. Immortalized cell lines behave differently in cell culture compared to primary cells, therefore, they could provide less relevance. Primary cells and tissue biopsies are therefore the most advantageous cell sources for construction of the physiological models, which can undergo relevant phenotypic changes within the *in vitro* conditions. However, due to ethical and regulation considerations, the primary cell source is not reliable for developing organ-on-a-chip models. Therefore, adopting human pluripotent stem cells (hPSC) as a cell source is a promising technique. This technique requires a better understanding of the cell microenviroment and cues that promote specific differentiated phenotypes.

(iv) Vascularization and common connecting fluids (cell culture media)

The validity of *in vitro* models depends on how cells or tissue interact with other cells or tissue within the same model and how well this mimics the organ-organ interaction *in vivo*. In the cell culture system, cells are supplied with artificial media that provide the necessary nutrients and growth promoting factors to maintain the cells in an active state. In current cell culture practice, there is no unified cell culture medium and different types of cells require different cell culture media. The challenge of finding a common cell culture media or blood substitute must be solved to allow construction of multi-organ model on a chip. This could pave the way to realizing "*in vivo*-like" integrated organs on a chip with a vascular connecting network for better cell-cell interaction and authenticity of the biological models.

(v) Need for specific sensors for cell culture control

Tissue engineering is a multidisciplinary field of science that integrates knowledge from engineering, biology, chemistry and medicine aiming at the development of functional tissues or organs. Unlike the conventional cell culture in the culture dishes, the cell populations in the artificially constructed tissues and organs are integrated in a complex 3Dmulti-cellular architecture in their ECM. With the recent advances in lab-on-a-chip technology and its application in cell-based analysis and currently in the organ-on-a-chip field,it becomes clear that the cellular, sub-cellular and molecular level cues in the surrounding microenvironment of the cells have an important effect on their growth, differentiation, morphology and metabolic state [192]. These cellular structures need to be constantly monitored in terms of various physiologically relevant parameters to evaluate their integrity and functionality. A considerable limiting factor of cell interaction platforms is the minute quantity of patient-derived samples that is available, which moreover are often difficult to be analysed using conventional detection systems. This will ideally require 'miniaturization' of the sensing aspects and the need for reliable and sensitive tools to assess the artificial tissue environment has become important.

The significant progress which has been made over the recent years in microfluidics-based biosensors and point-of-care applications can be highly beneficial for the tissue engineering and organ-on-a-chip applications. On the other hand, the typically small number of cells in a microchip system produces extremely low concentrations of analyte, when compared to conventional standard culture and this low amount of analyte is embedded in the very complex matrix of the cell environment. As a result, the biosensor has to be very accurate and sensitive to detect the analyte among a huge population of molecules. Recently, a number of studies have reported incorporating sensing tools within microfluidics-based culture devices such as integrating a set of microelectrodes for monitoring the TEER [173,193].Huang et al, 2013 [194] reported an integrated high-throughput microfluidic system which featured30microbioreactors for cell culture-based assays, which was able to control the cell culture parameters, such as temperature, cell loading, and media perfusion and detection. Hu et al., 2013 [195] developed a microfluidic system for studying the mechanism of cellular metabolism and drug effects. The concept of the system was based on the photocurrent amplification of a light-addressable potentiometric sensor. This system was able to rapidly detect the concentration change of cellular acidic metabolites in the extracellular microenvironment.

Summarizing, biosensors, including those based on optical, electrical, magnetic, acoustic and piezoelectric detection principles, are extensively used in microfluidic biochips. However, the translation of this success to develop tools for real-time monitoring of analytes in tissue engineering and in *in vitro* models is still at an early stage and need to be accelerated. Availability of such sensors would significantly enhance the systems functionality and be of benefit for the translation of the model from the lab to the point of interest.

As a result from all these challenges, there will be many technology developments required to transform this technology into readily usable and industry-adoptable platforms and it is therefore unlikely that animal models will be completely replaced in the near future. But the considerable potential of the organ-on-a-chip technology justifies the investment in further developments, possibly leading to some industrial applications on the longer term.

5. Concluding remarks and future outlook

The study of the immune system and of organ-immune system signaling is one of the outstanding challenges in modern medicine and becomes key to understanding the origin and progression of diseases. Microphysiological*in vitro* models and integrated multi-microphysiological models have a great potential to provide a deeper understanding of the physiological events that

characterize the immune system responses to endogenous andexogenous stimuli. These devices allow unprecedented control of the cellular microenvironment, by providing *in vivo*-like fluid flows, mechanical shear stress, and enhanced cell-cell and cell-matrix interactions due to the ability to control a precise position of cells in their niches and 3Dorientation of tissue-tissue interfaces. The individual microphysiological models can be connected in physiologically relevant order through endothelial cell-lined microchannel to emulate their order and interaction in the human body, giving rise to a "human-on-a-chip". The potentially low cost of the device technologypermits high-throughput screening of a large number of compounds having different stimuli (e.g. chemical, drug, food ingredients, etc.) in ashorter time comparedto the use of animal and conventional *in vitro* models. Therefore, it becomes possible to monitor the organ-organ interactions and signaling in real time. These unique capabilities give the micro-physiological models strong potential to create a platform technology for a compact immune analysis system for disease modeling, immune-toxicology, food allergy and skin allergy.Collaborative research between physiologists, bioengineers, stem cell technologists and computational biologistswill be needed to accelerate this translational research and narrow the large gap existing between the lab bench and industry.

Many challenges are still ahead and need to be overcome before adopting these tools as alternatives to animal models and current *in vitro* models. The target of the "human-on-a-chip" system is still far awaydue to the complexity and lack of physiologically accepted/relevant single organ models so far. Therefore, individual micro-physiological models should be established and the current challenges, including the reliability, robustness, cell source and scalability need to be resolved for each individual model before embarking on the more complex multi-organs system. However, we strongly believe that the "human-on-a-chip" concept will prove to bea very ambitious technology, which could revolutionize many aspects in translational medical research, particularly in human immunology, disease pathogenesis, and toxicology.

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Table 1: Recent miniaturized *in vitro* models of human organs.

Organ/tissue model	Cellular structure	Investigated functions	Significant results
Gut	• Confluent layer of Caco2 cell line [33, 34, 35, 36].	• Long-term culture and monitoring of polarized transport activity of the intestinal tissue [33, 34, 35]. • Integrating the intestinal epithelium with other organ models [36].	• The cells could be cultured for more than two weeks, and forming a monolayer [33,35, 36]. • Transport of rhodamine 123 was monitored by on-line fluorescent measurement [33]. • *Lactobacillus rhamnosus* GG co-culture on the epithelial cell layer survived for 1 week [34].
Lung	• Confluent layer of human alveolar epithelial-like cell line (A549) [36, R3]. • Confluent layer of NCI H441 alveolar epithelial cell line co-cultured with Clonetics™ Lung microvascular endothelial cells, human neutrophils [37]. • Human bronchial smooth muscular thin films cultured on top of an elastic polymer layer [38]. • Airway epithelial, lung fibroblasts and polarized microvascular endothelial primary cells [40]. • Human nasal epithelial stem/progenitor cells (hNECs) [42].	• Alveolar-micro-vascular capillary interface; responses to bacteria and inflammatory cytokines; the role of mechanical cyclic strain (breathing) on nanoparticle uptake; inflammatory response to bacteria and cytokines [37]. • Mimicking asthmatic inflammation by exposing the engineered tissues to interleukin-13 which result in hyper-contractility and altered relaxation in response to cholinergic challenge [38]. • Mimicking the airway mucosa complex microarchitecture using primary cells [40]. • Alveolar micro-injury due to gastric refluxes [41]. • Gaseous formaldehyde toxicity via airway delivery and monitoring of cilia beating [42].	• Demonstration of air-liquid interface culture on a suspended membrane [36]. • Mechanical strain enhances epithelial uptake of nanoparticles and stimulates their transport into the underlying microvascular channel [37]. • Demonstration of the lung model to exposure to bacteria and inflammatory cytokines. The model showedresponses to drug treatments for evaluation of safety and efficacy of new drugs [38]. • Demonstration of triple co-culture with well-differentiated primary human tracheo-bronchial epithelial cells at an air-liquid interface [40]. • Demonstration of lung epithelial wounding [41]. • Demonstration of *in vitro*differentiation of hNECs and a microfluidic chip for the real-timemonitoring of cilia beating and their reactivity function [42].
Blood vessel	• Human lung fibroblasts, human umbilical vein smooth muscle cells, and human umbilical vein endothelial cell lines [43]. • Mouse mesenteric artery segments (*ex vivo*) [44]. • Human umbilical vein	• The effects of matrix composition and multilayer configurations on 3D cell-cell interactions; cell migration [43]. • Long-term culture; smooth muscle function of mesenteric arteries in a spatially heterogeneous microenvironment [44].	• Demonstration of microscale hierarchical “neotissues” with 3D configurations of matrix materials and multiple cell types [43]. • Artery constriction is spatially restricted to the site of stimulation with phenylephrine [44]. • Shear stress exerted by flowing blood attenuates endothelial cell sprouting and interstitial flow

	endothelial cells (HUVEC) [45]. • HUVECs lined in a PDMS microfluidic channel [46]. • ECM hydrogels that are lined with monolayers of HUVECs in 3D lumen structure [47, 48]. • Human promyelocytic HL-60 cell line [50]. • Brain endothelioma cell line (bEnd.3) and murine neutrophils that are differentiated from myeloid progenitors [51].	• The impact of shear stress exerted by flowing blood and vascular endothelial growth factor (VEGF) gradient on the endothelial morphogenesis [45]. • Microvascular occlusion and thrombosis formation and the effect of the drug hydroxyurea on the microvascular obstruction [46]. • Mimicking vessel structures and angiogenesis assay [47]. • Formation of interconnected 3D vascular networks *in vitro* [48, 49]. • Transendothelial migration of neutrophils [50]. • Leukocyte adhesion and trafficking [51].	directs endothelial morphogenesis and sprout formation [45]. • Shear stress influences microvascular thrombosis/obstruction and the efficacy of the drug eptifibatide, which decreases platelet aggregation, in the context of hemolytic uremic syndrome (HUS) [46]. • Demonstration of patterning of endothelial-lined lumens in a variety of geometries [47]. • Demonstration of a vascular lumen network with good cell adhesion capability and adaptive response to shear stress [48]. • Vascular channels sustained the metabolic function of primary rat hepatocytes in engineered tissue constructs [49]. • Measuring the three-dimensional transmigration of neutrophils during the inflammatory process [50]. • Demonstration of leukocyte extravasation and interstitial migration in a 3D microfluidic environment [51].
Liver	• Primary rat hepatocyte cultured on a porous membrane [52]. • L2 (rat lung Type II epithelial cells), H4IIE (rat hepatocytes), and human hepatocarcinoma celllines (HepG2/C3A) [53]. • Human hepatocarcinoma cells (Hep G2) [54]. • HepG2/C3a cells [55]. • Caco2,mucin-producing cells (TH29-MTX) and HepG2/C3A cell co-culture [57]. • HepG2 and NIH-3T3 cell co-culture [58].	• Influence of perfusion on cell behavior [52]. • Toxicity testing [53]. • Xenobiotic's metabolism [55]. • Liver injury by ingested nanoparticles [57]. • Drug screening [58]	• Demonstration of significant increase in albumin secretion by the cultured primary rat adult hepatocyte in perfusion-based culture comparing to static cell culture [52]. • Demonstration of naphthalene toxicity on chip [53]. • To achieve longer and healthy cultures in microfluidic bioreactor for large-scale cultures, including an oxygen chamber is necessary [54]. • Cell culture in the microfluidic biochip for 96 h, including 72 h of perfusion, provoked a 24 h delay in cell growth [55]. • The biochip environment contributed to maintain theprincipal enzymes involved in the xenobiotic metabolism after 96 h of culture [55]. • After crossing the gastro intestinal tract epithelium, nanoparticles that were administered in high doses induced liver cell injury [57]. • Demonstration of an array of individually addressable, free-floating hydrogel-based microtissues and monitoring the

			enzymatic activity and hepatotoxicity in the co-culture [58].
Kidney and urinary tract	• Primary rat inner medullary collecting duct (IMCD) [59]. • Madin Darby Canine Kidney (MDCK) cells [60]. • Human kidney proximal tubule epithelial cell line (HK-2) and primary renal proximal tubule epithelial cells (RPTECs) [61].	• Influence for shear stress [59]. • Toxicity testing [60]. • Barrier function on various membrane pattern types [61].	• Applying fluidic shear stress of 1 dyne/cm^2 for 5 hours showed enhanced cell polarization [59]. • Ammonium chloride significantly reduced the cell growth ratio [60]. • HK-2 and RPTEC aligned in the direction of ridge/groove topography of the membrane in the device [61].
Blood-Brain barrier (BBB)	• Immortalized human brain endothelial cell line hCMEC/D3[63]. • Human brain capillary endothelial cells (SV-HCEC) and rat astrocytes [12, 64].	• Mechanical and biochemical modulation of the BBB function [63].	• Shear stress positively influenced barrier tightness and increased the transepithelialelectrical resistance (TEER) values with a factor 3, while TNF-α decreased the TEER with a factor 10 [63]. • Demonstration of the BBB on a silicon nitride membrane [12, 64].
Skin	• *Ex vivo* skin tissue model and skin biopsy (EpiDermFT^{TM}) [66].	Effect of mechanical shear stress on cell signaling [66].	• Epidermal barrier function was preserved better in the dynamic culture compared to static culture [66].
Pancreatic islets	• Rat primary pancreatic islet cells, primary rat hepatocytes and adipose-derived stem cells [62].	• Co-culturing single primary islet cells with adipose-derived stem cells (ADSCs) to create potent pancreatic islets for transplantation [62].	• ADSC exposed islet spheroids showed significantly different morphologies, higher viability, and enhanced insulin secretion compared to mono-cultured islet spheroids [62].

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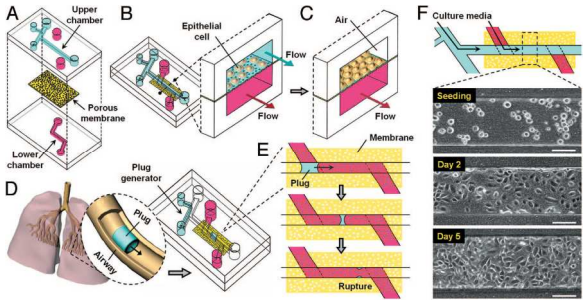

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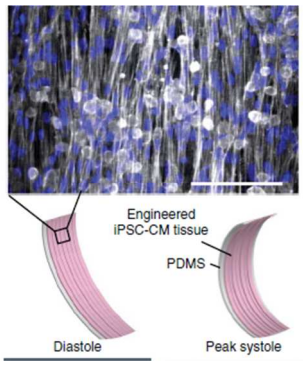

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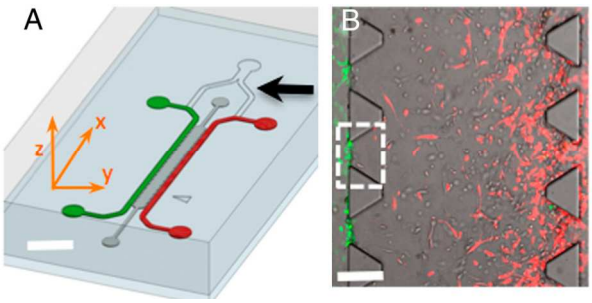
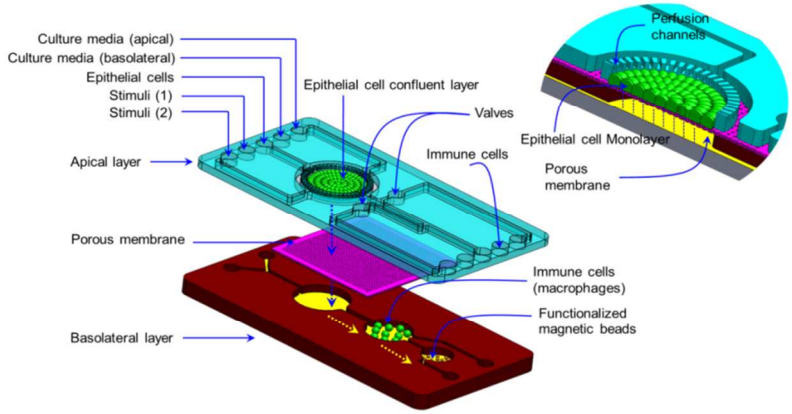


(c)

Figure 1: (a) Compartmentalized microfluidic airway system.A:The microfabricated small airways are comprised of PDMS upper and lower chambers sandwiching a porous membrane. B: Primary human small airway epithelial cells (SAECs) are grown on the membrane with perfusion of culture media in both upper and lower chambers until the cells become confluent. C:Once confluence is achieved,media are removed from the upper chamber, forming an air–liquid interface over the cells. D: Physiologic airway closure is recreated in the microfluidic system by exposing the differentiated cells to plug flows. E: Liquid plugs created in a plug generator progress over a monolayer of the epithelial cells and rupture in the downstream region, reopening the *in vitro* small airways. F:Attached SAECs.(b) *In vitro* model of cardiomyopathy of Barth syndrome (BTHS). Tissue constructs exhibit depressed contractile stress generation. Top:α-actinin-stained image of a muscular thin film. Bottom:iPSC-cardiomyocytesseeded onto thin elastomers with patterned lines of fibronectin self-organize into anisotropic myocardial tissues. Cardiomyocyte stress generation reduces the radius of curvature of the construct as it contracts from the diastole to the peak systole of the cardiac cycle. (c) Microfluidic tumor-vascular interface.A:Schematic drawing of the microfluidic tumor-vascular interface model, indicating the endothelial channel (green), the tumor channel (red), and the 3D ECM (dark gray) between the two channels. B: Phase contrast

image showing the fibrosarcoma cells (HT1080, red) invading through the ECM (gray) toward the endothelium (MVEC, green). A single 3D ECM hydrogel matrix region is outlined with the white dashed square.(a) From Ref [78]. Reprinted with permission from PNAS, Copyright (2007) National Academy of Sciences, U. S.A. (b) From Ref. [85].Reprinted with permission from Nature Publishing Group(c) From Ref. [82]. Reprinted with permission fromPNAS,National Academy of Sciences, U. S.A.



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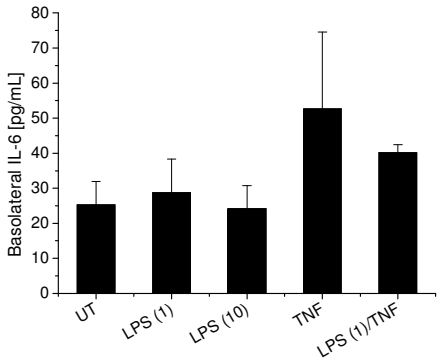

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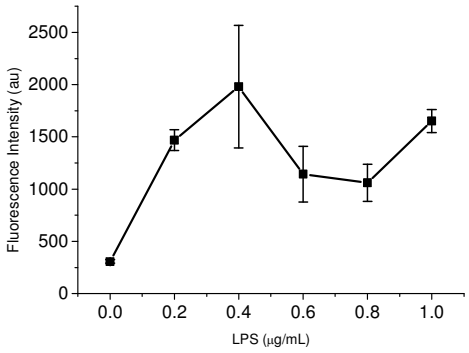

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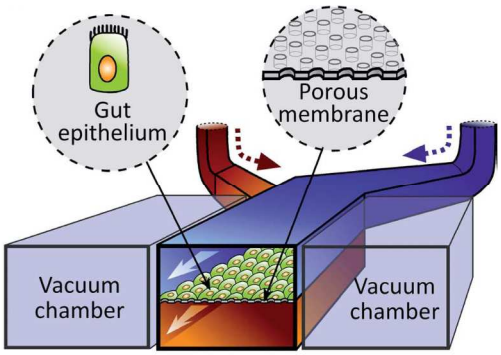

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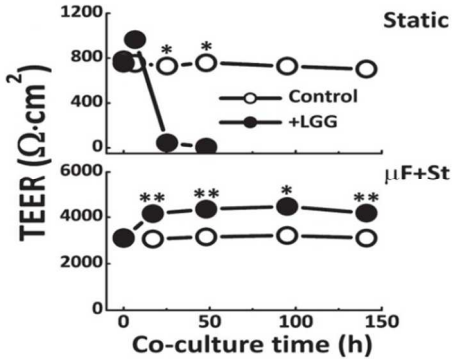


(e)

Figure 2: (a) Schematic diagram of the human GIT biological model consisting of a monolayer of confluent epithelial cells interacting with immune cells. (b) IL-6 expression measured at the basolateral side of the confluent Caco2 layer as a response to apical stimulation with LPS and TNF-α, or combination of both stimuli. (c) Increase of IL-6 secretion after treating differentiated monocytes (U937) with LPS, as measured using an immunomagnetic assay. (d) Schematic of agut-on-a-chip device showing the flexible porous ECM-coated membrane covered by gut epithelial cells; the membrane is placed in the middle of a microchannel, and full-height vacuum chambers on both sides allow to induce mechanical deformations on the cell layer. (e) TEER measurement of the barrier function of aCaco2 monolayer cultured in the absence (open circles) or presence (closed circles) of LGG cells in

aTranswelldevice (Static) or microfluidic gut-on-a-chip device with cyclic strain (μF+St). (a-c) From Ref. [35].Reprinted with permission from Royal Society of Chemistry. (d-e) From Ref. [34].Reprinted with permission from Royal Society of Chemistry.

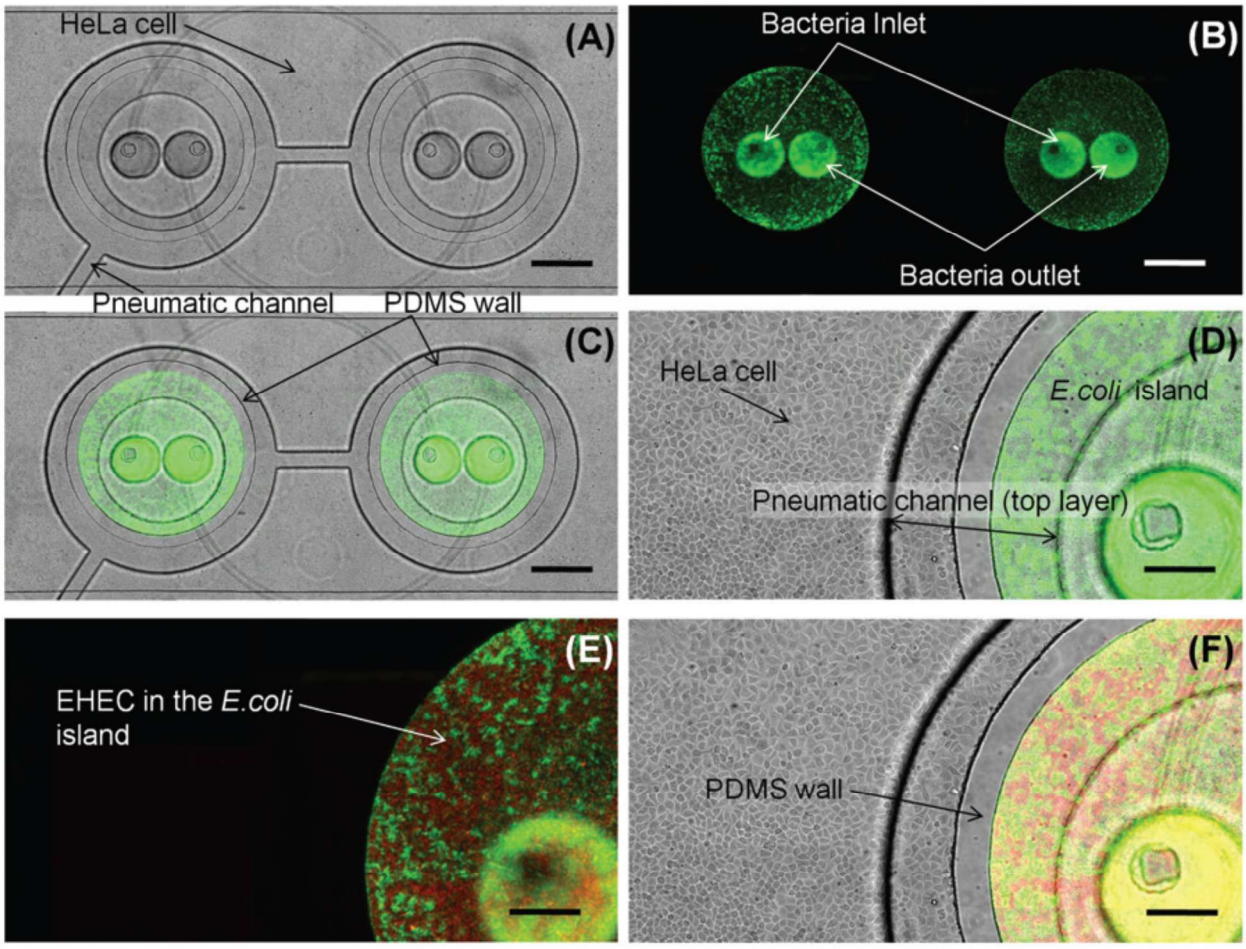
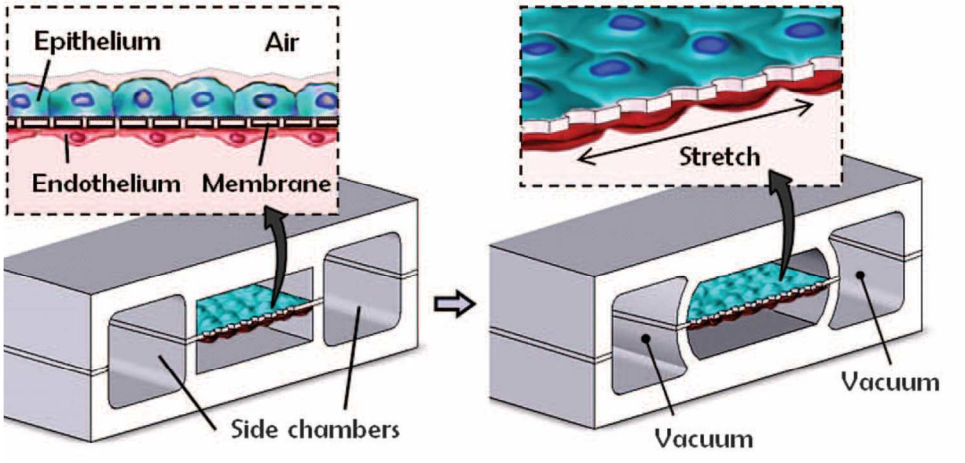


Figure3: Co-culture of HeLa cells and bacteria in a microfluidic chip. (A): Transmitted light image of a HeLa cell monolayer. (B): Fluorescence image of GFP-expressing *E. coli* BW25113 localized in bacterial islands. (C): Overlay of transmitted and green fluorescence images showing co-culture of HeLa cells and *E. coli* BW25113 for 48 h. (D): Close-up view of HeLa cells and *E. coli* BW25113 in a bacterialisland after 48 h. (E): Fluorescence image of RFP-expressing EHEC and GFP-expressing *E. coli* BW25113 in an island. (F): Overlay of transmitted, green, and red fluorescence images in the device. Scale bar represents 500 μmin panels (A)–(C) and 200 μm in panels (D)–(F). From Ref. [148].Reprinted with permission fromRoyal Society of Chemistry.



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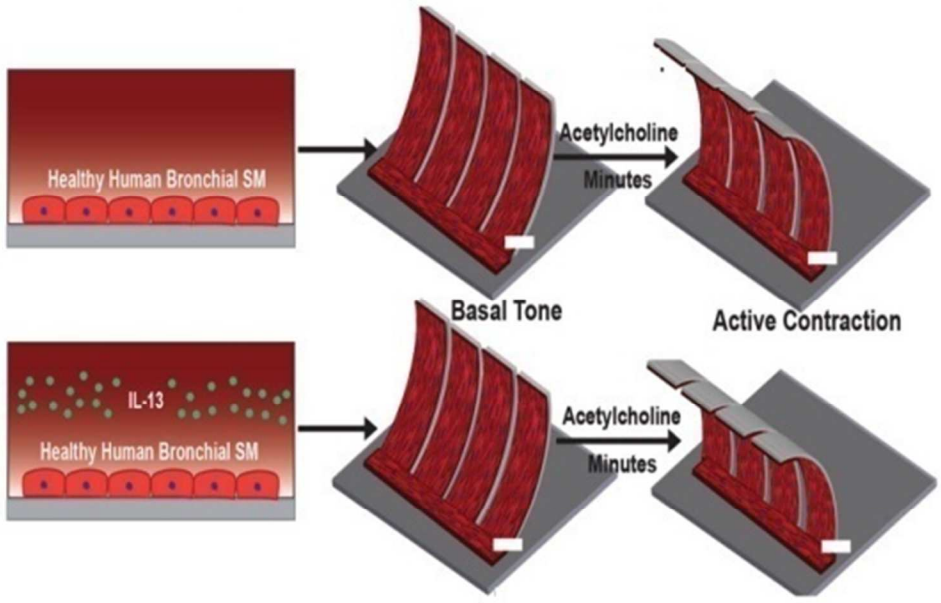
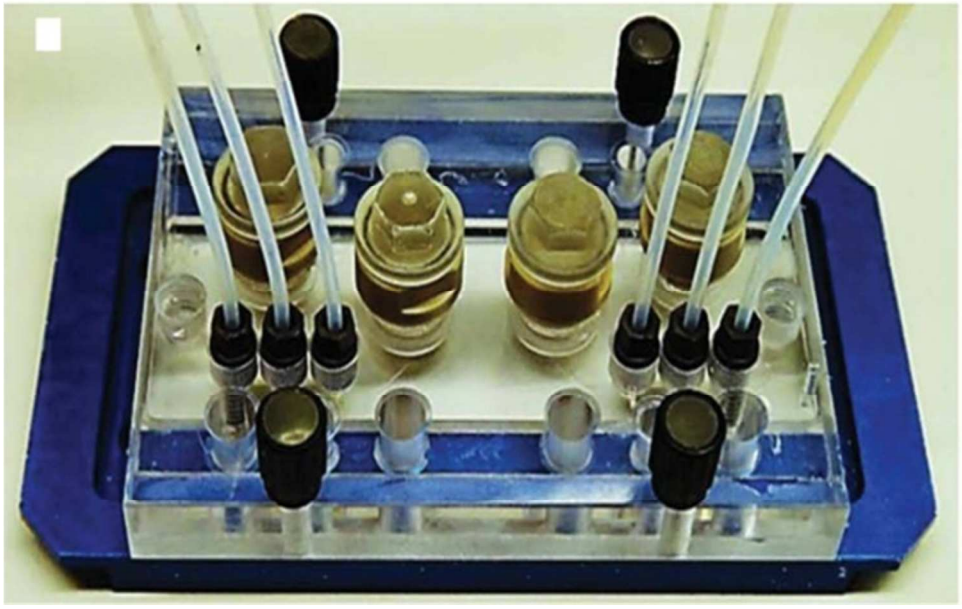


(b)

Figure 4: (a) A microfabricated lung-on-a-chip device uses compartmentalized PDMS microchannels to form an alveolar-capillary barrier on a thin, porous, flexible PDMS membrane. The device recreates physiological breathing movements by applying vacuum to the side chambers and causing mechanical stretching of the PDMS membrane forming the alveolar-capillary barrier. (b) Schematic diagram depicting an airway musculature on a chip, which was recapitulated by engineering anisotropic, muscular lamella on thin films. The bronchial smooth muscular thin film contraction was induced by administering acetylcholine. (a) From Ref. [38].Reprinted with permission fromThe American Association for the Advancement of Science (b) From Ref. [39].Reprinted with permission fromRoyal Society of Chemistry.



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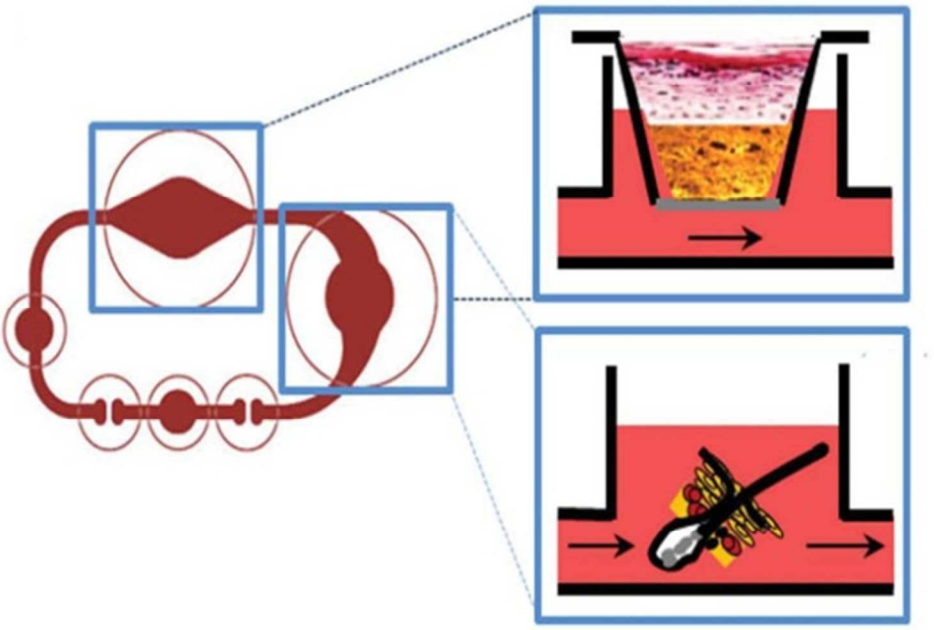
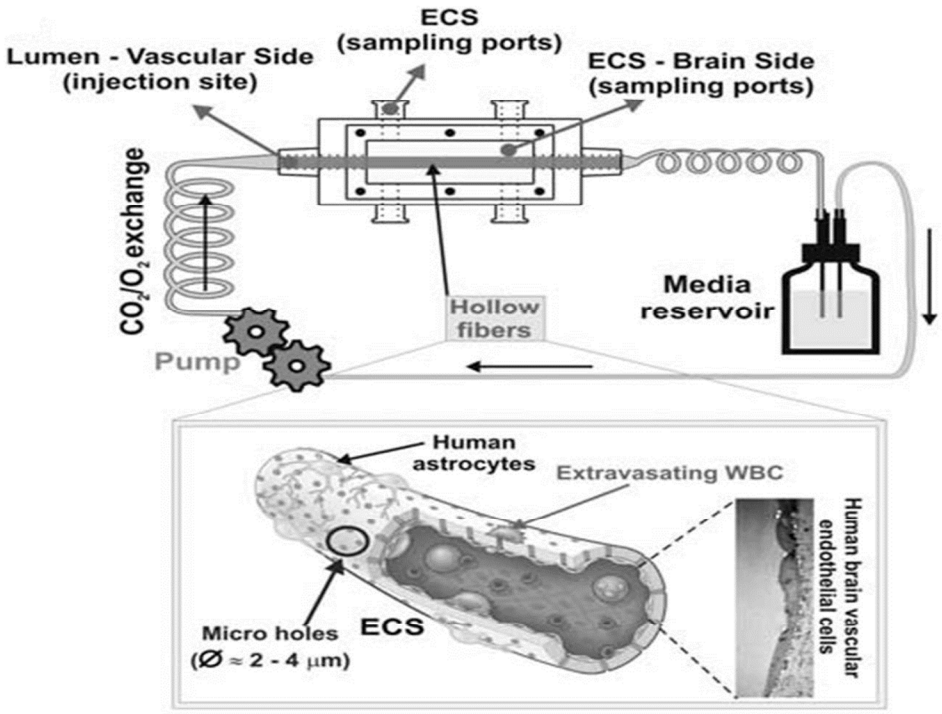


(b)

Figure 5: Multi-organ-chip for hosting a skin model. (a) Chip with built-in micropump providing a pulsatile flow of culture medium. (b) Separate chip componentsare used for culturing ex vivo skin biopsies and in vitro skin equivalents using either Transwell-type culture or follicular unit extracts that are directly placed in the stream. From Ref. [66].Reprinted with permission fromRoyal Society of Chemistry.



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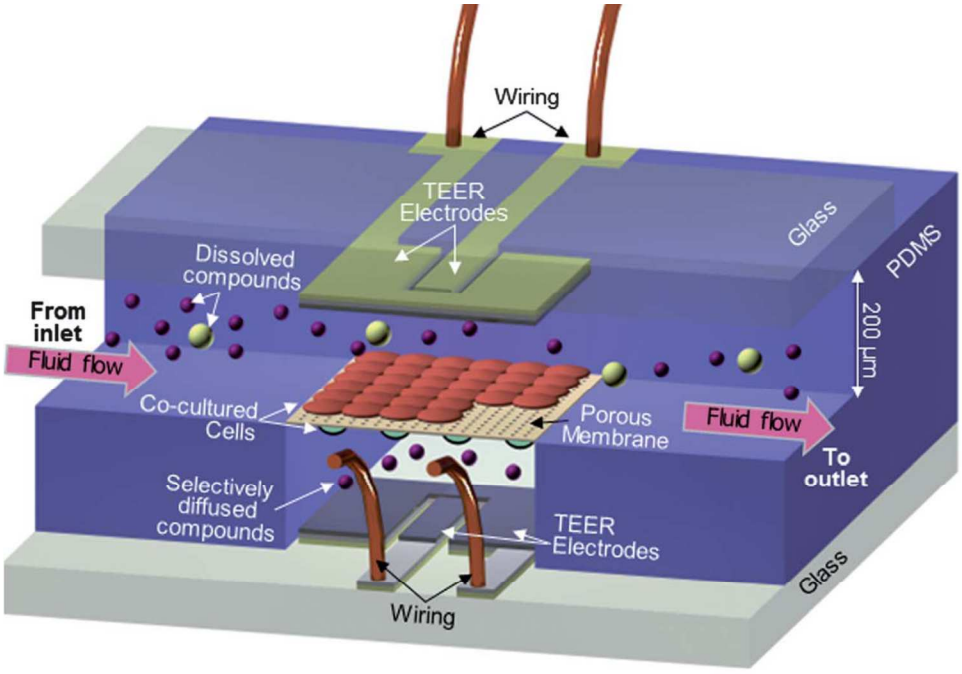

(b)

Figure 6: (a) Schematic representation of adynamic *in vitro* BBB model. A bundle of porous polypropylene hollow fibers is suspended in a chamber and isin continuous contact with a medium source through a flow path consisting of gas-permeable silicone tubing. (b)Micro-BBB system comprising two perpendicular flow channels with endothelial/astrocytes co-culture. The system also incorporated a pair of electrodes in the upper and lower chamber to measure TEER. (a) From Ref. [170].Reprinted with permission from Nature Publishing Group. (b) From Ref. [173].Reprinted with permission fromRoyal Society of Chemistry.

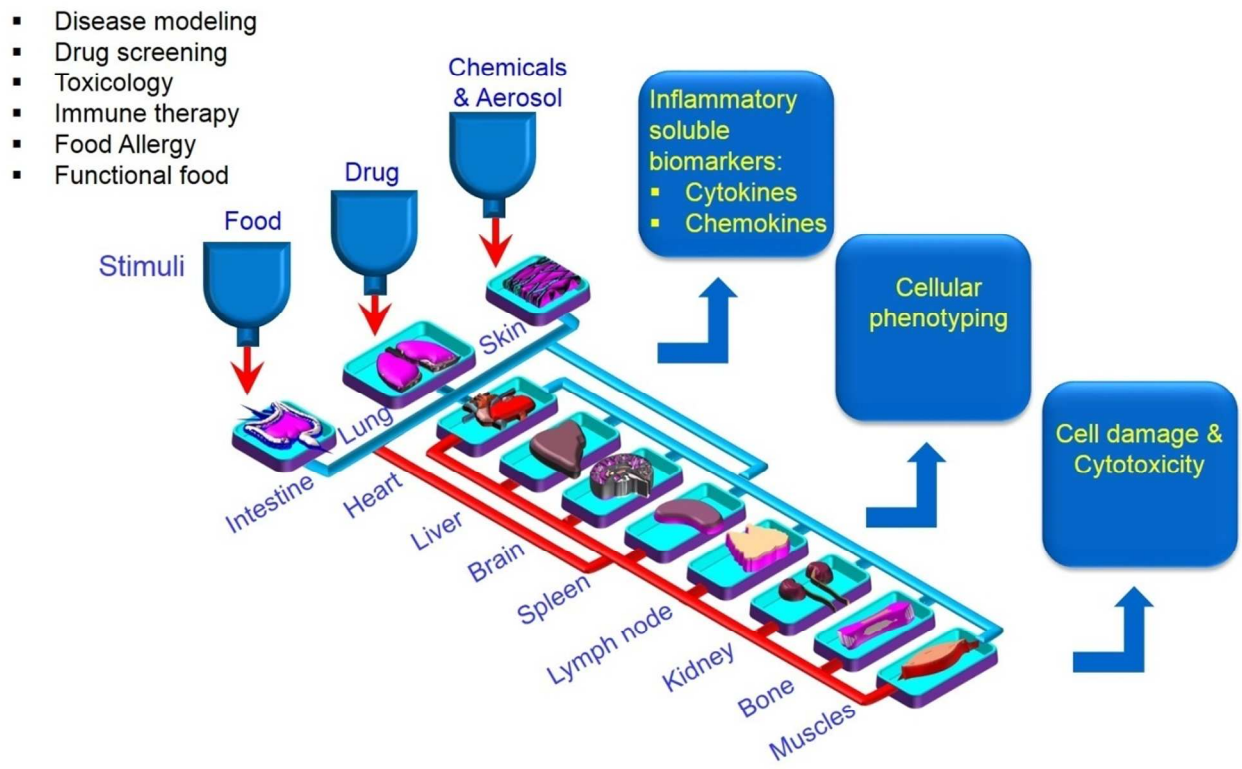

Figure7:A schematic diagram of a microfluidic approach for establishing a 'whole-body'-type immune-screening platform (multiple organs-on-a-chip), which can be exploited for different applications, such as disease modeling, drug screening, toxicology, food allergy studies, immune therapy development, functional food development, etc..