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REVIEW ARTICLE

Engineering mechanobiology through organoids-on-chip: A strategy to boost therapeutics

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Abstract

The mechanical environment of living cells is as critical as chemical signaling. Mechanical stimuli play a pivotal role in organogenesis and tissue homeostasis. Unbalances in mechanotransduction pathways often lead to diseases, such as cancer, cystic fibrosis, and neurodevelopmental disorders. Despite its inherent relevance, there is a lack of proper mechanoresponsive in vitro study systems. In this context, there is an urge to engineer innovative, robust, dynamic, and reliable organotypic technologies to better connect cellular processes to organ-level function and multi-tissue cross-talk. Mechanically active organoid-on-chip has the potential to surpass this challenge. These systems converge microfabrication, microfluidics, biophysics, and tissue engineering fields to emulate key features of living organisms, hence, reducing costs, time, and animal testing. In this review, we intended to present cutting-edge organ-on-chip platforms that integrate biomechanical stimuli as well as novel multicellular culture, such as organoids. We focused on its application in two main fields: precision medicine and drug development. Moreover, we also discussed the state of the art for the development of an engineered model to assess patient-derived tumor organoid metastatic potential. Finally, we highlighted the current drawbacks and emerging opportunities to match the industry needs. We envision the use of mechanoresponsive organotypic-on-chip microdevices as an indispensable tool for precision medicine, drug development, disease modeling, tissue engineering, and developmental biology.

KEYWORDS

drug screening, mechanobiology, microfluidics, organ-on-chip, organoids, personalized medicine

1 | INTRODUCTION

Mechanical stimuli play a pivotal role in organogenesis, tissue homeostasis, and diseases. Cells can interpret and translate mechanical forces (e.g., stiffness, roughness, and softness) into biochemical signals (Schwarz, 2017). The translation of mechanical forces into biochemical signals is mainly mediated by integrins, actin cytoskeleton, and focal adhesion, hence, regulating several signaling events, including cell fate, migration, differentiation, and apoptosis (Figure 1a) (Schwarz, 2017).

Mechanical changes in living-cell environments often lead to the onset of diseases. Tumor progression, for instance, is mainly characterized by its extracellular matrix (ECM) stiffness that can achieve up to tens of kPa compared to healthy tissue (Deptuła et al., 2020). In mammary tumors, for example, the constant deposition of collagen-I

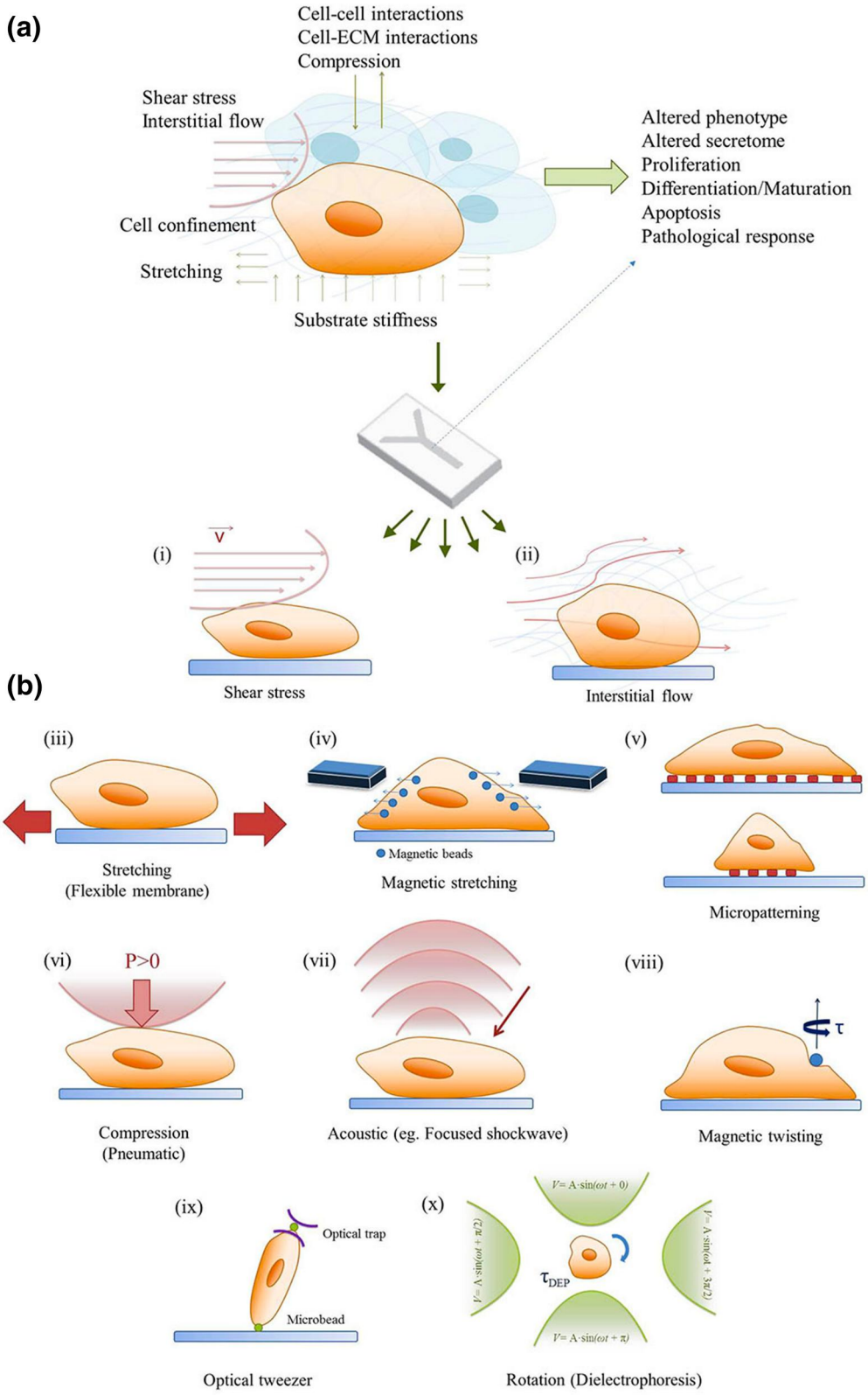

FIGURE 1 Biomechanical stimuli. (a) Different biomechanical stimuli can regulate cell behavior. (b) A microfluidic device as a strategy to mimic in vivo biomechanical stimuli: (i) Shear stress, (ii) interstitial flow, (iii) stretching, (iv) magnetic stimulation, (v) micropatterning, (vi) compression, (vii) acoustic stimulation, (viii) magnetic twisting, (ix) optical tweezers, and (x) rotation (dielectrophoresis). Reprinted from Ergir et al. (2018) under the terms of the Creative Commons Attribution License (CC BY) [Colour figure can be viewed at wileyonlinelibrary.com]

by cancer-associated fibroblasts leads to an increase in ECM stiffness and density. The mammary tumor is approximately 5 times stiffer and the tumor stroma can reach 20 times more stiffness than the healthy mammary tissue (Kumar & Weaver, 2009).

Likewise, the biomechanical unbalance of ECM contributes to neuro disorders such as schizophrenia and epilepsy (Goriely et al., 2015; McRae & Porter, 2012). Interestingly, it has been shown that alterations in the stiffness or elasticity of the substantia nigra are related to the development of early Parkinson's disease symptoms (Berg, 2010). Other disorders such as osteochondral pathologies, sexual dysfunction, and rheumatologic are also influenced by biomechanical disturbances (Mammoto et al., 2013).

Due to the significance that biomechanical stimuli have at the tissue and cellular levels, studies have tried to integrate them to closely resemble the in vivo conditions (Dieffenbach et al., 2018; Yang et al., 2020). For instance, bioengineering anisotropic structures, such as knee meniscus, are still a hurdle mainly because these constructs lack proper load bearing and shock absorption stimuli. The engineered graft usually results in long-term joint degeneration (Bilgen et al., 2018). To surmount this challenge, Zhang and co-workers combined a customized dynamic tension-compression loading system with biochemical clues to reconstruct the native meniscus tissue. The bioengineered meniscus demonstrated long-term chondroprotection of the knee joint in a rabbit model (Zhang et al., 2019). Likewise, efforts have been made to integrate mechanobiological stimuli in bone (Uda et al., 2017), lung (Huh et al., 2010), vasculature (Mazzocchi et al., 2018), and pathological study models (Armistead et al., 2020).

Although there have been remarkable improvements in the mechanobiology field in the last decades, many studies remain lacking proper experimental techniques (technical challenge) and representative multicellular model systems (biological challenge). It is known that traditional monolayer (2D) in vitro static culture lacks to recapitulate the morphology, phenotype, biophysics, and dynamism of cells within the body. Events such as drug diffusion kinetics as well as the predictability of side effects are unrealistic when scaled to patients (Fontoura et al., 2020). Moreover, despite being a gold-standard practice, animal testing leads to high costs, ethical issues, and dubiety in the interpretation of the data acquired (Skardal et al., 2016).

In this context, there is an urge to develop innovative, robust, and reliable organotypic technologies to better connect cellular processes to organ-level function. Microscale technologies, such as microfluidics devices, have been proved to be an astonishing approach to surpass these challenges (Rothbauer et al., 2019). Within these devices, it is possible to control, monitor, and provide biomechanical signals simultaneously with enhanced spatiotemporal precision (Rothbauer et al., 2019). These advantages have allowed an augmented understanding of in vivo processes that were not possible in traditional assays. Shear flow, compression, stretch, and strain are among the main mechanobiological stimuli emulated in microfluidic devices (Figure 1b) (Kaarj & Yoon, 2019).

Reported for the first time in 2010 by Hu and co-workers, a mechanically active microdevice able to reproduce the microarchitecture of the human alveolar–capillary unit was developed. Measuring only 1–2 cm in length, the pioneer breathing lung-on-a-chip microdevice was able to reproduce complex responses triggered by pathogens and nanoparticles introduced into the alveolar space (Hu et al., 2010). Despite being a proof-of-concept, this device paved the way towards disruptive microsystems, such as intestine-on-chip, stroke-on-chip, neurovascular-unit-on-chip, heart-on-chip, and body-on-chip (Firoozinezhad et al., 2019; Maoz et al., 2018; Oleaga et al., 2018; Sakamiya et al., 2020; Sticker et al., 2019).

Whereas microfluidic devices mimic environmental clues of living tissue, organotypic multicellular constructs (e.g., organoids) can be synergically combined within these devices providing enhanced biological responses (Park et al., 2019). These constructs reproduce key multicellular, anatomical, and functional properties of real organs at the micrometer–millimeter scale. Organoid technology has a wide range of applications and when combined with microfluidic devices has real potential to leverage crucial sectors such as drug development, personalized medicine, tissue engineering, and developmental biology (Park et al., 2019). Moreover, this synergism can also be a powerful tool to unravel the mechanistic knowledge of life-threatening conditions (i.e., cancer, obesity, diabetes, and cardiac diseases) as well as outbreaks such as the new coronavirus.

Due to its physiological relevance to human settings, the effective implementation of organoid-on-chip also decreases the limitations of traditional organoids culture, by promoting automated control of biochemical, biophysical, and nutrients supply, while reducing variability and providing high-throughput manipulation and analysis of organoids crosstalk (Park et al., 2019).

In this review, we intended to present cutting-edge organ-on-chip platforms that integrate biomechanical stimuli as well as novel multicellular culture, such as organoids. We focused on its application in two main fields: precision medicine and drug development. Moreover, we also hypothesize the state of the art for the development of a mechanically active microfluidic model to evaluate the metastatic potential of tumor organoids from patients' biopsies. Finally, we highlight the current drawbacks and emerging opportunities to match the industry needs.

2 | ORGANOTYPIC ON-CHIP MODELS: BRIDGING THE GAP BETWEEN TRADITIONAL IN VITRO CULTURE AND ANIMAL TESTING

Advances in 3D cell culture systems have resulted in the development of more physiologically relevant in vitro models, such as organoids. Organoids have the capability of recapitulating intrinsic embryogenesis steps, reproducing key structural and functional features of those found in vivo (Park et al., 2019).

Organoids can be established from embryonic stem cells (Finkbeiner et al., 2015), induced pluripotent stem cells (iPSCs) (Tao et al., 2019), adult stem cells (Drost & Clevers, 2017), and be co-cultured with a variety of cell types (Skardal et al., 2017, Yu et al., 2017). Although previous studies extensively used immortalized cells (Bertaux-Skeirik et al., 2016; Ma et al., 2009), they are often

derived from cancer cells and have lost their original functional activity. Hence, current studies have adopted iPSCs, human primary cells, and progenitor cells (Tao et al., 2019; Yu et al., 2017; Zhang et al., 2017).

The use of organoids as a preclinical reliable model system is already a reality (Derouet et al., 2020; Ganesh et al., 2019; Gao et al., 2018; Lancaster et al., 2013; Weeber et al., 2015, 2017). Due to its capacity to retain key properties of native tumors, biobanks composed of different types of primary cancer organoids have been created (Calandrini et al., 2020; Sachs et al., 2018; Yan et al., 2018). They represent a collection of well-characterized models that facilitate identifying patient-specific drug sensitivities, boosting personalized medicine and drug development.

Despite the advantages aforementioned, organoids technology still has some limitations (Park et al., 2019). In order to properly mature, organoids demand coordinated activation of morphogenetic signaling. Accordingly, adequate perfusion of the culture medium is crucial to prevent hypoxia of cells, waste removal, and nutrient supply (Yu et al., 2019). The traditional formation of brain organoids, for example, consists of laborious steps beginning with the encapsulation of embryoid bodies (EBs) into Matrigel, followed by their transfer to static petri dishes or spinning bioreactors (Peng et al., 2018). Although these models have shown remarkable results toward the initial human brain development, they often fail to achieve proper tissue complexity and maturation seen in the adult brain (Lancaster et al., 2013). Nonetheless, the unpredictable growth patterns in traditional organoids culture result in heterogeneity and variability, hampering its effective translatability for drug discovery and personalized medicine (Weeber et al., 2015).

In this context, the synergism between microfluidic-based systems and organoid culture provides a powerful in vitro platform with several advantages, such as (i) tissue-like construct with cellular and environment fidelity, (ii) control of nutrient supply and waste removal, (iii) real-time monitoring, and (iv) control of the biophysical and biochemical environment (Figure 2a) (Park et al., 2019). Moreover, the continuous perfusion of the culture medium allows a constant infusion of oxygen, avoiding necrotic centers within the organoids and promoting a consistent supply of nutrients and biomolecules, hence extending their lifespans toward a fully mature in vitro model (Yu et al., 2019). Accordingly, within these systems, it is also possible to design multicompartments to recapitulate the endogenous stromal components (e.g., fibroblasts and immune cells) as well as the native microbiome, surpassing another limitation of conventional organoids culture (Weeber et al., 2015). The synergistic effect of mechanically active microfluidic devices and organoids goes even further. The biomechanical clues can also improve the differentiation and maturation of stem/progenitor cells within the organoids. Kidney organoids on-chip under high shear stress, for instance, showed enhanced vascularization and maturity compared with organoids in static culture (Figure 2b,c) (Homan et al., 2019). The effects of shear stress and interstitial fluid flow were also reported for the pancreas and intestine (Tao et al., 2019; Workman et al., 2017). Thus, microfluidic-based systems and organoids are complementary technologies that combined have the potential to surpass current technical and biological challenges. While organoids are superior in vitro models, that recapitulate key steps of initial human development events, microfluidics can mimic the biological microenvironment, providing mechanical clues and vascularization-like, resulting in a sophisticated and more reliable in vitro platform (Park et al., 2019).

It is noteworthy that several physiological functions are dependent on the interaction with multiple tissues. Likewise, pathological conditions need tissue crosstalk to begin and aggravate, for example, lupus disease (Stojan & Petri, 2018). In this context, different organoids (e.g., liver, lung, heart) can also be fluidically connected, resulting in multi-organoids-on-chip or body-on-chip platforms (Yu et al., 2019). Connecting key representative organoids—such as the liver, kidney, intestine—also allows one to evaluate the biological response of a drug candidate systematically, including possible side effects (Skardal et al., 2016).

Indeed, the physiological relevance of the multi-organoids-on-chip platform to evaluate drug efficacy and safety in humans has shown startling results. Skardal and co-workers investigated the response of a multi-organoid platform containing liver, heart, lung, vascular, testis, colon, and brain with recalled drugs. These drugs were withdrawn from the market due to the adverse effects on the liver and heart in humans. The safety evaluation using the multi-organoid platform indicated similar toxicity levels, demonstrating the predictability and reliability of the platform. Moreover, the authors also demonstrated the importance of including liver organoids to evaluate drug toxicity. It was seen that this type of organoid is responsible to coordinate the downstream response to other organs, resembling in vivo conditions (Skardal et al., 2020).

Organotypic-on-chip models are an evolving field that holds great promise. These models have real potential to fulfill the technical and biological challenges seen in traditional in vitro systems and animal models. In recent years, groundbreaking mechanically active organoids-on-chip platforms have been reported (Table 1). In the next sections, we will discuss their applicability and functional hallmarks in different tissues and pathologies.

3 | PATHOLOGICAL CONDITIONS OF MECHANOBIOLOGY: HOW MICROFLUIDIC DEVICES ARE HELPING TO UNDERSTAND THE ROLE OF MECHANO-STIMULI IN ONCOLOGY

Cancer is a serious public health issue worldwide. According to the World Health Organization, cancer is the second cause of death globally with an economic impact estimated at US$ 1.16 trillion per year (Stewart & Wild, 2014). Accordingly, there is an urge to develop new strategies to unravel the mechanistic knowledge behind cancer development and metastasis as well as to develop technological platforms able to emulate this pathological condition to test new drug solutions.

It is known that mechanical forces display a pivotal role during cancer development. The malignant progress is composed of acellular

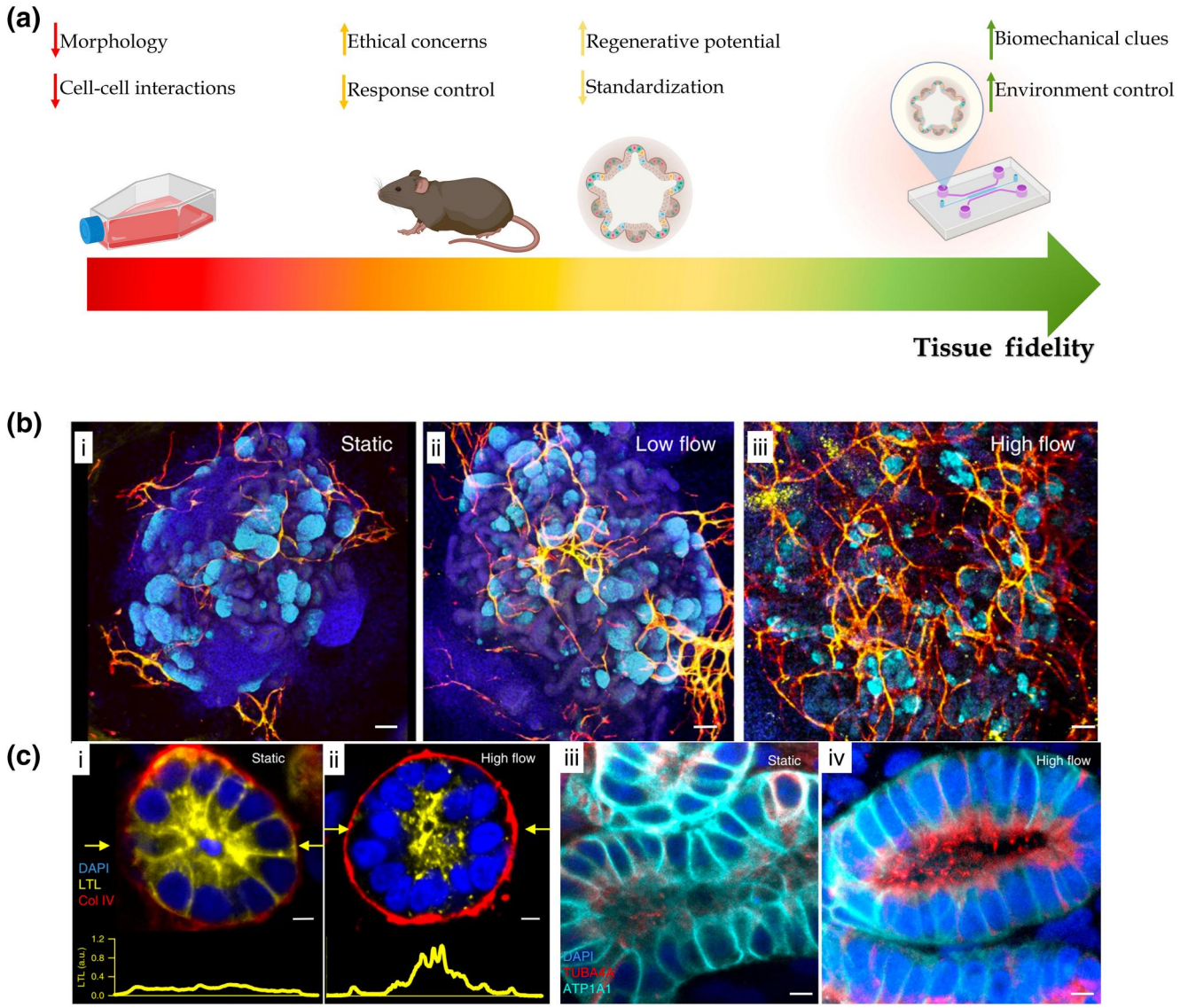

FIGURE 2 Mechanically active organoids-on-chip as a strategy to achieve tissue fidelity. (a) Schematic representation of different study models and their proximity to the human physiological system. (b, c) To notice the influence of shear stress on culture kidney organoids. Imposing shear stress (high flow) in kidney organoids improved the differentiation and maturation of cells (b): fluorescent microscopy of kidney organoids under (i) static, (ii) low flow, and (iii) high flow culture condition. Scale bars: 100 μm. (c) Expression of collagen IV and LTL (lotus tetragonolobus lectin) at day 21 under static (i) and high flow (ii). Yellow: Shows the intensity of LTL staining. (iii, iv) Staining of tubule cross-section for TUBA4A (ciliary marker) and ATP1A1 under static (iii) and high flow (iv) at day 21. To notice the difference of cellular organization in static condition versus high flow. Scale bars: 5 μm. (b, c) Adapted from Homan et al. (2019), with permission from nature methods [Colour figure can be viewed at wileyonlinelibrary.com]

(e.g., ECM remodeling, collagen content, oxygen levels) and cellular factors (immune cells, mesenchymal stem cells, tissue-resident cells) (Lynch et al., 2020). The pathological biomechanical scenario for cancer to perpetuate is often characterized by (i) the uncontrollable proliferation of tumor cells in a limited space generating stress in the surrounding tissue, (ii) augmented ECM deposition leading to its abnormal stiffness, and (iii) interstitial fluid flow caused by increased angiogenesis within the tumor (Lynch et al., 2020). Indeed, it has been shown that tensile forces are directly related to the invasiveness and dissemination of tumor cells to other organs (Kopanska et al., 2016).

These biomechanical forces in the tumor microenvironment can be grouped as solid stress and fluid stress. The solid stress is the mechanical forces, mostly from the resident cells and the ECM. The combination of uncontrollable cell proliferation coupled with the increase of collagen fibers in the ECM leads to augmented solid stress governed by compressive and tensile forces (Jain et al., 2014). The differences in the ECM can impact the disordinate formation of new capillaries, leading to fluid stress. The fluid stress is caused by the pressures of microvascular fluid, interstitial fluid, and shear stress, employed by the blood flow and interstitial flow (Bordeleau et al., 2016). The fluid stress generated within the tumor modulates endothelial cell function, affecting their barrier function, tubule formation, morphology, and sprout formation (Bordeleau et al., 2016; Song & Munn, 2011).

TABLE 1 Mechanically active organotypic-on-chip devices for dynamic cell culture

Organotypic-on-chip models						
Emulating	**Biomechanical clues**	**Cell type**	**Kinetics**	**Scaffold type**	**Outcomes**	**Reference**
Eye	Blink-induced mechanical forces	Human keratocytes, corneal and conjunctival epithelia	16 days	PDMS*	Blink-induced physyological mechanical forces may have significant contributions in the differentiation of human corneal epithelial cells	Seo et al. (2019)
Kidney	Shear forces and interstitial fluid flow	Human glomerular microvascular endothelial cells, Human umbilical vein endothelial cells, human neonatal dermal fibroblasts, induced pluripotent stem cell, and human embrionic stem cell	21 days	PDMS	Vascularized kidney organoids cultured under flow had more mature podocyte and tubular compartments with enhanced cellular polarity and adult gene expression compared with the ones static condition	Homan et al. (2019)
Stomach	Peristaltic luminal flow, shear forces, and geometric confinement	Human pluripotent stem cells	≅ 15 days	PDMS	A steady-state luminal flow was achieved, recapituling in vivo luminal flow within the stomach	Lee et al. (2018)
Brain	Shear forces	Human embryonic stem cell	20 days	PDMS	Two opposite forces from the cytoskeletal and the nuclear expansion of cells in the perifery of the organoids were identified as the main regulators in the appearance of surface wrinkles in brain organoids	Karzbrun et al. (2018)
Neuronal network	Shear forces, surface topography, and geometric confinement	Rat neural stem/progenitor cells	5 days	PDMS	Neurons were generated and produced functional and recyclabe synaptic vesicles, and the connections of the neural network were also confirmed	Liu et al. (2018)
Intestine	Shear forces and interstitial fluid flow	Human primary epithelial cells and intestinal tissue-specific microvascular endothelial cells	12 days	PDMS	Transcriptomic analysis indicates that the intestine chip closely mimics the whole human duodeno in vivo, compared to the duodenal organoids off chip	Kasendra et al. (2018)
Organs interaction and cancer (liver, heart)	Fully automated shear forces	Human primary hepatocytes, human induced pluripotent stem cell-derived	5 days	PDMS	The platform were able to reproduce the chronic drug responses and acute toxicity	Zhang et al. (2017)

TABLE 1 (Continued)

Organotypic-on-chip models						
Emulating	**Biomechanical clues**	**Cell type**	**Kinetics**	**Scaffold type**	**Outcomes**	**Reference**
		cardiomyocytes, and hepatocellular carcinoma cells			similar with in natura	
Intestine	Shear forces, surface topography, and geometric confinement	Human-derived small intestine cells	8 days	PDMS	The architecture, tissue polarity and luminal accessibility were successfully reproduced	Wang et al. (2017)
Midbrain	Randomized gravitational vectors and shear forces	Human induced pluripotent stem cell	84 days	Thermoplastic polymers	Crucial events of ZIKA virus infection (e.g., increased cell death and reduced proliferation, resulting in decreased neuronal cell-layer volume resembling microcephaly) were recapitulated	Qian et al. (2016)
Heart	Pneumatic actuation system to induce homogeneous uniaxial cyclic strains	Human and rat induced pluripotent stem cell-derived cardiomyocytes	6 days	PDMS	Stimulated μECTs* showed superior cardiac differentiation, as well as electrical and mechanical coupling, which promoted early spontaneous synchronous beating and better contractile capability	Marsano et al. (2016)
Retina	Shear forces	Retinal cell types derived from human induced pluripotent stem cells	180 days	PDMS and PET	The interaction of mature photoreceptor segments with retinal pigment epithelium was recapitulated for the first time	Acherger et al. (2019)

Abbreviations: μECTs, micro-engineered cardiac tissues; PDMS, polydimethylsiloxane; PET, polyethylene terephthalate.

Due to the nature of tumorigenesis, organotypic on-chip models are effective platforms to mimic the solid and fluid stresses. In order to investigate the correlation between biomechanical forces and drug resistance, Pang et al. (2019) developed a microfluidic device able to analyze the formation of single-cell-derived tumor spheres. After applying a deformation force in single glioblastoma cells, the tumor spheres were formed and the drug resistance was evaluated within the device. Interestingly, it was seen that tumor-spheres derived from more deformable cells presented enhanced resistance to anticancer drugs. A comparison between 2D cell culture and tumor-spheres was also made. The results showed that tumor-spheres had higher drug resistance than the traditional 2D culture method. These finds indicate that (i) it is possible to produce tumor-sphere according to tumor cell-specific biomechanical properties, (ii) biomechanical forces displayed in a cancerous environment may influence the anticancer drug efficiency, (iii) tumor-sphere provides a physiologically relevant tumor model than 2D culture (Pang et al., 2019).

The integration of multiple sensor systems to achieve automated in situ monitoring of biophysical and biochemical parameters in organoids-on-chip is still a hurdle. Attempts have been made to achieve multiparametric measurements nonetheless the models still lack multiple sensors and the automation of monitoring (Chen et al., 2011; Lockery et al., 2012; Ma et al., 2009; Midwoud et al., 2010; Sung et al., 2010; Xu et al., 2012, 2013; Ye et al. 2007).

In this context, Zhang et al. (2017) reported a fully integrated microfluidic controlling breadboard. The platform was equipped with physical sensors, electrochemical immunobiosensors, and miniaturized microscopes. The platform was able to provide data regarding the extracellular microenvironment (e.g., pH, O_2, temperature), soluble protein biomarkers, and morphology of the organoids. As a proof-of-concept, the evaluation of short-term and chronic drug response was made using dual-human liver-cancer-and-heart-organoid-on-chip and liver-and-heart-organoid-on-chip. All the experiments were fully carried out uninterruptedly by a computer for 5 days. Moreover, the reported real-time in situ monitoring platform is also compatible with existing organ-on-chip devices and could be coupled to boost data acquisition (Figure 3a,b) (Zhang et al., 2017).

The most related cancer deaths are caused by metastasis. Metastasis is a multistage mechanochemical process where cancer cells acquire invasive phenotypes and colonize distal organs (Yoshii et al., 2016). The increase in matrix stiffness, caused by enhanced stromal collagen deposition, contributes to the mechanotransduction pathways, leading to metastatic behavior (Yoshii et al., 2016). To date, it is not yet well understood the key events that guide cells to colonize specific distal organs.

The elucidated events that occur in metastasis, such as transendothelial migration, circulation in the vascular system, extravasation, and colonization can be better represented in microfluidic devices. In these devices, it is possible to control hydrostatic pressures, fluid flow, and to engineer different regions to mimic specific in vivo microenvironments, for example, the migration of tumoral cells to the bloodstream. Thus, providing an in vitro model with physiological relevance (Skardal et al., 2016). Moreover, it is known that compression, interstitial pressure, and flow are important biomechanical forces that regulate tumoral cells in vivo (Shieh, 2011). Hence, to engineer mechanobiology through microfluidics is a valid strategy to unravel the mechanistic knowledge behind several diseases and their features, such as the metastatic potential of tumoral cells. In this context, our research group hypothesizes the development of a mechanically active microfluidic model to evaluate the metastatic potential of tumor organoids. The state of the art consists of a microfluidic device able to apply a compression force on organoids derived from patients' tumors Figure 4. The proposed device combines mechanical clues such as compression, interstitial pressure, and flow resembling the tumor microenvironment. Moreover, to use organoids derived from a patient's biopsy as a biological model within this device promotes more realistic results, allowing to test specific drugs and treatment.

Cancer is a ubiquitous disease that has a severe socio-economic impact. In a statistical study coordinated by Miller and co-workers, it was observed that leukemia, breast, colorectal, and melanoma was the most commonly diagnosed cancer in the group aged 20 to 39 years (Miller et al., 2020). This data emphasizes the need to invest time and resources to develop more reliable in vitro methods to change these statistics. Mechanically active microfluidic devices can be a resourceful platform not only to assess tumor behavior but also to be a stepping stone to select effective biomarkers present in the early stage of cancer. Hence, leveraging drug development and precision medicine.

4 | MECHANICALLY ACTIVE ORGANOTYPIC-ON-CHIP DEVICES FOR DYNAMIC CELL CULTURE

4.1 | Toward a reliable heart beating study model

During the contraction/relaxation phases of the heartbeat, cardiomyocytes are constantly under strain stress. The cyclic uniaxial strain, caused by the deformation, is perceived by the ECM which leads to a mechanotransduction pathway signaling; hence, modulating heart behavior (Krueger et al., 2020). To closely emulate heart conditions in vitro, mechanical stimuli are mandatory. The synergism among microfluidics, organotypic culture and mechanical stimuli were reported by Marsano et al. (2016). To replicate the mechanobiology of the heart, they developed a microfluidic device able to transform pressure signals into a controlled uniaxial cyclic strain submitted to the cells (Marsano et al., 2016).

The inclusion of mechanobiological stimuli within the device promotes enhanced cardiomyocyte differentiation, with phenotypic similarity within in vivo conditions. Moreover, the stimulus can result in spontaneous synchronous beating and greater contractile capability in response to electric pacing. Interestingly, the authors also used a finite element model of the microfluidic platform to evaluate whether the compression applied to the cells was uniaxial (Marsano et al., 2016). Mathematical modeling has become, in recent years, an important tool to predict biological responses of complex and heterogenous in vitro models (Sung et al., 2019). The computational analysis enables the design without the need to create a material experiment, to test

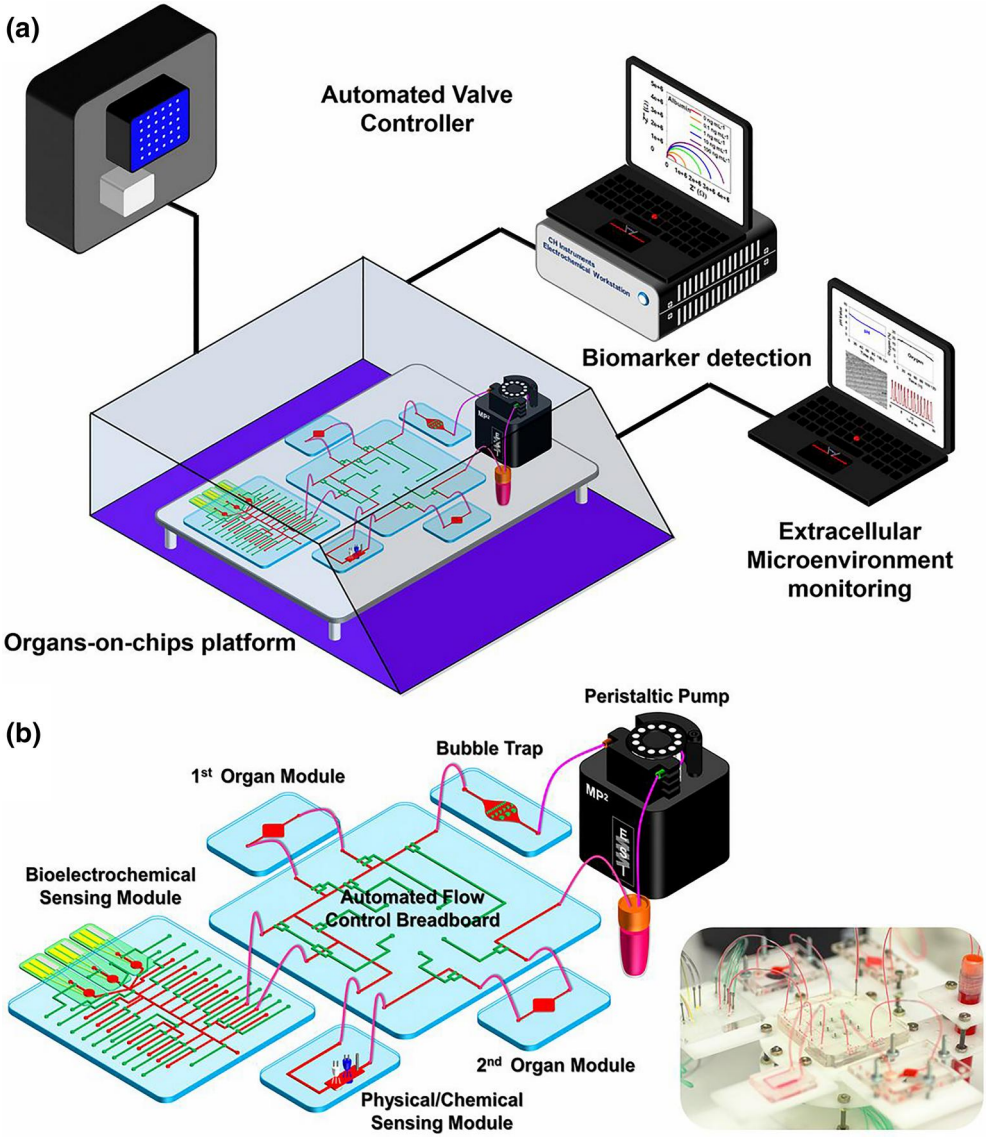

FIGURE 3 Integrated microfluidic platform. (a) Illustration of the entire integrated system with the microfluidic device in an incubator, an automated pneumatic valve controller, potentiostat to measure the electrochemical signals, and a computer-based real-time monitoring system. (b) Illustration of the microfluidic platform with modular units, peristaltic pump, bubble trap, bioelectrochemical, and physical-chemical sensing module. Reprinted from Zhang et al. (2017), with permission from National Academy of Science [Colour figure can be viewed at wileyonlinelibrary.com]

complex geometries, to model local mechanical effects (e.g., deformation, stress), and to test several materials types. The use of finite element analysis to simulate stress propagation within microfluidic devices was also reported for in vitro vasculature (Ahn et al., 2020).

These innovative platforms, that combine biomechanical stress and mathematical modeling, pave the avenue to more predictable and reliable in vitro models to study, for example, hypertrophic changes seen in cardiac diseases as well as vascular abnormalities.

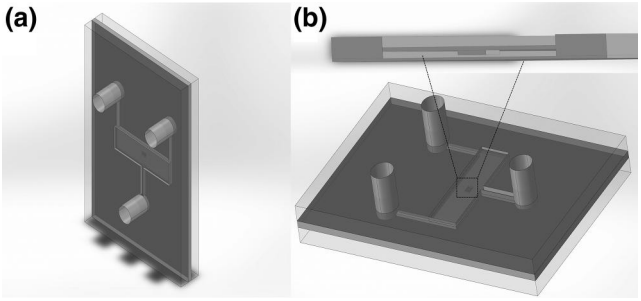

FIGURE 4 State-of-the-art for a mechanically active microfluidic model to evaluate the metastatic potential of tumor organoids. (a, b) Concept of the microdevice: (a) Microfluidic device; (b) microfluidic device with the mechanically active region in detail (i.e., compressive plate)

4.2 | Looking through the glass: ocular models

Retinopathies such as macular degeneration, diabetic retinopathy, glaucoma, and cataracts have no cure and are amongst the main

causes of vision loss (Flaxman et al., 2017). Furthermore, the ocular tissue is a common site for drug side effects, leading to local toxicity (Becerra et al., 2020). Due to the neuroretinal organization, heterogeneity of cell distribution, and wide blood supply, developing appropriately in vitro study models is still a hurdle.

Innovative studies have been held in microfluidics intending to mimic the ocular environment. Aiming to emulate blink-induced mechanical forces, a blinking-eye-on-chip was developed (Seo et al., 2019). The multilayered device was composed of a dome-shaped 3D cell culture scaffold connected to a perfusion chamber, a tear channel, and an eyelid able to slide on the scaffold surface. Overall, the device is an elasto-hydrodynamic model with (i) a blink-induced flow, (ii) deformation of the engineered ocular surface, and (iii) a moving eyelid.

The study showed that the insertion of biomechanical forces led to significant phenotypic and functional similarity within in vivo conditions. To understand the biomechanical-guided pathological processes in eye-dried disease, the authors tested the efficacy of lubricin as an ophthalmic lubricant. For the first time, it was possible to see its effects on ocular surface inflammation. These data reinforce the relevance to include biomechanical clues into a controlled microenvironment, such as microfluidic devices, combined with representative cell culture models Figure 5. These ocular organotypic on-chip cultures could also be an important tool to evaluate the pharmacodynamic and pharmacokinetics of new nano delivery systems that have been developed for currently untreatable eye diseases (Sánchez-López et al., 2017).

A human retina model, named retina-on-chip, was also reported to investigate the biomechanical influence on retinal organoids (Achberger et al., 2019). The authors used retinal organoids and retinal pigment epithelium as cell models. The study captured for the first time the interaction of mature photoreceptor segments with retinal pigment epithelium in vitro. The vasculature-like perfusion in the microdevice provided mechanical clues that were essential for these events to occur. Other biological events, such as the formation of outer segment-like structures and calcium dynamics, were also influenced by the biomechanics promoted in the model Figure 6a.

Ocular innervation displays an important role in the homeostasis of the eyes. The nerves promote sensing and trophic support to epithelial and stromal cells through chemical and biomechanical interactions (Al-Aqaba et al., 2019). Likewise, it also has a crucial homeostasis role in several organs, for example, heart, intestine, and brain. Therefore, integrating innervation-like within organs-on-chip has the potential to enhance the accuracy of acquired biological responses, providing a more dynamic environment for drug screening and basic research.

4.3 | Brain and innervation organoids-on-chip: integrative modular units to enhance the complexity of existing study models

The limited access and the complex physiology of the human brain, as well as the lack of proper in vitro models to reproduce its biology, hamper the development of therapeutic strategies to cure neuro disorders, such as Alzheimer's disease.

It is known that mechanical forces regulate brain-related events, for example, synaptic signaling, neuronal plasticity, and neuropathologies (Tyler, 2012). Indeed, dysregulation of mechanical clues can lead to reduced cortical folding and wrinkling, which is associated with neurodevelopmental disorders (Karzbrun et al., 2018). In this context, Karzbrun et al. (2018) used human brain organoids-on-chip as a predictive model to investigate the physics of the fold in the brain. Notably, the brain organoids have undergone self-organization into a shell-like structure with a lumen and convolutions, resembling the early development of the cortex.

After achieving a physiologically relevant model, the authors investigated the biomechanical forces underlying lissencephalic disease (i.e., smooth brain). To produce the diseased organoids, CRISPR/Cas9 genome editing was used as a strategy. It was observed that lissencephalic organoids showed reduced convolutions, modified scaling, and reduced elastic modulus compared with the control. These differences were attributed to the mechanical forces displayed by the cytoskeleton and it was only possible to investigate due to the microfluidic environment that the organoids were exposed to (Karzbrun et al., 2018). Therefore, the convergence between brain organoids and microfluidics shows it is possible to replicate complex and unseen biological events. Moreover, it is noteworthy to mention that, in order to complex future study models, integrative and interdisciplinary approaches such as the CRISPR genome editing tool might be recommended.

Innervation is a key component to properly mirror in vivo-like conditions. To date, restoring or creating innervation in vitro is one of the major drawbacks (Park et al., 2020). Aiming to surpass this limitation, Liu et al. (2018) developed a synaptic innervation-on-a-chip platform. The platform was able to support neural stem/progenitor cell differentiation and neurite outgrowth. The neurospheres produced functional synaptic vesicles and an electrical network that was validated with impedance measurements (Liu et al., 2018). The significance of this biochip goes beyond the biological achievements, it can be used as a modular unit to enhance the fidelity of information of existing organ-on-chip models. For instance, the nerves that innervate the aortic arch stretch and compress to regulate heart muscle contraction. Thus, modular units that provide mechanical active innervation would be an outstanding addition to (i) reproduce in vivo conditions and also (ii) to understand how neurons remain viable after these continuous cycles.

4.4 | Digesting-on-chip

The small intestine has a crucial role in human body homeostasis. In addition to digesting and absorbing nutrients, it has a close relationship with the endocrine and immune systems (Durel & Nerurkar, 2020). Ergo, imbalances in this organ, can reflect systematically and lead to the onset of diseases. The increase of stiffness, for instance, has been

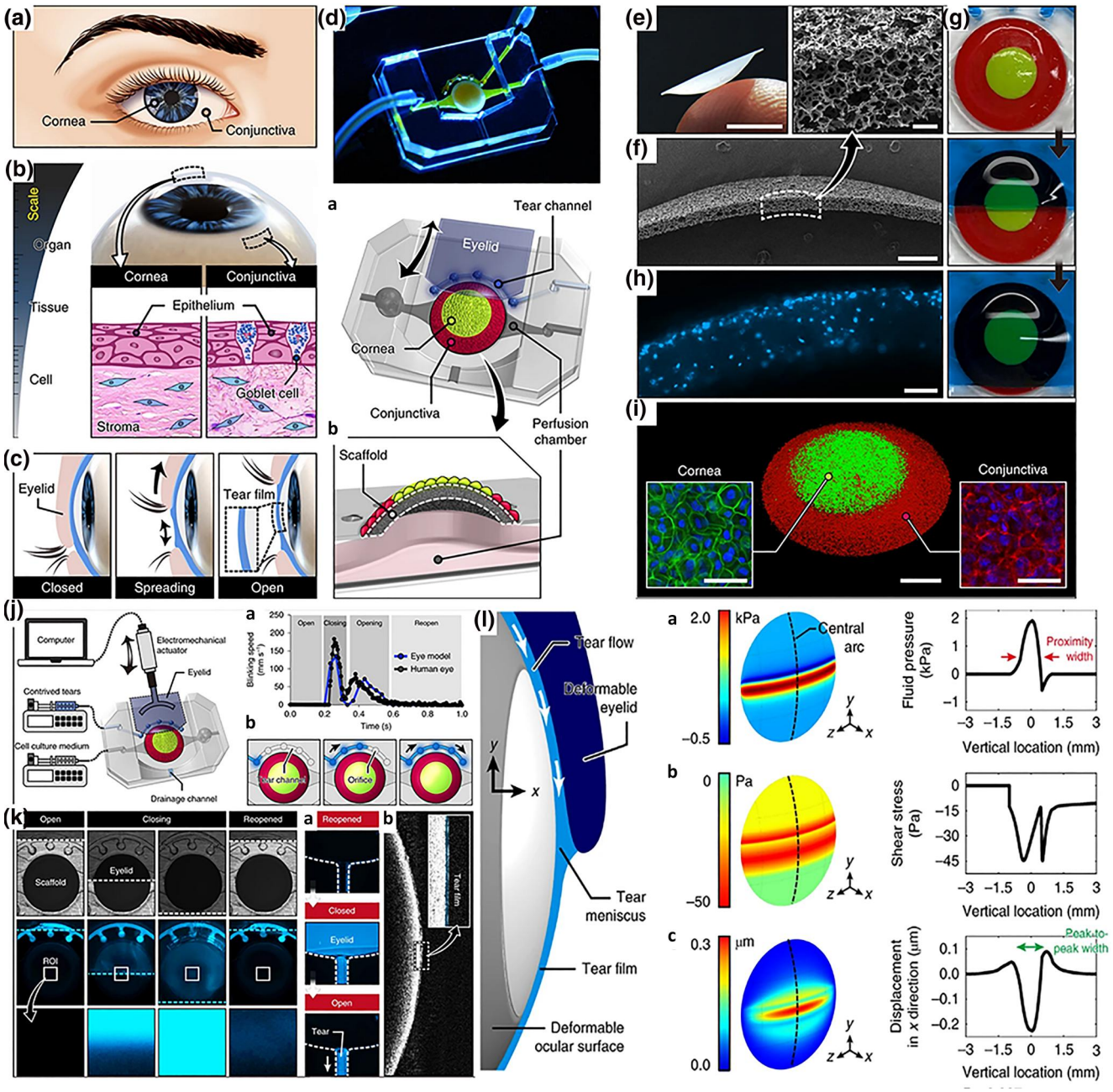

FIGURE 5 Blinking-eye-on-chip. (a) The cornea and conjunctiva tissue. (b) The ocular surface and its environment. (c) The formation of a thin film during eye blinking. (d) The device and its schematic illustration. (e) Dome scaffold. Scale bar: 3 mm. (f) Scanning electron microscopy of the scaffold. Scale bars: 500 μm (lower micrograph) and 50 μm (upper). (g) The hydrogel eyelid sliding over the engineered ocular surface (device). (h) Primary human keratocytes seeded in the scaffold. Scale bar: 100 μm. (i) Micrograph of human corneal and conjunctival epithelial cells on the surface of the scaffold. Scale bars: 1 mm and 50 μm. (j) The eye-blinking movement is controlled by a computer electromechanical actuator: (a) graphical representation of human eye blinking (grey) and the device (blue); (b) illustration of the tear channel with the tear injection and flow. (k) phase contrast (top) and fluorescent (bottom) microscopy show the blinking and tear fluid (blue): (a) fluorescent microscopy of the tear excess being cleared and directed by the eyelid into the drainage channel during blinking movement. Dotted lines: The channel walls. (b) shows a thin tear film (blue) on the ocular surface. (l) Representation of the engineered ocular surface: (a) shows the distribution of the tear fluid pressure predicted by the theoretical model. Red arrows: region where pressure is positive and higher than 0.6 kPa; (b) heat maps of fluid shear stress; (c) vertical displacement of the engineered ocular surface. Green arrows: Width of depression. Adapted from Seo et al. (2019) with permission from nature medicine [Colour figure can be viewed at wileyonlinelibrary.com]

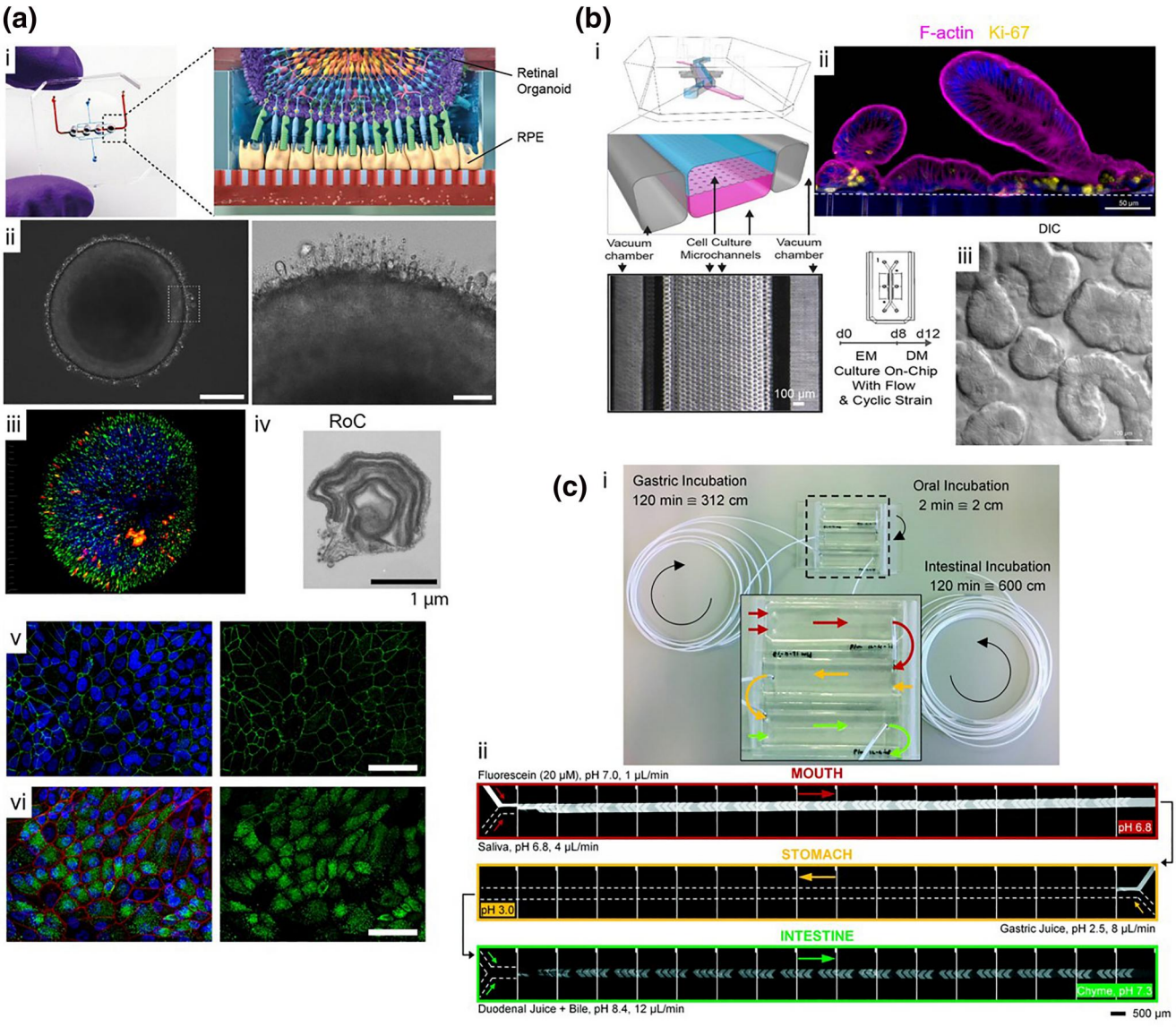

FIGURE 6 Mechanically active organoids-on-chip. (a) Retina-on-chip microdevice and the representation of retinal pigment epithelium. (ii) Phase-contrast microscopy of a day 180 retinal organoid and the inner and outer segment-like structure zoomed. Scale bar: 250 and 50 µm, respectively. (iii) Fluorescent microscopy of retinal organoids at day 180 (red, rhodopsin; green, ROM1, blue, DAPI). Scale bar: 20 µm (iv) Micrograph of a retinal organoid with organized morphology. Scale bar: 1 µm. (v, vi) Immunostaining for relevant markers of retinal pigment epithelium, at day 14 (green, ZO-1; blue, DAPI; red, ZO-1; Melanoma gp100 (green), respectively. To notice the organized morphology through the kinetic of culture. (Reprinted from Achberger et al. [2019]). (b) (i) Human intestine-on-chip geometry and fabrication (ii) Confocal immunofluorescence micrographs of human duodenal organoid-derived epithelial cells. (pink, F-actin; blue, DAPI-stained nuclei; yellow, Ki67). Scale bars: 50 µm. (iii) Human intestinal epithelium cultured on-chip for 12 days within a microfluidic device. To note the finger-like protrusions of the organoids. Scale bars: 50 and 100 µm, respectively. (Reprinted from Kasendra et al. [2018]). (c) Microdevices could act as a modular unit to mimic the human digestive system. (i) Representative image of the device. Red arrow: Represents the mouth. Yellow: Stomach. Green: Intestine. (ii) The three digestive modules in series (mouth, stomach, and intestine, respectively). A fluorescent dye named fluorescein—visible between pH 5–9—was used as a model. First, the fluorescein was mixed with artificial saliva (mimicking the mouth), after it was mixed with artificial gastric juice (mimicking the stomach), and then mixed with artificial duodenal juice and bile (mimicking the intestine). The fluorescein is not seen in low pH such as the stomach. It is only seen after the neutralization by gastric juice. (Reprinted from Haan et al. [2019]). All the figures were reprinted under the terms of the Creative Commons Attribution License (CC BY) [Colour figure can be viewed at wileyonlinelibrary.com]

correlated to precede inflammatory bowel disease (Stewart et al., 2018).

To closely resemble the small intestine's true nature, a novel approach combining mechanoresponsive microdevices and organoids was reported by Kasandra and co-workers. First, organoids composed of human epithelial cells were dissociated and cultured on a porous membrane within a microfluidic device and human intestinal microvascular endothelium cells were also cultured in a parallel microchannel. The mechanobiology of the intestine was emulated by constant luminal fluid flow and peristalsis-like cyclic deformations.

Using this approach, the researchers were able to reconstitute anatomical and functional characteristics seen in vivo such as 3D villi-like structures, cell differentiation, epithelial barrier function, enzymatic response, and mucus production. The transcriptome analysis also showed genes related to digestion, cell proliferation, and host defense response to infection. The reproduction of villi-like structures was crucial to reach the aforementioned results and, unlike other studies, it was formed by the cells naturally without scaffolding. The approach of dissociating the organoids and culturing them allowed a pre-conditioning of the cells for further inclusion of it within the device. Otherwise, if the organoids were cultured directly, these structures would be unable to form efficiently (Figure 6b) (Kasendra et al., 2018).

Intestine-on-chip has made great advances over the years (Firoozinezhad et al., 2019), and the next step is to complex these systems considering modular units to emulate the entire digestive system. Efforts in this area have been made (Haan et al., 2019). Haan et al. (2019) developed a bioinspired digestive system microdevice. It was composed of three micromixers connected in series to reproduce the digestive features of the mouth, stomach, and small intestine. Each chamber had a specific pH, buffer, mineral composition, and chemical clues. The system was able to continuously process nutrients and generated a constant flow of digested samples (Haan et al., 2019). Hence, it would be an interesting tool to assess the safety of orally administered compounds. Moreover, if used as a modular unit, it has the potential to improve the biological responses acquired from pre-existing devices, such as intestine-on-chip (Figure 6c).

To date, prescribed treatments for cancer and some rheumatological diseases are frequently based on the rate of success of a drug, without considering how a specific patient may respond to it (Falzone et al., 2018; Koźmiński et al., 2020). Although key mutations from the patient can be acquired from genetic profiling, the potential side-effects and the drug efficacy still remain unclear (Nguyen et al., 2015). Despite several in vitro models having been developed for drug screening, only a few are engineered with tissue-specific mechanical stimuli and targeting patients for precision medicine.

Mechanically active organotypic on-chip devices can narrow the gap in the trial and error process that occurs in many therapies. Within these devices, organoids derived from the patient can be analyzed in a physiologically relevant environment (Skardal et al., 2017). Thus, mechanically active devices resembling patients' specific tissues and conditions, as mentioned in the topics above, are prospective tools to optimize drug development pipeline and also to enhance drug screening predictability for precision medicine.

5 | REMAINING CHALLENGES: ARE WE CLOSE TO A PHYSIOLOGICALLY REPRESENTATIVE IN VITRO MODEL FOR CLINICAL DEPLOYMENT?

Mechanically active organoids-on-chip technology represents a versatile and predictive stepping stone as preclinical models. Nonetheless, to achieve practical clinical translation, several challenges such as (i) microfabrication process, (ii) cellular and culture medium-fidelity, (iii) sensing, (iv) data collecting, and (v) validation need to be overcome (Probst et al., 2018).

Although several models of organoids-on-chip have been proposed during recent years, most of them still lack scalability. The success in the construction of organotypic systems depends on their functional complexity. The microfabrication process should achieve the required scaling dimension (that will depend on the tissue/microenvironment that one is researching) without compromising the mechanical stimulus that is being imposed on the cells. Moreover, the microfabrication process should not have multiple and laborious assembly steps, favoring reproducibility and high production yield. Thereby, 3D printing technology may be a promising alternative to conventional microfabrication methods. This technology allows rapid manufacturing of complex microarchitectures with scale-up as well as customizable topographies at a submicron scale (Nouri-Goushki et al., 2019; Waheed et al., 2016).

Likewise, the formation of bubbles within microchannels is a common concern (Pereiro et al., 2019). These bubbles can impair the fluid flow and damage the cells at the liquid-gas interface. Hence, bubble traps should be added to the device. The surface chemistry of the material to compose the device should be carefully considered. Polydimethylsiloxane (PDMS) is widespread due to its several advantages (e.g., biocompatible, easy to handle, affordable, and optical transparency). Nonetheless, PDMS is gas permeable and can absorb small molecules, and may affect the performance of pharmaceuticals/toxicology studies (Meer et al., 2017). In this context, surface engineering techniques combined with substitute materials such as glass, thermoplastics, and other silicones have been explored (Meer et al., 2017).

The type of cells that will be used should also be suitable for large-scale production without the loss of its phenotype. Studies have been using primary and iPSCs rather than immortalized cell lines to better mimic the in vivo physiology (Ramme et al., 2019). However, the differentiation and maturation protocols of iPSCs organoids are still poorly standardized and laborious to reproduce (Matthys et al., 2020). In order to evaluate drug side effects as well as systemic metabolic cross-talk, a platform composed of multi differentiated organoids—such as liver, kidney, heart, lung—is necessary. Considering these integrated models, an optimal composition of culture media is required in order to maintain the different organoids viably without compromising their biological functions.

Additionally, to measure the responses acquired online, sensors that provide a reliable real-time readout on a cellular level are demanding. Microengineered organ-on-chips with miniaturized optics and sensors have been explored, in particular for tumor prognostic, which is a relevant area of organ-on-chip (Shamsipur et al., 2018). Nonetheless, considering body-on-chip platforms—with multiple representative organs—and multiple simultaneous real-time responses, it is essential the development and integration of more sophisticated sensors, able to capture all the complex biological phenomena. The human body is constantly under different biomechanical stimuli. To emulate these diversities, as well as to interpret

the output generated in one microfluidic device, still represents a technical challenge that is going to require even more effort from different fields of knowledge.

Finally, to achieve high throughput analysis with repeatability and robustness, several tests on a single chip should be able to be performed. Then, the large data generated needs to be collected, interpreted, and validated against current industry standards before organoid-on-chip can be translated as a valid method for the industry.

Mechanically active organoids-on-chip holds great promise to be a paradigm shift in several fields. However, in order to achieve practical clinical translation, these models still need to overcome some real challenges, ranging from standardization, scalability, bubble prevention, media composition, cell maintenance, and engineering. Likewise, more studies should be performed considering the allometric scaling as well as comparisons between in vitro models and in vivo systems should be carried out. It is imperative to have a profound understanding of the interaction of cells, their metabolism, and the environment to achieve rational applicability. In addition, coupling these platforms with machine learning and artificial intelligence can open a new scenario to simulate more complex biological phenomena. Accordingly, the platform should also be affordable and easy to handle for a broad scientific community.

Despite the prodigious efforts and results acquired from organotypic on-chip devices, its effective translation into the pharmaceutical industry still faces real challenges that need to be overcome before its implementation. Nonetheless, the continuous synergism of different fields of knowledge seen toward this goal is going to make organotypic on-chip devices an indispensable platform not only in the drug development pipeline but also to augment our understanding in several human conditions.

6 | CONCLUSION

Mechanotransduction regulates physiological and pathological conditions. Although substantial biological information has been acquired from traditional in vitro cell culture, there is a need to develop more reliable models that can mimic the biophysics of tissues and organs. The synergism between microfluidic devices and organoids is a promising strategy to achieve this goal. Mechanically active organoid-on-chip devices can mimic biomechanical forces such as shear stress, interstitial fluid flow, compression, and stretching. Hence, these platforms can surpass the technical and biological limitations found in traditional in vitro models by enabling a dynamic and predictive environment for precision medicine and drug development.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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