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Organ-on-a-Chip: A Preclinical Microfluidic Platform for the Progress of Nanomedicine

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Despite the progress achieved in nanomedicine during the last decade, the translation of new nanotechnology-based therapeutic systems into clinical applications has been slow, especially due to the lack of robust preclinical tissue culture platforms able to mimic the in vivo conditions found in the human body and to predict the performance and biotoxicity of the developed nanomaterials. Organ-on-a-chip (OoC) platforms are novel microfluidic tools that mimic complex human organ functions at the microscale level. These integrated microfluidic networks, with 3D tissue engineered models, have been shown high potential to reduce the discrepancies between the results derived from preclinical and clinical trials. However, there are many challenges that still need to be addressed, such as the integration of biosensor modules for long-time monitoring of different physicochemical and biochemical parameters. In this review, recent advances on OoC platforms, particularly on the preclinical validation of nanomaterials designed for cancer, as well as the current challenges and possible future directions for an end-use perspective are discussed.

1. Introduction

Nanomedicine has emerged in the last few decades as a field that can significantly impact the diagnose and therapy of human diseases.[1,2] Based on the outstanding properties that materials acquired at the nanoscale, such as high surface-to-volume ratio, high physicochemical stability, high charge carrier mobility and biocompatibility, a variety of nanoformulations have been developed to be applied in medicine by tailoring their size, shape, charge, and surface functional groups.[2,3] Based on those properties, the design of multifunctional nanoparticles (NPs) for nanomedicine is one of the most promising and exciting research areas that is expected to revolutionize the medical field in the next few decades.[4] Some of these multifunctional NPs have the potentiality to combine both diagnosis and therapy, the so-called theranostics, which is one of the ultimate goals of this field to achieve personalized and precise medical care (**Figure 1**). Among the therapeutic techniques, nanomaterials developed for drug delivery purpose have been widely investigated as smart drug nanocarriers capable to target tumor cells, protect drugs from degradation, enhance drug solubility, improve biodistribution, extend drug life cycle, and prevent lethal side-effects to healthy tissues and organs.[2,3] The design of these smart drug delivery systems can be engineered to target a specific location by taking advantages of the host environment, using for instance antibodies, aptamers or peptides; and then react autonomously as stimuli-responsive drug release agents, triggered by endogeneous chemical reactions (e.g., enzymes, pH, hydrolysis) or exogeneous stimuli-sensitive mechanisms (e.g., near infrared light, temperature raise induced by an alternating magnetic field, among others).[5] Comprehensive reviews on the topic of smart nano-based drug delivery systems can be found elsewhere.[5,6]

Further complex functionality is represented by smart theranostics, which hold high promise for the nanomedicine of the future. Next, recent representative examples from the research arena are described.

Cai et al.[7] make use of enzyme-responsiveness to design a cathepsin B-sensitive theranostic agent. They synthesized a biodegradable conjugate composed of a Gd chelate (Gd-DOTA) as a T1-magnetic resonance imaging (MRI) contrast agent,

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DOI: 10.1002/smll.202003517

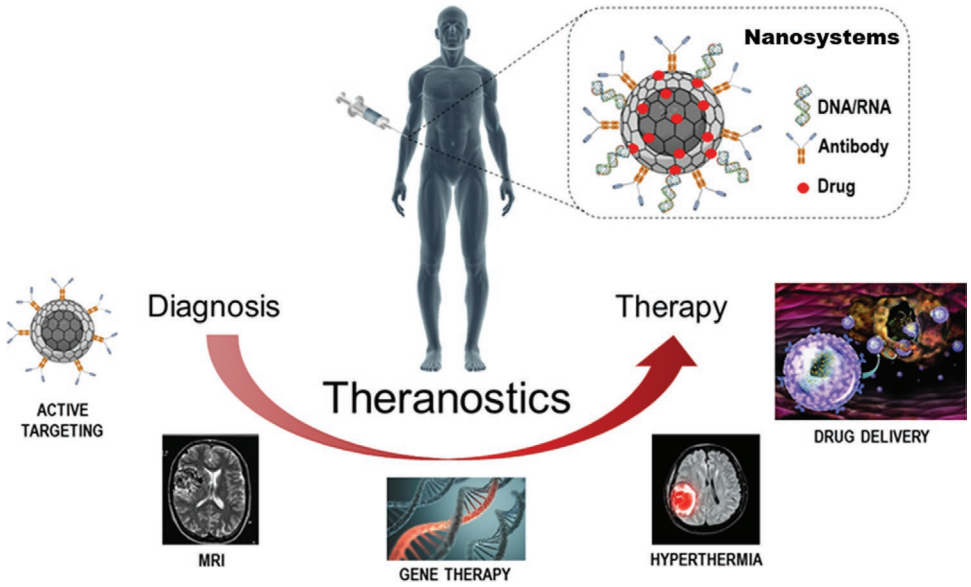

Figure 1. Schematic diagram of added value multifunctional nanoparticle that combines in the same nanosystem diagnosis and therapy (theranostics).

the chemotherapeutic drug paclitaxel (PTX), the fluorescent dye cyanine 5.5 (Cy5.5) and a cathepsin B-sensitive peptide. A pHPMA-DOTA-PTX copolymer was labeled with Cy5.5 and complexed with Gd^{3+}. The final conjugate self-assembles to form nanoparticles induced by hydrophobic interactions. Results showed a much higher PTX release (90%) in a rich cathepsin medium at low pH (5.4) compared to that in a medium without the lysosomal enzyme (20%). The imaging and therapeutic performance of the final nanostructure was validated in 4T1 breast carcinoma xenografts. In vivo MRI showed an enhanced T1 effect compared to Gd-DOTA after 24 h after injection, ascribed to the longer blood circulation time of the theranostic probes and their preferential accumulation at the tumor site. As the therapeutic index is concerned, the enhanced antitumoral properties of pHPMA-Gd-DOTA-PTX were evidenced by a tumor growth inhibition of 95%, whereas the free PTX administration led to only 17%. Suitable OoC devices would constitute a more realistic model for the validation of advanced responsive theranostic agents in their way toward the clinical translation, allowing for lower development costs and accelerating the time-to-market.

Recently, Wang et al.[8] designed a pH-responsive theranostic nanoprobe for targeted dual therapy (photothermal-PT, and photodynamic-PDT) of triple negative breast cancer. The nanosystem was composed of a core of superparamagnetic iron oxide nanoparticles and IR780 dye and a matrix shell made of stearic acid, polyethylenimine (PEI) and hexahydrophthalic anhydride (HHPA). This matrix responds to the lower pH of the tumor microenvironment (pH = 6.5) by breaking the amino bond that joins the PEI and HHPA, thus exposing the positively charged amino groups and promoting the electrostatic interaction with the cancer cells that results in an enhanced cell uptake of the nanosystems. The magnetic/dye cores and their pH-induced high accumulation into the tumor cells allow for enhanced magnetic resonance and near infrared imaging, respectively, whereas the therapeutic performance comes from the combined PT and PDT by light excitation. A significant reduction of the tumor volume in 4T1 mice models by one order of magnitude was observed in a 20 days window after laser excitation for 5 min compared to the same excitation after treatment with PBS. This highlights the interest of having OoC models able to mimic the tumor microenvironment for preclinical validation of advanced nanomedicines that enable cancer therapies based on other mechanisms of action different than those involved in chemotherapy.

As a proof-of-concept, Guldris et al.[9] orthogonally functionalized a magnetic core of iron oxide with several ligands for a multifunctional performance, namely targeting of cancer cells through specific recognition of biotin receptors, MRI and induced drug delivery under the remote application of a magnetic field. On the one hand, specific accumulation in cancer cells was observed by MRI thanks to a biotin moiety covalently attached to the nanoparticle surface. On the other hand, a temperature-responsive ligand bound to a fluorescent drug model through a temperature-sensitive Diels–Alder bond enabled the cleavage of the drug induced by magnetic hyperthermia. The medical translation of this type of innovative theranostic drug carriers would greatly benefit from the development of advanced OoC able to recapitulate, not only the endogenous tumor stimuli, but also to recreate functional behaviors after external stimulation.

Successful examples of tumor-targeting nanomedicines, designed as drug nanocarriers and already in use for the clinical treatment of breast cancer, are Doxil and Abraxane.[10] Doxil, a PEGylated liposomal nanoformulation containing the anticancer drug doxorubicin, was in 1995 the first FDA-approved nanodrug.[11] Doxorubicin is a chemotherapeutic drug used for a wide-range of cancer types, including breast cancer treatment; however, its cardiotoxicity is recognized as an adverse side effect.[10] The advance of nanotechnology and cancer research made possible to create nanodrugs, such as Doxil, which is able to provide intratumoral drug concentrations by enhanced permeability and retention (EPR) passive targeting effect that result

in tumor growth inhibition and improved survival rates. Additionally, this nanodrug leads to a major reduction of the typical cardiotoxicity of doxorubicin, threefold lower, when compared with free doxorubicin (standard chemotherapy).[10] Abraxane is another well-known drug-delivery nanoformulation composed of an albumin nanoparticle, bound noncovalently to the anticancer drug paclitaxel. Similarly, Abraxane is able to improve accumulation of paclitaxel at the tumor site (33% higher than its free version) and avoid typical solvent/surfactant-related adverse side effects that appear when it is administered as a free drug.[10] Other examples of US FDA-approved nanotechnology-based products are Eligard, a leuprolide acetate (a testosterone inhibiting drug) associated to a polymer nanoparticle (PLGA, a degradable polymer), which allows controlled delivery of payload with longer circulation time for the treatment of prostate cancer. This nanoformulation respond after being injected in a solid implant, where the polymers precipitate and slowly release the leuprolide acetate by hydrolysis.[12] Ontak was the first active targeting nanomedicine being approved by FDA. In this case an engineered protein combining IL-2 receptor antagonist and diphtheria toxin, designed to treat the aggressive form of non-Hodgkin's peripheral T-cell lymphomas, by targeting T-cells that overexpress the IL-2 receptor. This nanoformulation when combined with CHOP (the first-line drug for the disease) improves the survival rate to 63.3% in comparison with 32–35% when CHOP is applied alone.[12] These are just few examples that highlight the potential that nanomedicine-based drugs have in the advance of medicine, and their advantages in comparison with the standard treatments. Moreover, the perspective to combine in the same nanocarrier several therapeutic approaches, such as chemotherapy, hyperthermia,[3,13–15] and/or gene therapy,[16–18] with imaging technologies, such as MRI,[19,20] creates added value to the final nanosystem. These unique multifunctional nanosystems are expected to be the next big revolution in modern medicine.

Although the progress and potentiality of nanomedicine, the rapid bench-side developments have not been translated neither to commercial or clinical applications. In fact, just a few nanoformulations as those abovementioned have been approved by official health agencies, such as FDA or European Medicine Agency.[1,2] The main problem of this slow pace from the in vitro proof-of-concept to clinical application is attributed to the lack of robust preclinical platforms able to mimic and predict with high accuracy the in vivo conditions found in the human body.[1] This is especially relevant to determine in the early stages of the development of a formulation, where it needs to meet the requirements to be accepted for phase I of clinical trials, which will avoid costly and long-term screening procedures. Currently, preclinical studies are mainly based on 2D cell culture and animal models.[21] Although there is some merit in these studies, none of these models are able to accurately predict the effect of the nanoformulations within the human body, including their degradation and clearance in circulation, half-life cycle, and toxicity caused by immune response stimulation.[17] As an alternative to these models, microfluidic devices have been explored as useful tools capable to give new insights over the chemical, physical, and biological response of cells.[22] Recently, a new class of microfluidic devices, called organ-on-a-chip (OoC), has emerged alongside with the development of tissue engineering with the purpose to fulfil the limitation of animal studies in predicting clinical outcomes.[23] Hence, the purpose of this review is to provide a comprehensive perspective on OoC platforms designed for nanomedicine with the potentiality to accelerate their clinical application, particularly those that are being developed for cancer theranostics. Wherein, recent advances on OoC platforms for the validation of multifunctional drug nanocarriers, as well as the current challenges and future directions for an end-use perspective, including the importance of the integration of biosensor modules for long-time monitoring and automation of these advanced microfluidic devices, will be discussed. First, it is outlined the importance of the design and physicochemical properties of NPs for nanomedicine and the limitations of the standard methodologies, as testing models for their screening. Then, it is described the main applications of advanced microfluidic devices as innovative alternatives and more reliable platforms to mimic the physiological conditions of a human body. For that, it starts by reviewing the importance of single-cell microfluidic devices as the first screening studies devoted to NPs, their evolution to single OoC devices and lastly, the potential of multiorgan/human-on-a-chip for nanomedicine. To conclude this review, the importance of sensors and their current challenges is also discussed. This last topic is a vital requirement for a long-term monitoring of OoC as a standard preclinical screening platform for nanomedicine and clinical research.

2. Design and Properties of Nanoparticles for Medicine

By definition, a NP is a nanomaterial or nanocomposite with dimension between 1 and 100 nm,[24] cf. **Figure 2**.

The advance of nanotechnology has allowed the development of a variety of nanomaterials that includes, gold and silver,[25,26] liposomes,[27,28] carbon-based NPs (fullerenes, nanotubes, quantum dots, graphene),[13,29,30] iron oxides,[31,32] silica,[33,34] polymeric micelles,[35] among others. At reduced sizes, these nanomaterials acquire special properties that at macroscale do not possess. One of the most extraordinary examples of this phenomenon is the superparamagnetism that magnetic material, such as iron oxides, exhibits at nanosized scale. Superparamagnetism takes place in ferromagnetic or ferrimagnetic NPs that present a single magnetic domain with sizes generally below 100 nm. Under an external alternating magnetic field, the magnetic moment of these magnetic NPs is quickly reoriented, leading to a loss of energy that heats the surrounding environment. This phenomenon is called as magnetic hyperthermia and can be used to locally destroy tumoral cells. After the magnetic field is removed, the particles tend to be re-dispersed and act like a non-magnetic material.[36] Due to this fact, magnetic nanoparticles (MNPs) have received much attention in the last decades as ideal candidates for the treatment of cancer with the potential to be also used as contrast agents in MRI, fulfilling the theranostic application. Among different magnetic nanomaterials, magnetite (Fe_3O_4) was the first magnetic nanomaterial used to obtain a magnetic fluid in 1960 by NASA.[37] Afterward, other ferrites, such as MFe_2O_4 (M = Fe,

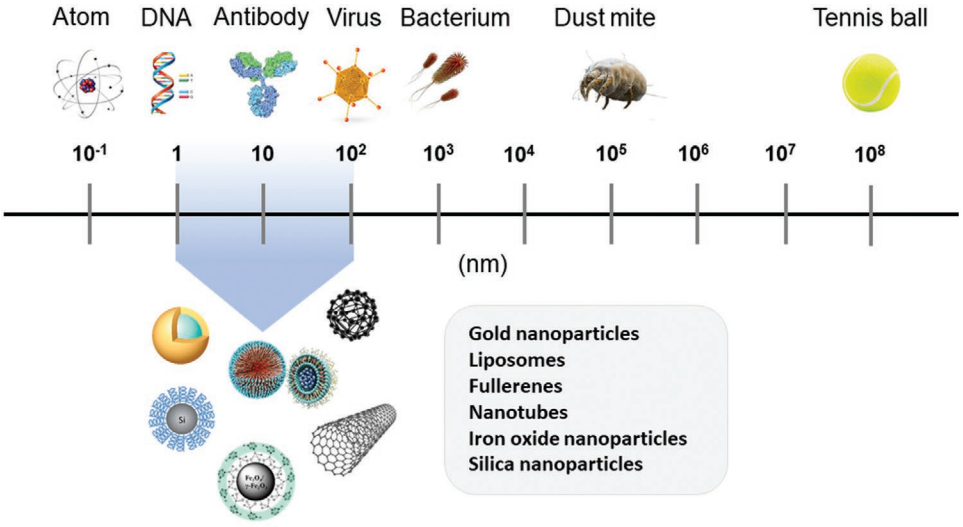

Figure 2. Nanometer scale comparison of nanoparticles with other life-structures and materials.

Mn, Ni, Co, Zn), some alloys as FePt, NiPt, and NiPd, and pure metals (Fe, Co, Ni), were synthesized and tested especially for hyperthermia application.[37–40] However, bare MNPs present several limitations. For instance, they have inherent tendency to aggregate and precipitate when introduced inside blood vessels, showing low colloidal stability, and low biocompatibility.[41] To achieve a safer application of these nanomaterials in medicine, it is highly recommended that MNPs are covered by an organic or inorganic biocompatible coating. Alternatively, bio-inspired nanomaterials, such as liposomes, lipid NPs, protein NPs, and others, are gaining increasing attention for cancer therapy, due to their intrinsic biocompatibility and biodegradability.[42] Nevertheless, these nanoformulations in general present lower versatility for smart designs and poor drug delivery efficiency when compared to MNPs or other NPs, such as carbon-based ones. To suppress this limitation, bio-inspired nanomaterials are being combined with inorganic nanoparticles, such as gold, silver, silica, iron, or zinc oxides, achieving a wider range of biomedical applications, including cancer theranostics.[42]

These evidences show the importance of physicochemical properties of nanomaterials. It should be noted that these properties are highly dependent on the selected strategy of synthesis and functionalization of the nanomaterials, which determine their shape, particle size and distribution, surface charge, and biocompatibility. Generally, these nanoformulations are well described in literature and characterized in terms of their physicochemical and surface chemistry. However, the lack of representative preclinical screening assays limits the clinical translation of these nanomaterials. As shown in **Figure 3**, a metadata analysis made in Scopus database with the searched keywords "nanoparticles + nanomedicine," between 2002 (year of the first work reported in literature) and 2019, shows a total sum in this period of 12.137 published papers and 3.445 patents.

In spite of this increasing research effort during the last decade, just a few nanoformulations were able to reach the clinical application and commercial purpose.[24,43] Particularly, in May 2020, 98 clinical studies with the keywords "nanoparticles + cancer" were listed as "active" or "recruiting" on *clinicaltrials.com*. Although the numbers of nanoformulations approved by FDA have increased in the recent years, which shows the clinical relevance of these nanomaterials for medicine, their clinical translation remains residual.[44] This is a clear indicator of the need to develop advanced preclinical platforms that enable the screening of important biotoxicological criteria, namely: (1) biostability and biocompatibility, (2) specificity to target specific cells, and (3) ability to enter diseased tissues and remain inside them.[24]

3. Limitations of Current Gold Standard Methodologies for Nanomedicine Screening

As previously mentioned, the standard toxicological screening of nanomaterials is based on two main models, the 2D cell culture platform and animal models. The main limitation of the in vitro 2D cellular models is their inability to mimic the complex 3D in vivo microenvironment, wherein the cells and extracellular matrix (ECM) exist in well-organized architectures.[45] Alternatively, small animals (e.g., mice, rats, rabbits, among others), beside the ethical concern that rises from the use of animals, show to be inefficient to predict human response due to the genomic interspecies differences.[46] Also, this methodology is extremely time-consuming and expensive, with poor imaging resolution for the whole-animal, making the visualization of the target-tissues limited.[1] Due to these restrictions, animal models are typically complemented with in vitro assays, such as the 2D cell cultures tests. In these static in vitro models, cellular uptake and biocompatibility of imaging and therapeutic agents are assessed by applying directly the nanoformulations mixed with culture media on the cell monolayers. Looking for better models, an effort has been made to develop 3D models of tissues and organs that better replicate their in vivo functions. The progress of biotechnology, tissue engineering, and bioprinting technology has boosted the development of new 3D models, such as multicellular spheroids. These 3D biomodels are induced to grow in spontaneous aggregation of cells that

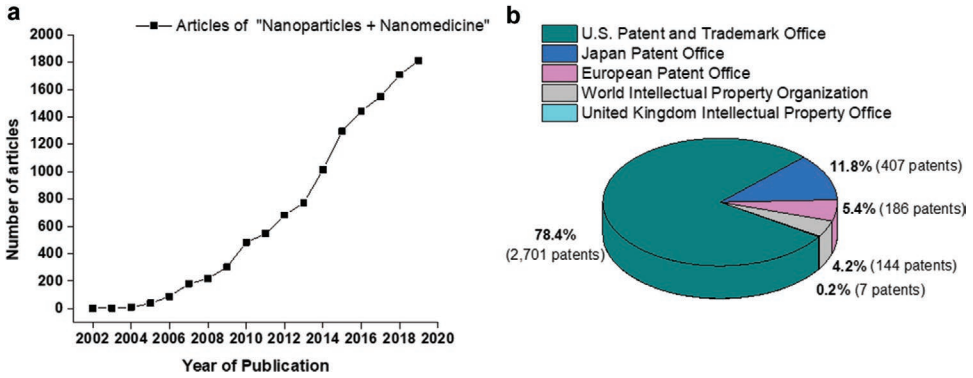

Figure 3. Metadata analysis of the keywords "nanoparticles + nanomedicine" between 2002 and 2019. a) Number of publications per year, summing 12.137 in the reported period; b) Total number and percentage of patents. Source: Scopus (www.scopus.com).

are held by ECM secreted by residing cells,[1] and can better replicate the in vivo environment found in the human body.

An example of a human 3D microtissue system developed as a valuable tool for cost and time-efficient screening of nanomaterials was reported by Kabadi and co-workers (2019).[47] In this work, it is described the construction of human lung microtissues comprised of lung epithelial cells and fibroblasts, co-cultured with macrophages. This model was used to evaluate the toxicity of multi-walled carbon nanotubes (MWCNTs) that have been identified as a toxic nanomaterial that activates the release of inflammatory and pro-fibrotic mediators, related to several changes observed in the lungs of the exposed animals. The results have shown the advantage of this 3D microtissue system as a toxicity testing platform, by bridging the technological gap between gold standard in vitro and in vivo methodologies, allowing the display of morphological and molecular signatures of fibrosis in just four days, compared to the weeks or months that lung fibrosis usually takes to be detected in animal models.[47] With proven advantages over the gold standard methodologies, such as the possibility of long-time usage and recapitulation of the physiological microenvironment found in the human body, 3D microphysiological systems have been increasingly gaining the attention for both pharmaceutical studies and regenerative medicine. Indeed, this field represents a big opportunity for pharmaceutical companies to significantly reduce the high cost of drug development. It is estimated that the development of a new drug takes an average of 12 years from start-to-market, with a minimally cost of 1 billion euros/drug.[48,49] The non-efficient drug development pipeline causes a high rate of drug fails, with special impact during the clinical trial stages, which costs almost two-thirds of the drug development budget.[50]

In the last decade, the efficient fabrication of uniform and reproducible 3D models of organs and representative tissues has been emerged and reported. Among these models, tumoral organoids,[51–53] cardiac organoids,[54,55] vascular organoids,[56] and liver organoids [57–59] are the most representative organoid models. Organoids are small, self-organized 3D tissue cultures that in general derived from stem cells. This in vitro tissue construction mimics the corresponding in vivo organ, to generate physiological and relevant aspects of the complex organ architecture, namely nutrients, gas, and morphogen gradients that are transferred through diffusion.[60] However, these static 3D models are unable to be maintained in culture for long periods of time. Taking advantage of the well-established microfluidic and microfabrication technologies, a new advanced microfluidic device emerged to support these biomodels, which allowed their long viability and study, and naming these new advanced preclinical platforms as "organ-on-a-chip." Thus, OoC can be described as engineered 3D tissue models, or in some cases 2D culture cells, combined with a microfluidic system (microdevice), to simulate the mechanics and physiology of entire organs.[61] The microfluidic channels provide a system that mimics the cardiovascular structure for nutrient and gas feed, as well as for waste disposal, by applying similar mass transport mechanics encountered in in vivo conditions.

4. Organ-on-a-Chip as Potential Solution to Address Unmet Need in Nanomedicine

More than a decade ago, Beebe and co-workers[62] stated that, "microfluidics has the potential to significantly change the way modern biology is performed." This optimism around microfluidic systems was supported by several remarkable advantages that this microscale tool has over traditional assays used in cell biology, such as portability, cost-effective, low reagents and samples need, long-term monitoring, and a better representation of the physiological and pathological conditions of complex biological systems.[63,64] In addition to this biomimetic capability, microfluidics allows the possibility to integrate micro(bio)transducers, automation systems, and cell culture. The versatility of microfluidic devices promotes the investigation of a variety of biological systems from single-cell biophysical characterization to miniaturization of an entire laboratory onto a single chip (i.e., lab-on-a-chip, LOC),[65] and more recently, the recapitulation of the organ physiological parameters into a chip (i.e., OoC).[21,66] **Table 1** summarizes the main advantages, challenges, perspectives, and potential impact of the use of microfluidics for screening developed NPs in the advance of nanomedicine.

In this context, OoC has emerged as a LOC platform combined with tissue culture techniques that are able to generate fluid shear stress, biochemical concentration gradient, and

Table 1. Main screening applications of microfluidic devices, advantages, challenges, perspectives, and potential impact of the use of nanoparticles (NPs) in the advance of nanomedicine. Adapted under the terms and conditions of the CC BY license.[22] Copyright 2019, The Authors. Published by MPDI.

Screening applications of microfluidic devices	Advantages	Challenges	Perspectives	Potential impact
• Hemo- and biocompatibility	• Biological conditions similar with in vivo microenvironment	• Contamination issues	• Single-cell microfluidic device	• High-throughput assays regarding toxicity, efficacy, targeting and organ distribution of NP
• Toxicity	• Reduce the sedimentation effect of static cultures by flow control	• Demands complex operation for the integration of screening modules	• Blood-vessels-on-a-chip platform	• Rapid characterization and optimization of NP in human organ models
• Uptake of NPs by cells	• Low reagent and cell need for long-time monitoring	• Expensive fabrication of the microfluidic platform	• Organ-on-a-chip platform	• Real-time tracking of NPs distribution in organ models
• Accumulation	• Screening of large number of NPs at different concentrations	• Lack of standard methods to translate data from the lab-scale to organisms	• Tumor-on-a-chip platforms	• Evaluation of chemotherapeutic drugs efficacy carried by NPs
• Target studies	• Suitable for test personalized medicine	• Difficult to determine biodistribution and pharmacokinetics	• Multiorgan-on-a-chip platforms	• Improvement between in vivo-in vitro correlation
Treatment efficacy	• Suitable for real-time imaging and transport studies		• Screening system standardization	• Advanced platform for personalized medicine in diagnosis and treatment
• Contrast agent enhancement	• Low number of cells and NPs sampling is required			
• Transport	• Possibility to integrate monitoring modules			

other physical stimuli with the aim to recapitulate human physiology at low cost and high reproducibility.[67] Additionally, this novel approach overcomes the ethical concern regarding the use of animals for human testing products, which is in line with the 3Rs animal principle, i.e., reduce, refine, and replace animal testing.[68]

Generally, OoC is composed with four key components, namely (i) microfluidics, (ii) cell tissues/organs, (iii) stimulation, and (iv) sensor systems.[69] The ability to integrate in the same platform all these components makes OoC a unique platform for the screening of nanomedicines. Particularly, these advanced microfluidic devices allow to embed in perfusion chambers, 3D culture cells (single cell lines or co-cultured cell lines), which are generally developed by the incorporation of a biocompatible material, such as hydrogel, so the in vivo ECM can be recreated. Also, the microfluidic system is able to mimic a microvasculature system that connects and feeds organ models, allowing to model and study nanodrugs administration, extravasation from vasculature and targeting tissue under study. Another advantage of OoC is the fact that these systems allow to control and monitor the external and internal cell environments, i.e., physiological parameters such as fluid shear force, concentration gradient, dynamic mechanical stress, and cell patterning can be accurately simulated to fully reflect the in vivo processes.[69] Because the advance of nanomaterials for biomedical applications is inherently linked with their ability to cross the vascular system and targeted pathological tissue, OoC is an ideal preclinical system to improve nanomedicine designs and create new strategies for more effective therapeutic outcomes.

Regarding microfabrication, OoC adopts in general the same techniques, e.g., photolithography and soft-lithography, and structure principles as LOC and other basic microfluidic cell culture devices, for the fabrication of the microfluidic chip.[70] However, as mentioned before, OoC goes beyond the basic microfluidic cell culture devices, where single cells or cell monolayers are inserted in the microfluidic chip. For this reason, in OoC, the microfabrication requires further processes to implement other key-elements, such as biomodels, sensors, and stimulus loading components to mature the tissues and organs.[71] These additional steps make the fabrication of OoC expensive and time-consuming.[71] Recently, other strategies, such as bioprinting, are being used and improved for OoC fabrication. Bioprinting allows a layer-by-layer printing and thus, is capable to print different materials and build 3D complex constructs in a rapid and customized manner. Comprehensive revisions on this topic can be consulted elsewhere.[70–72] Usually, the most common material used for the fabrication of the microfluidic device is polydimethylsiloxane (PDMS). PDMS is a widely used polymer for microfluidic chips fabrication due to its biocompatibility, highly gas permeability, transparency, and flexibility. However, PDMS presents a serious limitation, since it has a strong interaction with small hydrophobic molecules that are absorbed by the polymer. This drawback is especially serious for drug

studies. Thus, other materials, such as fluoroelastomer, are being proposed to advance OoC as platforms for more reliable pharmacological studies.[73]

Another important aspect for the advance of OoC is the design of suitable biomodels. Since the pioneering work, in 2010, of the research group led by Donald Ingber,[74] where the concept of OoC was introduced with the development of a lung-on-a-chip, several other organ models have been described in the literature, showing the potential of these advanced microfluidic devices for diagnosis and treatment of human diseases. For that, a variety of human organs have been developed to mimic different human physiological conditions and organs, such as bone,[75] brain,[76] eye,[77–79] heart,[80] liver,[57,81,82] lung,[83] skin,[84] vasculature system,[85–88] among others. Despite the advantage that single OoC can have to test and predict a specific organ response in a triggered microenvironment, many biomedical applications, such as pharmacology tests, still need further development of multiorgan-on-a-chip systems to recapitulate the complexity, functionality, and interconnection of different organs and tissues.[68] Alongside with the usual drug screening application, OoC platforms can play an important role in the development of nanomaterials for nanomedicine,[45] once these tools can replicate the in vivo human tissue-level structures and their highly complex 3D environments, in a faster and robust manner.

At the end of this section, an overview over advanced microfluidic devices developed for the evaluation of NPs will be summarized with special focus on the study of its transport, uptake, toxicity, accumulation, and drug efficacy or their potentiality to be used in diseases research studies. In a timeline perspective, the following sub-sections describe the evolution of advanced microfluidic platforms to perform research in nanomedicine, where the first studies started with the incorporation of single cells to understand the basis of NP–cell interactions and blood vessel models to study the NPs' transport phenomena, and then the integration of single-organ models and more recently, multiorgan models to reflect the physiological complexity found in vivo.

4.1. Single Cells in Microfluidic Devices

Biophysical phenomena of single cells have been widely studied by means of microfluidic devices.[89–91] Among these studies, the blood flow behavior in microcirculation has been extensively investigated. Red blood cells (RBCs), the most abundant blood cells, are subjected to large external flow forces and their inherent deformability can be used as biomarker to determine many RBCs-related diseases (e.g., malaria, diabetes, sickle cell disease, leukemias, among others).[92] Envisioning more insights over the complex NP–RBCs membrane interaction, a microfluidic extensional approach was proposed by Rodrigues and co-workers,[90] as an indicator of hematological disorders caused by MNPs in comparison with a conventional hematological test (cf. **Figure 4**a). This new methodology has shown the ability to detect small increments in the rigidity of RBCs in contact with MNPs, corroborating the numerical study performed by Curtis and his colleagues,[93] and which pointed out the uptake of surrounding MNPs by the RBC membranes as the main reason for the increasing rigidity observed in these blood cells. More recently, a novel microfluidic drop chip combined with time-resolved inductively coupled plasma mass spectrometry (ICP-MS) was proposed to determine zinc in single HepG2 cells, via a microflow nebulizer.[94] This approach shows the ability to quantify zinc and ZnO NPs uptake/adsorption in single HepG2 cells, exhibiting potential to monitor the content and distribution of trace elements/NPs in a single cell (cf. Figure 4b). Both studies have shown the aptitude of microfluidic devices to screen and gain new insights on NPs–cell interactions in a precise and simple manner that standard methodologies cannot do.

Additionally, studies like the ones described in this subsection, due to their simplicity and mimicking controlled physiological conditions, are a good starting step to understand the complex biological impact of NPs developed for clinical applications. By understanding NPs–cell interactions phenomena, such as NPs hemocompatibility, transport, uptake, toxicity, and targeted accumulation in single cells, extrapolations in more

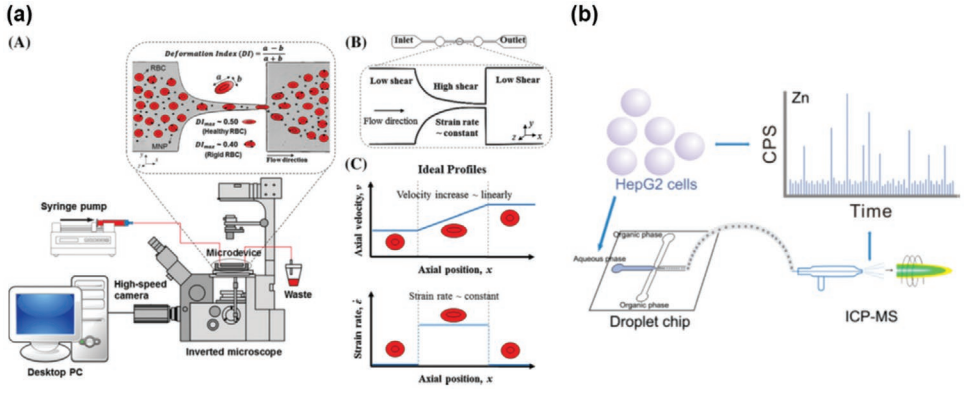

Figure 4. Microfluidic devices for single cell analysis: a) Hemocompatibility of red blood cells in contact with MNPs through deformation index assessment, A) schematic view of microfluidic setup, B) Microchannel device geometry with the zoom of the hyperbolic channel, C) graphical representation of the fluid-induced condition profiles that occur in the hyperbolic channel. Reproduced with permission.[90] Copyright 2016, Springer Nature. b) Microfluidic drop chip combined with ICPMS system for determination of zinc in single cell. Reproduced with permission.[94] Copyright 2017, ACS Publications.

complex, and representative human models, such as OoC, can be easily accomplished.

4.2. Blood Vessels-on-a-Chip

As aforementioned, vascular platforms are important models for the study of NPs interactions in the blood stream, namely their vascular transport and hemotoxicity. Additionally, these blood vessel models are critical platforms to understand the mechanism of delivering nutrients, removing metabolic products and drug transport via the circulatory system.[1,66] Among the vascular structures, straight channels,[86] bifurcations,[87] or a mixture of more complex structures[88] have been investigated. The formation of these vascular models can be achieved by the seeding of endothelial cells onto a mold structure created via injection molding, 3D printing techniques, sacrificial networks, or even by embedding cells directly into hydrogel structures.[66] Generally, nanoformulations designed for medicine are developed to be injected directly into the bloodstream. It is known that the physical properties of NPs, such as size and shape, are directly related with their capacity to escape from the reticular endoplasmatic system (RES) and successfully reach their target. Although the importance that hemodynamics (blood shear rate and vessel size) and hemorheology (blood hematocrit) studies have for the optimization of nanoformulations, there is a lack of these kinds of studies. This is mainly because the current gold standard in vivo models are too complex to give comprehensive understanding of these phenomena and the basic microfluidic studies are too simplistic to give conclusive results. Thus, blood vessels-on-a-chip, which combine the simplicity of microfluidics with the integration of endothelial tissue and blood cells, can be an optimal platform for a first stage evaluation of NPs designed for biomedical applications. Indeed, published works of Namdee and co-workers,[95] have shown that these physical properties can influence their accumulation inside the microchannels/microvascular structures, where microparticles tend to show higher marginal accumulation than NPs. Other studies have shown the influence of the NP shape in the specific targeting and accumulation, e.g., rod-shaped NPs showed higher specific targeting compared with sphere-shaped ones.[96] Recently, a vascular OoC was developed by using patient blood-derived cells, which is also known as blood outgrowth endothelial cells (BOECs), cf. **Figure 5**. In this study, a vascular-on-a-chip was used to model vascular pathologies with the potential to serve as a preclinical tool for personalized assessment and drug discovery.[97] In another study, a human blood vessel organoid, created from stem cells, was generated as a model of diabetic vasculopathy and successfully transplanted into a mice.[56] This work describes a vessel organoid containing endothelial cells and pericytes that self-assemble into capillary networks enveloped by a basement membrane. This vessel organoid model can represent a new strategy for drug development and drug nanocarrier studies, for diseases like cancer. Overall, vessels-on-a-chip represents an important model to study: (i) system dysfunctions caused by specific diseases (e.g., diabetes, tumors, etc.), (ii) effect of inflammation on vascular integrity (e.g., thrombosis), (iii) NPs interaction with blood components, (iv) drug delivery systems, as well as to (v) connect with other organ models for body-on-a-chip applications. Due to these advantages, these models can be a desirable preclinical platform for the translation of theranostic NPs to clinical applications.

4.3. Single Organ-on-a-Chip

The main goal of an OoC platform is to recapitulate the physiology of the organ system to be studied, with potential to be applied in nanomedicine discovery and development. In this way, the maintenance of simplicity with sufficient biological fidelity of the tissue/organ models is the fundamental basis of

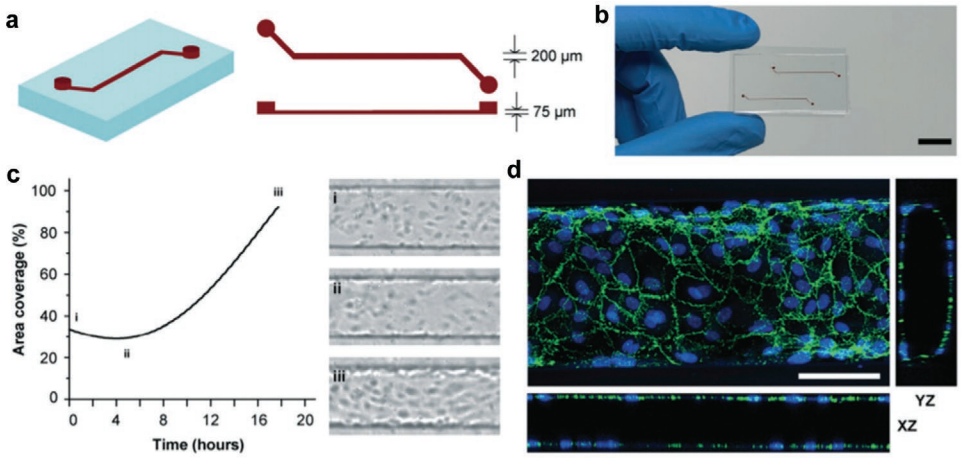

Figure 5. Vessel-chips lined with blood outgrowth endothelial cells (BOECs): a) Representation of the vessel-chip with an inlet, a 200 µm wide and 75 µm high straight duct. b) Photographic representation of the vessel-chip made of polydimethylsiloxane (PDMS) containing two independent microchannels on a collagen-coated glass slide (scale bar: 10 mm). c) Quantification of BOEC growth and spreading in microchannels with time (left); snapshots (right) show BOEC coverage at (i) seeding, (ii) initial attachment, and (iii) confluence (scale bar: 100 µm). d) Confocal micrograph showing a section of the endothelial lumen formed by BOECs in the microchannels (green: VE-cadherin; blue: nuclei; scale bar: 100 µm). Reproduced with permission.[97] Copyright 2019, Royal Society of Chemistry.

tissue engineering.[66] To accomplish this goal, the field of biomimetic OoC has quickly expanded with the replication of several organs including, liver, kidney, heart, gut, breast, and lungs. Some of the most representative models for nanomedicine and theranostic studies are briefly discussed in this section.

4.3.1. Liver-on-a-Chip

Liver is the largest metabolic and detoxifying human organ responsible for drug metabolism, plasma protein synthesis, and glycogen storage.[59,66] As a result, liver is susceptible to suffer from abnormal metabolism, resulting in accumulation of toxic substances and liver diseases. This is the main reason for drug withdrawal, due to hepatotoxicity that is caused by unsafe drugs. Therefore, there is a huge demand for robust in vitro liver models to assess drug metabolism and test drug hepatotoxicity.[57,98] In nanomedicine, these models can have a dramatic impact in the clinical translation of NPs developed as drug delivery systems, where animal models often fail in predict the drug outcomes in humans.[1] So far, several liver-on-a-chip platforms have been developed, including 2D/3D mono- and co-cultures with healthy and/or diseased cells. Among the microfabrication techniques to develop biomodels, 3D hepatic spheroids have been receiving great attention for drug testing.[57,58,82] Ma and co-workers have demonstrated the functionality of the hepatic spheroids in a novel platform with a significant improvement compared to conventional perfusion methods, i.e., hepatic polarity, liver-specific functions, and metabolic activity.[82] Bhise et al. have reported a liver-on-a-chip platform with cultured spheroids using a 3D-bioprinting technique that remained viable and active during the 30 days of culture period.[57] With this platform, the cellular response to acute acetaminophen (APAP) was able to predict similar drug toxicity when compared with in vivo conditions. In another study, a new concept to recapitulate a multiscale organotypic scaffold-free structure with feasible physiological structural hierarchy, complex drug clearance, and zonal physiology from cell to tissues was demonstrated for long-term culture liver-on-a-chip.[99] In this study, micro-engineering techniques to control the assembly of primary liver cells (PLCs) into an organotypic hierarchy was used. The strategy consisted in the deposition of PLCs to form a biological growing template on collagen-coated PDMS membrane and enclosed to form a culture chamber, providing a vertical cell anchorage with a hydrophilic flow diverter, as shown in **Figure 6**a. However, due to the limited lifetime and availability of primary cell lines, future of liver-on-a-chip platforms is closely related to the development of models using induced pluripotent stem cells (iPSC)-derived hepatocytes. These models have the advantage to perform personalized studies of drug treatment and toxicity on patients with different backgrounds.[66] Therefore, those liver-based OoCs represent one of the most important organ models for the theranostic screening in nanomedicine studies.

4.3.2. Kidney-on-a-Chip

Kidneys are vital organs responsible for the filtration of blood from waste products (i.e., food, medication, and toxic substances), with regulation of fluid balance and minerals.[66] Thus, this organ can be one of the most representatives for drug screening toxicity and NPs clearance studies, especially when administered intravenously.[1] Among the standard preclinical kidney's models, animal models have the disadvantage of species differences in blood flow, transporter expression, and plasma protein binding that cannot be extrapolated to humans. An alternative is ex vivo models, but they are limited for their short viability, just allowing studies for few hours after isolation/preparation.[100] Therefore, representative 3D models, such as kidney-on-a-chip, have a great potential to accelerate drug development and predict the toxicity and clearance effect of new NPs developed for biomedical applications. Presently, kidney-on-a-chip systems have been microfabricated by embedding or seeding renal cells on the interface of ECM or membranes surrounded by perfusable microchannels that provide nutrients, waste clearance, and stimulated flow.[101–103] Recently, a tubule-kidney-on-a-chip was created[104] for drug transport and nephrotoxicity assessment (cf. Figure 6b). This representative kidney model was developed to replicate renal conditions, i.e., fluid flow and shear stress, with luminal and tubular chambers, and designed in a PDMS platform separated by a porous membrane. Nevertheless, the future for more reliable kidney models demands the construction of multicompartmental 3D tubular structure of perfused human renal cells interacting with each other, which due to its complexity, still remains a challenge.[103,105] At the present moment, kidney OoC is being used essentially for drug studies. However, the ability of these 3D models to improve our understanding of the clearance phenomenon of NPs in the human body, put these models as one of the most representative for the clinical translation of nanomedicines.

4.3.3. Heart-on-a-Chip

Cardiovascular diseases are the leading cause of death in the developed countries, presenting high prevalence worldwide.[106] Additionally, cardiovascular toxicity represents the main issue for phase I drug failures.[107] Since nanomaterials can be designed as smart drug delivery systems that can avoid the interaction with healthy organs, heart has been a relevant model for nanomedicine development. Similar to other human organs, animal models are inefficient to mimic and accurately represent the outcomes and drugs effects on the human heart. Among the interspecies differences, animals have different heart rates compared to humans. Heart-on-a-chip platforms, based on induced iPSC-derived cardiomyocytes, can suppress this animal limitation and be used for personalized patient drug tests.[66] By using microfluidic OoC models, recent advances have been made in engineering the relevant physiological features of myocardial tissues at the microscale level. A technique developed by Annabi and co-workers, which consisted in seeded cardiomyocytes into methacrylated tropoelastin (MeTro) hydrogel, has revealed an improvement on the cell attachment, proliferation, and beating rate in comparison to gelatin methacryloyl (GelMA).[108] Nevertheless, a model that is able to fully recapitulate human in vivo dynamics has to consider the integration of the heart and microvasculature.

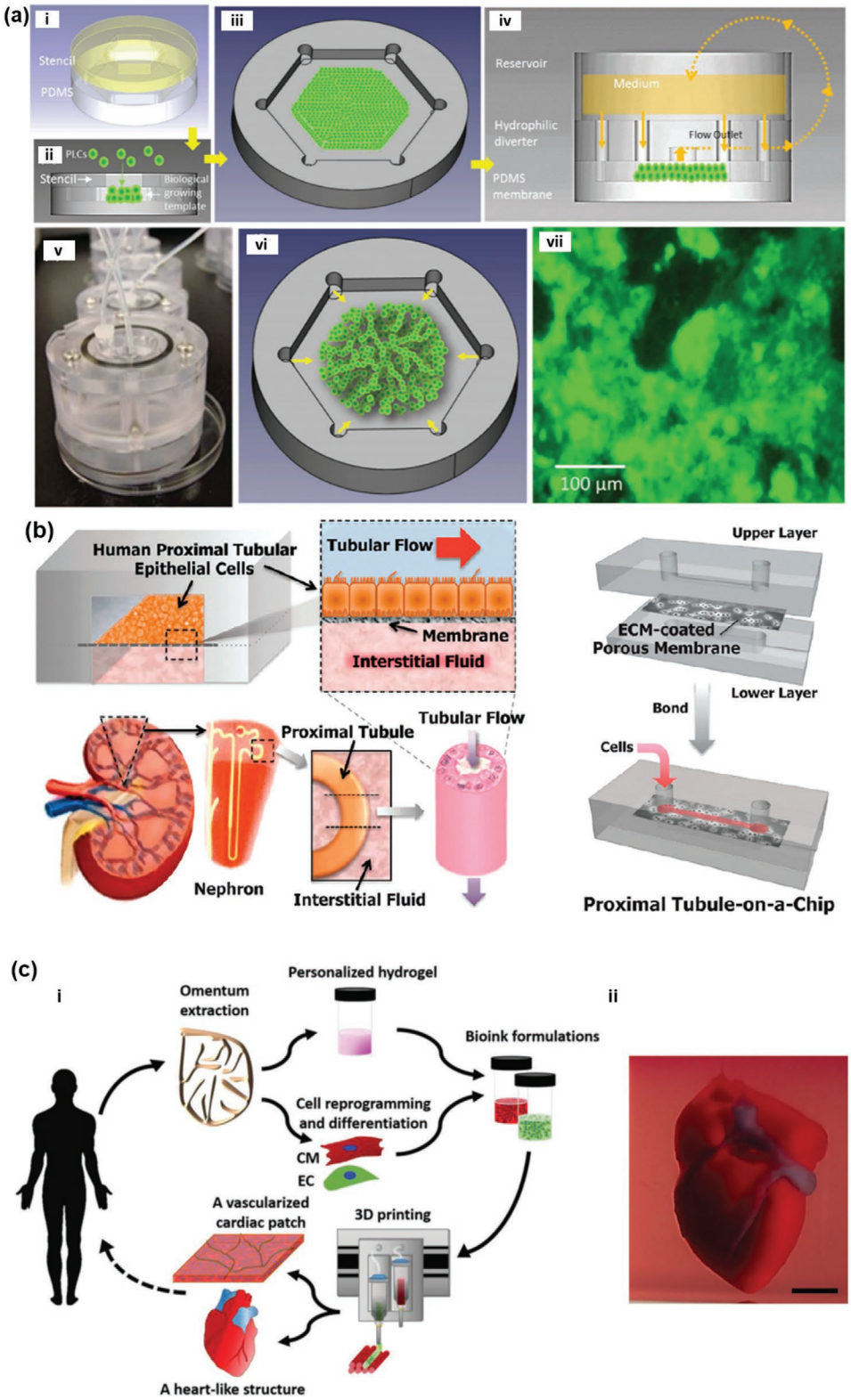

Figure 6. Representation of single-organ-on-a-chip platforms: a) Scaffold-free liver-on-a-chip cultured with multiscale organotypic cultures; i–iv) schematic diagram of design principles, v) entire device, vi) schematic diagram of radial flow, vii) good cell viability of the liver-on-a-chip model stained with Calcein AM (green). Reproduced with permission.[99] Copyright 2017, Wiley-VCH. b) Tubule-kidney-on-a-chip platform designed for drug transport and nephrotoxicity assessment. Reproduced with permission.[110] Copyright 2013, Oxford University Press. c) 3D bioprinting of personalized cardiac patches and heart, i) Schematic concept of the 3D bioprinting technique, ii) Printed heart within a support bath. Reproduced with permission.[111] Copyright 2019, Wiley-VCH.

Recent advancements in 3D bioprinting has shown great potentiality to achieve those complex models. In 2016, Zhang and co-workers have proposed a novel 3D bioprinting hybrid strategy to fabricate endothelialized myocardium to create a heart-on-a-chip model for drug screening and disease modelling.[109] They have concluded that the endothelialized-myocardium-on-a-chip model treated with a common anticancer drug, doxorubicin, presented similar results when compared to standard ones, i.e., dose-dependent responses that cause the decrease in the beating rate and increase in the toxicity. Additionally, the authors have shown that the developed platform was able to test nanomedicines, such as NPs–cardiac cells interaction. Another breakthrough was achieved by the study performed by Noor et al. (2019), where they have reported a 3D bioprinting technology to produce vascularized cardiac patches that fully recapitulate the anatomical heart structure, biochemical and cellular components of an individual, by using bioinks from patients (cf. Figure 6c). This customized technique allows the possibility to develop cardiac models that mimic individual heart organ using patient cells, creating an ideal platform for personalized medicine and drug screening tests.

4.4. Tumor-on-a-Chip

Albeit the effort that has been made in cancer and drug research, the number of diagnosed and untreatable cases is growing, representing a leading cause of death in many developed countries.[53] These indicators express the necessity for the development of more effective and advanced technologies, either to screen new chemotherapeutic drugs and drug delivery strategies, or to better understand the tumor microenvironment and cancer disease. In this regard, tumor-on-a-chip represents an ideal platform to accomplish these goals.[53,112,113] Tumor-on-a-chip systems, which are OoC platforms where healthy tissues are replaced by cancer ones, are seen as outstanding preclinical platforms for theranostic application with a possible impact in oncology.[45] The ability of OoC to closely mimic the in vivo cell biology and physiology allows these platforms to surpass the low accuracy of classical planar and static cell culture models. Furthermore, the potential to combine OoC platforms with patient cells makes these systems extremely attractive when compared with animal models, particularly due to the possibility to develop personalized medicine.[46,68] Therefore, it is expected that tumor-on-a-chip systems can bring significant advances in drug screening, therapy efficiency assessment, metastasis studies, personalized medicine, and nanomedicine.[45] This is an important subject, since adverse side effects still are severe issues in cancer therapy. Hence, the development of smart drug nanocarriers, which can be designed as stimuli-responsive drug systems, is being pursued by pharmaceutical companies and researchers to reduce chemotherapy into a minimal dosage with the maximization of the therapeutic efficacy by targeting cancer sites. In vitro screening studies have shown the great efficacy of smart NPs for this purpose. Unfortunately, in vivo trials have mostly failed to show the same great performance.[1] Tumor-on-a-chip preclinical platforms, due to the dynamic flow conditions and the integration of microfluidic networks that can represent the microvasculature system, offer a unique opportunity to study in detail, the efficiency of novel nanoformulations for drug delivery and, at the same time, their toxicological effect. Surprisingly, and although the remarkable potentiality of these bioplatforms to assess theranostic NPs, cancer-on-a-chip platforms have not been intensively implemented to perform preclinical studies with nanomedicines. One of the first studies that has involved a simple microfluidic device coated with a monolayer of cancer cells was performed in 2005, when Farokhzard and co-workers studied the dynamic interaction of different NPs sizes with cancer cells.[114] Since then, some successful prototypes of 3D tumor-on-a-chip platforms have been developed for the assessment of NPs.[113,115,116] In 2013, Albanese et al.[113] have reported a tumor-on-a-chip model to study transport phenomena of AuNPs in human melanona-spheroids immobilized in a PDMS chamber. This study has shown that the penetration of NPs into the tumor is directly affected by their diameter, and its retention can be improved by receptor targeting. More recently, Wang et al.[112] have designed a tumor-vasculature-on-a-chip platform to assess NP extravasation and tumor accumulation (cf. **Figure 7**). In this study, it was reported a microfluidic tumor-vasculature-on-a-chip (TVOC) that was able to mimic key biological barriers, namely the tumor leaky vasculature and 3D tumor tissue with dense ECM. By so, a TVOC model was created to study the efficacy of NPs to extravasate from the vasculature and infiltrate the target tumor tissue, following their accumulation. The researchers have subscribed once more the importance of the physicochemical properties of nanoformulations, such as size, composition, and surface properties, in opposition to stiffness that has shown a small effect on NP extravasation rate. These 3D tumoral models have shown the importance for a deeper understanding of NP–cells interactions, vascular permeability, tumor targeting, accumulation and anticancer drug efficacy, promoting the continued research, and development of NPs for oncological applications.[117] In another interesting work, Carvalho et al. (2019)[118] have published a colorectal tumor-on-a-chip system for the assessment and validation of the targeted delivery of drug-loaded NPs. In this study, the authors have shown the development and validation of a relevant 3D microfluidic model, emulating the human colorectal tumor microenvironment and physiological functions of a microvascular tissue. By integrating the tumor microenvironment with the physiological function of the microvascular tissue, the assessment of a more physiological in vivo microenvironment was achieved for the investigation of the drug delivery efficacy of drug-loaded nanoparticles (i.e., CMCht/PAMAM dendrimer nanoparticles loaded with gemcitabine) in a dynamic controllable gradient. As a result, the biomimetic model was able to recapitulate the beginning of the angiogenic sprouting process. By means of comparative and quantitative PCR data, the authors could also verify that the tested NPs were able to deliver the anticancer drug in a highly advantageous way compared to gold standard methodologies, resulting in lower undesirable side effects on the endothelial cells (healthy tissue). Although the advantages and potential that the presented model shows in comparison with other tumor-on-a-chip systems, this model does not comprise

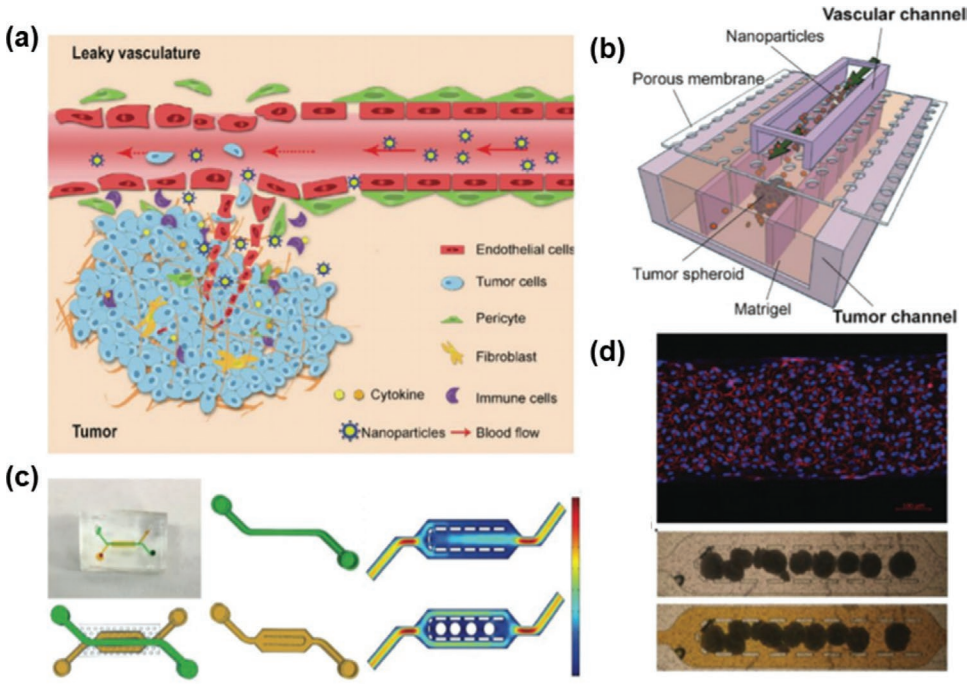

Figure 7. Tumor-on-a-chip platforms for nanoparticles research. a) Schematic of the in vivo tumor microenvironment of leaky vasculature and tumor tissue, b) Representation of tumor-vasculated-on-a-chip model, c) Photo of the tumor platform and computational simulation of the fluid velocity, d) Confocal images of the spheroids trapped into the microfluidic channel. Reproduced with permission.[112] Copyright 2018, ACS Publications.

an oxygen gradient, common in in vivo cancer microenvironments, which the authors refer to be part of future work. More recently, Nashimoto et al. (2020)[119] have described a vascularized tumor-on-a-chip to evaluate the tumor activities with intraluminal flow using an engineered tumor vascular network for drug delivery studies. The results in this study have shown that under perfusion condition the dose-dependent effect of anticancer drugs was not detected in contrast to the results under static conditions. By so, this study highlights the importance of the conjugation of the vascular network for the evaluation of tumor activities in drug screening studies, which are also relevant in nanomedicine. Additionally, other relevant studies, such as the one performed by Liu et al. (2019),[120] have incorporated tumor-on-a-chip devices with healthy tissues, creating multiorgan-on-a-chip, to address oncological questions of interest, such as the molecular mechanism underlying metastasis and/or the efficiency of drug therapies,[121,122] which with the goal that standard methodologies were unlikely to be accomplished.

Table 2 reviews some of the most representative OoC platforms that have been reported regarding the screening of drugs and nanomaterials for medicine.

Table 2. Representative organ-on-a-chip platforms designed for the screening of drugs and nanomaterials for biomedical applications.

Organ typefunctional unit)	Main application studies	Cell types[a)]	Readouts	References
Vasculature	Barrier functionality, vascular transport and hemotoxicity	Endothelial cell lines, human MSCs and iPSCs	Shear stress, permeability	[95,96,123,56]
Liver	Drug metabolism, hepatotoxicity, particles interaction	Mono or co-cultured cell lines, iPSC-derived hepatocytes	Drug-induced liver injury, detection of liver biomarkers, e.g., albumin	[58,57,82,66]
Kidney	Drug transport and nephrotoxicity, nanoparticles clearance studies	Mainly primary tubular epithelial cells	Filtration, urea concentration, fluid shear stress, expression of ATPase and aquaporin1, albumin uptake, and glucose reabsorption	[101,104,102,103,105]
Heart	Cardiovascular toxicity and drug screening tests	iPSC-CMs, CMs, cardiomyoblast, neonatal rat CMs, mono or co-cultured cell lines	Beating rate, production of cardiac biomarkers, cell viability	[109,124,111,125]
Cancer	Screening of chemotherapeutic drugs and theranostic studies	Several cell lines depending on the tumor in study	Tumor apoptosis, cell phenotype, tumoral biomarkers production	[115,113,116,126,112,118,119]

[a)]MSCs, mesenchymal stem cells; iPSCs, induced pluripotent stem cells, CMs, cardiomyocytes.

4.5. Multiorgan-on-a-Chip and Body/Human-on-a-Chip

The achievement of an advanced multiorgan-on-a-chip or a complete body-on-a-chip platform for drug development and personalized medicine, it is seen by many researchers and companies working in this field as a golden point. Despite the advantages that single organ models can have to test in a simpler manner a triggered physiological response and reduce the ambiguity in the interpretation of findings, other studies demand a more complex system that integrates several organ/tissue models that can adequately mimic the physiological function of the human body.[127] The most important and differential aspect of these microfluidic systems, compared to the above-mentioned single OoC, is the interconnection and communication between the different organs and tissues, offering a better recapitulation of the human body function. To achieve this goal, different organs and tissues should provide a scaled human physiology with proportional volume and mass, while maintaining the normal biological function. However, the achievement of body scaling and functional activity is an extremely complex task, due to the multi-dimensionality of the human body.[50] This body-on-a-chip tool can have a significant impact in the replacement of animal models for basic and applied research, such as drug testing development, as well as in creating a faster pace in the clinical translation of nanomaterials.[127,128] Presently, several successful prototypes have been proposed, patented, and commercialized, which have promoted the begging of several start-up companies, such as the ones listed in **Table 3**.

5. The Importance of Sensors in OoC Platforms

As previously discussed in this review, the design of OoC has evolved in the last decade at a remarkable speed. Most of the investigation and effort has been devoted to mimic relevant 3D culture techniques, development of representative microfluidic devices, and incorporation of smart biomatrices, which have been well described elsewhere.[129–131] Nevertheless, the necessity to autonomously monitor the culture environment and biomarkers secreted by the 3D organ models has also been the focus of a considerable amount of studies.[23,98,132] The monitoring of cells' viability and functionality still relies on the use of conventional analytical methods and biochemical assays (e.g., enzyme-linked immunosorbent assay kits, life/dead and/or viability tests), which are laborious, time consuming, and need high volumes of samples. The main advantages of alternatively using biosensors for replacing those conventional methods are: (1) the small working volumes, (2) low limit of detection of biomarkers (LOD, as low as pg mL^{-1}), (3) low-system disturbance, (4) suitability for miniaturization, and most important, (5) the possibility for the integration of sensors and automation of the OoC platforms—which ideally will run in continuous over extended periods of time.[132] Due to these features, microsensor systems are seen as essential tools for cell metabolic studies and standardization of advanced cell culture platforms. Even though the progress achieved in microfluidics and microsensors, the translation of both fields into OoC has been limited by the difficulty to achieve suitable integration of microsensors with the bioplatforms.[133] Despite the state of the art, microsensors and microbiosensors are being improved for low levels of detection, or squeezed down for reduced area, or as flexible-stretchable, or biocompatible, or for long-term usage or even being integrated with readout electronics, there is still a need for new designs and new fabrication methods able to combine all these features in an OoC platform. Herein, a broad perspective of the most relevant physical, chemical, and biochemical sensors published in literature, with the intent of being combined in OoC platforms, is discussed.

5.1. Physical Sensing Units to Monitor Culture Microenvironment—pH, Oxygen, and Temperature Sensors

Acid-base homeostasis (equilibrium of body pH) is one of the most important parameters for the maintenance of cell viability and metabolic activity. A small pH change can affect the normal function of many organs. Thus, pH can be used as an indicator of several diseases, including cancer, or even used for the evaluation of pH-dependent drug nanocarriers. Additionally, the accurate quantification of the pH is an important feature to be considered for long-term monitoring of the 3D

Table 3. Multiorgan-on-a-chip start-ups, selected products and representative devices.

Company	CEO/website	Selected products	Devices
HESPEROS THE HUMAN-ON-A-CHIP COMPANY	Dr. Michael L. Shuler (https://hesperosinc.com/)	Offers four body-on-a-chip systems for disease modelling and drug testing. Customized body-on-a-chip.	
TISSUSE Emulating Human Biology	Dr. Uwe Marx (https://www.tissuse.com)	Offers four multiorgan-on-a-chip systems, from two model organs to body-on-a-chip.	
CNBio innovations	Dr. David Hughes (https://cn-bio.com/)	Offers single-organ, two-organ (2-OC) and multiorgan.	
DRAPER	Dr. Kaigham J. Gabriel (https://www.draper.com/)	Offers Draper's human organ Systems (HOS) technology that can array 96 independent single organ models.	

biomodels. Typically, the pH monitoring is made through light-addressable potentiometric sensors (LAPS),[134] ion-selective field effect transistors (ISFET),[135] metal oxide-based potentiometric sensors,[136] or optical pH sensors.[137] Among these strategies, Zhang and co-workers have developed an optical pH sensor based in the absorption of phenol red (≈515 nm, a supplement of culture medium that indicates the pH value), in the pH range of 6.5–8.0 with linear response and sensitivity of 0.159 V pH^{-1}.[132] Nevertheless, potentiometric sensors based on metal oxides, such as iridium oxide, tungsten oxide, or ruthenium oxide, are often considered as the preferred technology to measure pH, since optical sensors have a small-range of pH detection and, in general, need the mixing of a second reference dye for the pH measurement.[98,133] However, there are several challenges related to the design and integration of such technology that still need to be overcome, such as long-term usability, readout electronics integration, reproducibility, among others.

In the case of absolute oxygen detection, crucial task for cellular metabolic functions, two types of sensors are in general adopted for OoC applications, namely optical and electrochemical sensors. Based on the reduction of oxygen molecules of a noble metal electrode, electrochemical oxygen sensors are the preferable technology, due to its robustness and reproducibility for online monitoring.[98] Also, this approach enables the simultaneous measurement of oxygen and local pH change caused by the oxygen reduction process using the same readout circuitry.[133] On the other hand, optical sensors are the preferable in the case of low oxygen levels and do not need physical or electrical contact of the electrode/detector inside the solution.[98]

Temperature is also an important parameter to be considered for OoC long-term culturing and testing. In 2014, Yu et al. have developed a platinum based grid micro-heaters fabricated on a glass substrate and assisted with a microthermal sensor with a control program at 37 ± 0.3 °C.[138] More recently, Zhang et al.[132] have developed a multisensor-integrated OoC comprising a stable temperature sensor implemented in a benchtop incubator for a period of 7 days (cf. **Figure 8**). Yet, there is a big space for the improvement and implementation of such sensors, specially within the bioreactors that contain the biomodels, for the direct and long-term monitoring of these organs.

5.2. Biosensors to Monitor Cell Behavior—Metabolic Products and Cell Viability

The information about cellular metabolic activity can be provided by biosensors that measure the concentration of certain cells products, namely glucose, lactate, glutamate, among others. In the case of drug tests, the screening of drug toxicity over tissues and organs is done by monitoring selective molecular biomarkers released by specific organs, such as albumin and transferrin for hepatotoxicity,[23] or creatine kinase-MB and troponin T for cardiac toxicity.[98,139] In general, these enzyme-based biosensors are designed as electrochemical sensors. Typically, these sensors are based in a target enzyme that is directed immobilized in a membrane or matrix on a working electrode. The analyte is converted by the enzyme, generating a by-product that is oxidized or reduced. Commonly, oxidase enzymes, such as glucose, lactate, or glutamate oxidase, produce a hydrogen-peroxide (H_2O_2) as a by-product, which is oxidized at a noble metal electrode, producing an electrochemical signal that is measured via voltammetry or amperometry.[98,133] Also, H_2O_2 is one of the reactive oxygen species (ROS) with important role in cellular process, which can be directly used to monitor cell proliferation. Therefore, selective and sensitive H_2O_2 biosensors can be used to detect both inside and outside amounts of H_2O_2, and also to infer on real-time conditions of cells.[98] In 2017, Shin and co-workers have developed a novel and reusable label-free microfluidic electrochemical biosensor able to perform continuous measurements of cell-secreted biomarkers from a cultured organoid of a human liver-on-a-chip,[23] cf. **Figure 9**. In this work, an impedance-based biosensor for the detection of albumin and GST-α was successfully developed and integrated in the OoC system achieving a LOD for these biomarkers as low as, 23 and 10 pg mL^{-1}, respectively. In the same year, the same research group showed the possibility to apply a similar

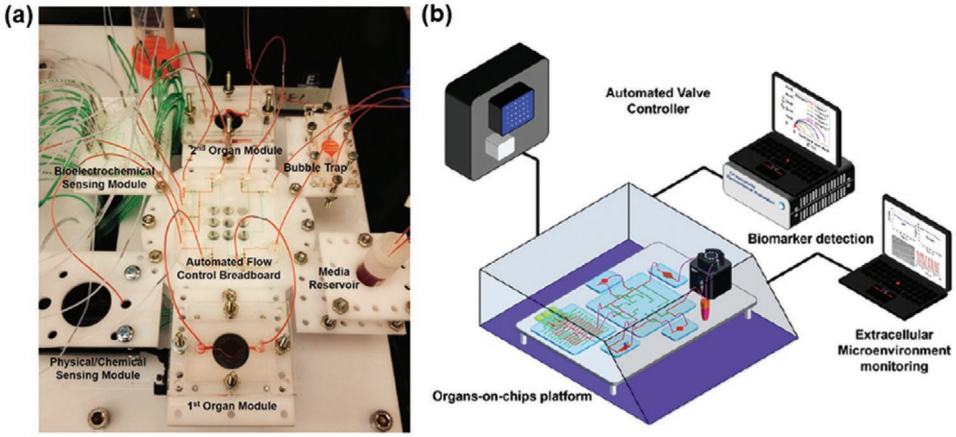

Figure 8. Multisensor-integrated organ-on-a-chip platforms for automated and continuous in situ monitoring of organoids. a) Photograph of the integrated platform including microbioreactors, breadboard, reservoir, bubble trap, physical sensor and electrochemical biosensors; b) Schematic of the full system organ-on-a-chip platform encased in an in-house designed benchtop incubator. Reproduced with permission.[132] Copyright 2017, PNAS.

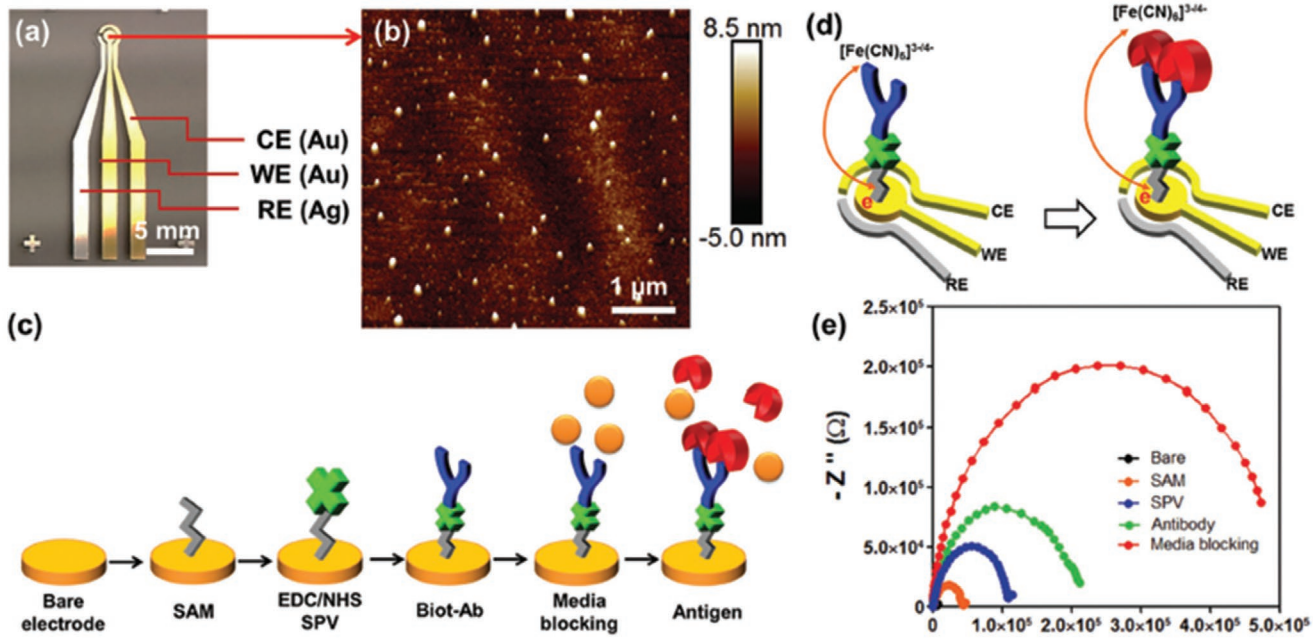

Figure 9. Label-free EC biosensing system for continuous measurement of cell-secreted biomarkers from an organoid cultured of a human liver-on-a-chip. a) Photograph of the microelectrode; b) AFM image of the bare working electrode surface; c) schematic illustration of the antibody immobilization on the surface of the microelectrodes; d) Nyquist plots after antigen binding upon antibody-antigen. Reproduced with permission.[23] Copyright 2017, Wiley-VCH.

strategy for the detection of alpha-1-antitrypsin (A1AT), a cardiovascular biomarker.[139] The high sensitivity and specificity of the immune response of the proposed sensing method was recognized as a new avenue to the development of highly sensitive electrochemical sensors for continuous and autonomous monitoring of OoC.

6. Challenges, Future Directions, and Conclusions

OoC platforms are promising tools with the capability to mimic complex physiological human body function, replace animal testing, improve and speed up drug testing and development, and change the standard medicine by steeply advancing toward the goal of personalized medicine. Along with these goals, these novel bioplatforms constitute a breakthrough in the screening and optimization of NPs developed for medicine. Nonetheless, due to the complexity and multidisciplinary that OoC platforms require, there are many challenges that still need to be surpassed to achieve robust OoC as preclinical platforms. These key challenges can be subdivided in two categories: biological and technical. Among the biological challenges are the: (i) appropriate organ scaling, (ii) development of a universal media, (iii) vascularization of tissues, (iv) co-culture of different cell types to form mimetic organ models, (v) control over cell density, and (vi) recapitulate the immunological response. On the other hand, technical challenges also need to be surpassed, namely the (i) drug and biomarkers adsorption and binding to typical polymers used as substrate for the construction of the bioreactor (e.g., PDMS), (ii) integration of cultured organoids and sensing modules, maintaining the sterility, reproducibility, and avoiding bubbles, (iii) similar shear stress and shear rates among different platforms, (iv) optimal oxygenation and nutrient supply for different organs, and (v) monitoring cell–cell interactions and cell viability by assessing with high precision the biochemical and physical parameters that occur in the culture environment.[21,50] This latter goal can be achieved by the optimization and implementation of microsensor systems, as well as, by applying microscopy analysis, enabling an automatic monitoring of these 3D models for extended periods of time with real-time analysis.[98] A major effort has been made during the last years regarding the optimization of human organoids to precisely recapitulate the complex physiological and biochemical microenvironment found in human tissues and organs. Although there has been a growing interest in developing sensors for OoC monitoring, in situ integration of micro(bio)sensor systems into OoC platforms faces several challenges that need to be continuously addressed. Additionally, it is crucial to overcome standardization and regulatory endorsement challenges in the next few years. The increasing collaboration of multidisciplinary groups, from engineers to clinicians, may provide innovative OoC platforms that could lead to several breakthroughs in screening drugs and nanomaterials for biomedical applications. Hence, OoC is promised to be a standard technology for nanomedicine studies and basic science investigation, such as cancer research, in the near future.

Acknowledgements

This work is the result of the project NORTE-01-0145-FEDER-029394, RTChip4Theranostics, and was supported by Programa Operacional Regional do Norte–Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement,

through the European Regional Development Fund (FEDER) and by Fundação para a Ciência e Tecnologia (FCT), IP, project reference PTDC/EMD-EMD/29394/2017. The authors also acknowledge the partial financial support by the projects UID/EEA/04436/2020, UID/EMS/04077/2020, UID/EMS/00532/2020 from FCT; and by the projects NORTE-01-0145-FEDER-030171 (PTDC/EMD-EMD/30171/2017), NORTE-01-0145-FEDER-000019, NORTE-01-0145-FEDER-031142, POCI-01-0145-FEDER-028237, funded by NORTE 2020 Portugal Regional Operational Programme, under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund and by Fundação para a Ciência e Tecnologia (FCT); and by 2014–2020 INTERREG Cooperation Programme Spain–Portugal (POCTEP) through the project 0624_2IQBIONEURO_6_E.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

biosensors, nanoparticles, organ-on-a-chip, theranostics, tissue engineering

Received: June 8, 2020
Revised: August 13, 2020
Published online: November 25, 2020

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