Analytical Methods

MINIREVIEW

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2018, **10**, 3122

Recent advances in an organ-on-a-chip: biomarker analysis and applications

Xian Li and Tian Tian *

In pharmaceutical research and development, improving the effectiveness of preclinical predictions is significant, thus *in vitro* and *in vivo* experimental models are needed to investigate human drug responses in organisms. As a microengineered model, an organ-on-a-chip (OoC) could provide reliable predictions of drug efficacy and safety by mimicking the complex biological functions of normal cellular interactions. It presents a novel platform with the advantages of high throughputs, low cost, and high efficiency for drug development. Here, we provide an outlook on the recent fundamental development of OoC studies and discuss the prospects of their applications in relation to drug discovery. In particular, we highlight a set of recently developed strategies that facilitate high-throughput analysis of biomarkers and drug metabolites on an OoC, including ELISA, PCR, LC-MS and lab-on-a-chip. A brief summary is also presented along with the prospects of this field in the future.

Received 30th April 2018
Accepted 24th May 2018

DOI: 10.1039/c8ay00970h

rsc.li/methods

Introduction

In pharmaceutical research and development (R&D), according to Eroom's Law,[1] getting a drug to the market currently takes more than 10 years and almost USD 2 billion on average, and the costs are increasing because drug failures are common in clinical trials.[2] Because obtaining sufficient clinical data is difficult, *in vitro* and *in vivo* experimental models are often used to investigate drug mechanisms in organisms.

Typically, two-dimensional (2D) models, such as standard cell culture and animal models, are used; these models offer multiple advantages. Cell culture is simple, inexpensive and easy to manipulate,[3] and it primarily focuses on single cell lines and cellular effects. However, obtaining extreme cell phenotypes or reproducing cell–cell and cell-extracellular matrix (ECM) interactions is difficult in static culture.[4–7] Hence, to gather more information, it is necessary to use model animals, for example nude mice or rabbits.[8–11] These *in vivo* models not only provide complex systems of living organisms but also recapitulate the dynamic interplay among various organs and tissues. Consequently, these models are often applied to drug R&D and tumor growth research, but their use is limited by ethical issues,[12] high cost and poor homology with humans.[13,14]

To generate reliable predictions of drug efficacy and safety in humans, organ-on-a-chip (OoC) models microengineered with miniaturized optics and sensors have been created. These OoC models include a functional vasculature network to transport nutrients or waste as well as various metabolites generated by cells and also mimic the human cellular microenvironment and express organ-specific characteristics. Hence, these models require biomimetic materials to recapitulate natural structures[15] and proper cells to replicate the tissue–tissue interface.[16] A variety of OoC models have been established, such as liver,[17] lung[18] and kidney,[19] thus providing great opportunities for *in vitro* absorption, distribution, metabolism, excretion and toxicity (ADMET) testing and human physiological studies.[7,15,20]

Trace analysis of metabolites is also a crucial step in the application of OoC models, especially the identification and quantification of biomarkers or target metabolites.[21] For the analysis of biological responses and pharmaceutical metabolism, low culture volumes and cell numbers in OoC platforms often give rise to technical issues associated with detection sensitivity. At present, this challenge can be met through coupling OoC with diverse analytical methods, such as liquid chromatography coupled with mass spectrometry (LC-MS), enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). Another novel strategy is leveraging advances in lab-on-a-chip (LoC) technologies to integrate OoC models with bioanalytical platforms that enable high-resolution biochemical analysis with substantially reduced sample volume requirements.

In this article, we focus on a set of recently developed strategies that facilitate high-throughput analysis of biomarkers and drug metabolites on OoC, and we review their applications in biological systems relevant to drug development. We also provide an outlook on OoC approaches which mimic biological microenvironments and systems, including specific examples of tissue culture chips. Our perspectives on future directions, opportunities and technical challenges for the field are also discussed.

Chongqing Key Laboratory of Natural Product Synthesis and Drug Research, School of Pharmaceutical Sciences, Chongqing University, Chongqing 401331, P. R. China. E-mail: tiantian@cqu.edu.cn

OoC

As microfluidic cell culture devices, OoC models mimic the critical microenvironment in a manner that has not been possible in cell-based and animal models, thus providing a useful platform to conduct real-time preclinical tests of disease in living organs (Fig. 1). Some models have been developed to recapitulate the structural and functional complexity of human organs such as the liver, heart, lung, intestine, kidney, brain and bones (Table 1). In addition to biocompatibility, mechanical stability and processability under physiological conditions, specific constraints should be considered in OoC design: (i) choice of scaffold materials. The use of polydimethylsiloxane (PDMS) is advantageous in comparison to many other materials, owing to its transparency, flexibility and permeability to gas. The optical transparency of OoC microdevices is a key advantage over animal models, because it enables direct real-time visualization and quantitative high-resolution analysis of diverse biological processes in ways that have not been possible in animal models. (ii) Three-dimensional (3D) microarchitectures defined by the spatial distribution of multiple tissue types. The geometry of each organ must be able to convey a certain number of cells, as defined by physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) rules. Specific ranges of shear stress to cells must be considered, depending on the type of cell cocultured for each organ tissue. (iii) Biomimetic structures constituted to mimic complex organ-specific mechanical and biochemical microenvironments. (iv) Functional tissue–tissue interfaces.

Donald E. Ingber *et al.* have described a human lung-alveolus-on-a-chip, which reconstitutes the mechanically active alveolar-capillary barrier in the human lung.[18,36] This is the first chip in this field, and it contains two microfluidic channels, each culturing human alveolar epithelial cells or pulmonary microvascular endothelium, to mimic the complicated physiological functions of the normal lung and the growth of orthotopic lung cancer. Beyond a lung-on-a-chip, a variety of chips for different uses have been created. Because hepatotoxicity is one of the main concerns in drug metabolism,[42] finding an effective tool for drug screening is urgently needed. A related OoC example is a biomimetic liver-sinusoid-on-a-chip, which maintains the bioactivity and function for at least 7 days. The chip contains a monolayer of human umbilical vein endothelial cells (HUVECs) formed through self-assembly of endothelial cells (ECs), and laminar flow has been used to perform toxicity tests of acetaminophen treatment.[17] Because the sinusoid is the basic functional unit in the liver, this chip may be an ideal tool for hepatotoxic drug screening. Recently, a glomerulus-on-a-chip has been used to reconstitute organ-level kidney functions in early stage diabetic nephropathy; this model recapitulates the glomerular microenvironment and the glomerular filtration barrier and reproduces high-glucose-induced critical pathological responses. Real time and high resolution imaging analysis have been used to monitor the *in vivo* responses under high glucose conditions, and hyperglycemia has been found to play a crucial role in the development of increased barrier permeability to albumin and glomerular dysfunction, which lead to proteinuria.[43] Thus, this model may become a novel and effective platform that can be applied to kidney or glomerulus diseases. Moreover, a novel tunable microfluidic atherosclerosis model has been reported recently. This 3D stenosis chip consists of two channels separated by a PDMS

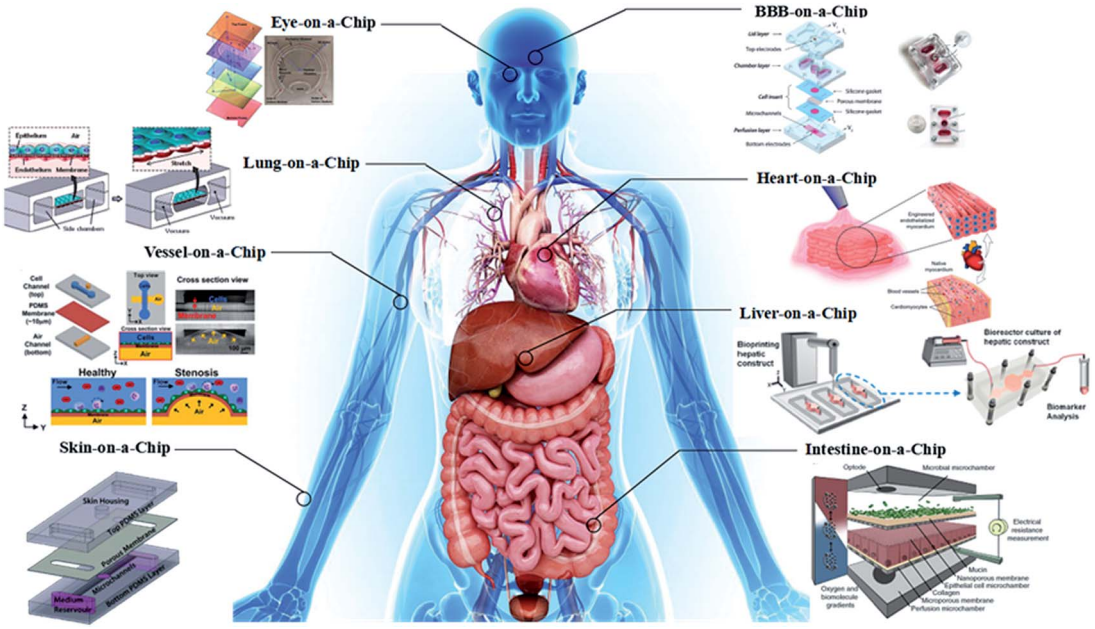

Fig. 1 Various OoC models reported to mimic certain physiological conditions in human organs or tissues, including eye-on-a-chip,[22] lung-on-a-chip,[18] vessel-on-a-chip[23] (https://creativecommons.org/licenses/by/4.0/), skin-on-a-chip,[24] BBB-on-a-chip,[25] heart-on-a-chip,[26] liver-on-a-chip[27] and intestine-on-a-chip.[28] The scheme of Skin-on-a-Chip is reproduced from ref. 24 with permission from the Royal Society of Chemistry.

Table 1 Representative OoC studies reported recently

Organ	Features	Functions	Ref.
Liver	Minimal handling No external power source	Quantitative and qualitative investigation of the paracrine effects of HSCs	29
	Two collagen layers with a clear borderline	Biomimetic structure and biomimetic functions of liver sinusoids	17
	Controllable and uniform distribution of discrete HUVECs	Albumin secretion and urea synthesis	
	Interfaced with a bioprinter to fabricate 3D hepatic constructs of spheroids	Drug toxicity analysis and high throughput screening	27
Brain	Medium recirculation at physiologically relevant perfusion rates with no pumps or external tubing	Physiological blood–brain barrier (BBB) functions Drug permeability studies	25
	Uniform neurospheroids Cell–cell interactions Slow interstitial level of flow	*In vitro* models of neurodegenerative diseases	30
	Built by a simple onestep UV lithography process	Brain-cancer-on-a-chip	31
Heart	Robustly reproducible in both embryonic and induced pluripotent stem cells	*In vitro* human cardiac microtissues Allowing development of beating 3D structures	32
	Induced pluripotent stem cells of human origin Automatic imaging method	Cardiotoxic tests	33
	Syringe pump for infusion and pressure generation	Evaluation of antihypertensive drugs	34
	3D bioprinting	Endothelialized-myocardium-on-a-chip for cardiovascular drug testing	26
Skin	No need for a pump	Long-term maintenance of full thickness human skin equivalents (HSE)	24
Intestine	Co-culture of human and microbial cells	Gastrointestinal human–microbe interface recapitulating *in vivo* responses	28
	Two microfluidic channels separated by a porous flexible membrane coated with ECM	Transport, absorption, and toxicity studies of the human intestine	35
Lung	Two microfluidic channels for cell culturing and mechanical stretching to mimic natural functions	*In vitro* human orthotopic models of nonsmall-cell lung cancer	18 and 36
Kidney	Microfluidic device lined with kidney epithelial cells	Nephrotoxicity tests	19
Vessels	Interaction between cell and fluidic media	Atherosclerotic models	23 and 37
Bone marrow	Co-culture of metastatic breast cancer cells	*In vitro* model of breast cancer bone metastasis	38
Eye	Retinal ganglion cell line to restructure the retina	Formation of silicone oil emulsion droplets in the eye cavity	39
Spleen	Two-layer microengineered device with closed-fast and open-slow microcirculations	Hydrodynamic forces and physical properties of the splenon	40
Multi-OoC	Intestine-liver-skin-kidney	Functionality of four organs over 28 days in co-culture Absorption, distribution, metabolism and excretion (ADME) profiling	41

membrane, and the top channel is used to culture cells and maintain a steady flow, while the bottom channel is orthogonally placed to allow air pumping to mimic a stenotic plaque. This model has been used to study vascular inflammation and leukocyte–endothelial interactions in 3D vessel stenosis by using fluid simulations and experimental bead perfusion, thus providing an alternative way to perform quantitative studies on hemodynamics and leukocyte–endothelial interactions.[23]

Clearly, it is far from meeting the needs to find new drug targets and therapies by using a single chip. An integrated multi-tissue platform can also help generate insights into complex physiological processes. To study organ–organ interactions and mimic the body system, a multi-OoC or human-on-a-chip has been created by use of a microfluidic cycle to connect several individual OoCs. A previous study has provided a successful example by integrating a gut-on-a-chip and a liver-on-a-chip to investigate the quantitative pharmacokinetics (PK) of diclofenac and hydrocortisone under different experimental perturbations.[44] In addition, Taylor *et al.* have evaluated the ADMET process of terfenadine, trimethylamine and vitamin D3 not only through simple liver-muscle coupling but also through functional intestine-liver-kidney-blood–brain-barrier (BBB) coupling.[45] One of the most inspiring results has been that a microfluidic system supporting murine ovarian follicles had been set up to produce the human 28 day menstrual cycle hormone profile. In this work, scientists have simulated the *in vivo* female reproductive tract and endocrine loops with a sustained circulating flow between tissues, including organ modules for the ovary, fallopian tube, uterus, cervix and liver, a model with significant advantages for studying organ–organ integration.[46] In summary, in terms of mimicking human biological responses, human, OoC or multi-OoC models are superior to either animals or simple *in vitro* systems. Thus, these novel models offer new potential approaches for pharmacological and physiological studies, such as therapeutic responses or side effects of drugs.

Detection of biomarkers and pharmaceutical metabolites

OoC-based technology has been used to design biomimetic microfluidic devices containing human cells to replicate fundamental functional units of human tissues and organs *in vitro*. However, for the application of OoC, finding a suitable biomarker is essential. An ideal biomarker based on OoC should have several characteristics, including: (i) high clinical sensitivity and specificity, (ii) quick release for early cell culture, (iii) capability to remain elevated for a reasonable length of time to allow for suitable analytical processes, and (iv) the ability to be quantitatively assayed in a cost- and time-efficient manner. Because no single marker has been found that satisfies all these characteristics, the simultaneous quantification of several biomarkers is of great interest. For example, L-arginine and asymmetric dimethylarginine in serum can serve as biomarkers of neonatal sepsis, and show a strong correlation.[47] Different biomarkers of diseases and organs are listed in Table 2. Continual monitoring of secreted biomarkers from OoC models is desired to understand their responses to drug exposure in a noninvasive manner. To achieve this goal, analytical methods capable of monitoring trace amounts of biomarkers are of particular interest, involving ELISA, PCR, LC-MS and LoC (Fig. 2).

Biological analysis

Because ELISA is often used as a traditional biosensing technology, scientists often apply it to biological analysis in OoC research. ELISA can be applied in detecting biomarkers in order to evaluate the functions or conditions of cells on chips. For example, because cells from orthotopic lung cancer often express mutations in the epidermal growth factor receptor (EGFR), an EGFR signaling antibody array kit has been used to evaluate a lung cancer-on-a-chip.[36] In addition, the viability of multi-OoC of the intestine, liver, skin and kidney has been monitored by measuring lactate dehydrogenase (LDH) by using an LDH cytotoxicity assay kit.[41] Moreover, multi-biomarker analysis can improve the specificity and accuracy of diagnosis. A previous study has reported development of a hepatotoxicity test of acetaminophen on a liver-on-a-chip, and the available ELISAs have been used to assess markers including albumin, urea, lactate dehydrogenase and α-glutathione *S*-transferase (α-GST). A multi-OoC of the liver, heart and lung has been developed, and the effects of bleomycin have been determined by using ELISA to quantify the levels of IL-8 and IL-1β.[67] In another study, albumin, ceruloplasmin, alpha-1 anti-trypsin and transferrin from the media of a liver-on-a-chip have been measured with ELISA kits.[27] Recently, more studies have reported the application of ELISA to other diseases and OoC models to obtain the concentrations of drugs or biomarkers, such as AKT, BCA, AMH, VEGF, glucose and insulin.[46,68] Beyond ELISA, PCR is also a suitable method to detect gene biomarkers, and real-time quantitative PCR has been used in OoC models.[68,69] As for the cell conditions, fluorescence or optical microscopy is commonly used.[31]

LC-MS technology

Cell-derived metabolites of chemical drugs provide complicated but detailed information on the reactions on organs, thus allowing for *in vivo* or *in vitro* pharmacokinetics to be studied through advanced analytical technologies. Because LC-MS not only provides better sample separation and high-throughput but also provides high sensitivity and efficiency for detection and diagnostic tests, it can be applied to identify metabolites. Previous research had been developed to find potential biomarkers of cancers.[70–73] Besides, a recent study had showed an integrated chip-MS device to provide an on-line pharmacokinetic analysis of paclitaxel. This microfluidic device could be divided into four parts, involving a chip to co-culture cervical carcinoma cells (CaSki cells) and human umbilical vein endothelial cells (HUVECs), a protein detection platform, micro-solid-phase extraction for pre-treatment and electrospray ionization coupled with quadrupole time-of-flight mass spectrometry (ESI-QTOF-MS), and it would be a potential tool for anti-cancer drug screening, as it is able to simplify the process of detection and shorten the analysis time.[74] In addition, other chip-MS platforms were developed for detection of lactate production from tumor cells and normal cells,[75] quantification of irinotecan and its active metabolite from HepG2 cells,[76] and monitoring of vitamin E from human lung epithelial A549 cells.[77]

As multi-OoC models provide more comprehensive system functions, scientists are focusing more on preclinical PK tests, and LC-MS is often coupled with OoC to profile metabolomics.[44,45] In a recent study, scientists have used a multi-OoC model involving liver, breast and lung cancer, and normal gastric cells, to characterize the dynamic metabolism of capecitabine and its intermediate metabolites 5′-deoxy-5-fluorocytidine and active metabolite 5-fluorouracil (5-FU).[78] Notably, LC-MS/MS is a suitable way to detect metabolites, and another study has used an LC-MS system to measure the concentrations of capecitabine and 5-FU in the medium of a multi-OoC composed of intestine, liver, cancer, and connective tissue cells.[79] Hence, this method provides an alternative means to identify and quantify appropriate metabolites in fluid medium samples with LC-MS.

LoC

After specific biomarkers are obtained, complex metabolomics data can indicate key factors relevant to disease and may make individual therapy possible. However, traditional biomarker detection methods have the drawbacks of limited sensitivity, selectivity and stability and require large working volumes, especially when cell culture medium is involved, which usually contains a plethora of nonspecific binding proteins and interfering compounds. Hence, novel analytical platforms are needed to provide accurate point-of-care information on the status of organoids at low working volumes. This challenge may be met by integrating OoC with LoC models,[80] which not only enable better sample preparation and a shorter detection time but also provide a way to achieve real-time and high-resolution biochemical analysis. LoC can be used to detect and analyze

Table 2 Analytical methods using biomarkers

Diseases	Markers	Analytical technique	Models	Ref.
Neonatal sepsis	L-Arginine Asymmetric dimethylarginine	ELISA	Clinical serum	47
Alzheimer's disease (AD)	Protein Tau	Magnetic particle-based digital ELISA	Plasma and clinical cerebrospinal fluid samples	48
Coronary artery disease (CAD)	Neural cell adhesion molecule-1 (NCAM-1)	ELISA	Clinical plasma	49
Chronic kidney disease (CKD)	Tryptophan Kynurenine	Capillary electrophoresis UV detection	Clinical serum	50
	Creatinine Citrulline Symmetric dimethylarginine *S*-Adenosylmethionine	LC-MS	Clinical plasma	51
	Tubular kidney injury molecule-1 (KIM-1)	ELISA	Urine	52
	Neutrophil gelatinase-associated lipocalin (NGAL)	ELISA	Clinical serum	53
	Cystatin C	Particle-enhanced immunonephelometry	Clinical serum	53 and 54
Cardiovascular disease (CVD)	Hs-CRP cTnI NT-proBNP	Bead-based ELISA (LoC)	Whole blood and whole saliva	55
Pulmonary arterial hypertension (PAH)	Endothelial progenitor cells	Immunofluorescence (LoC)	Clinical whole blood	56
Osteoarthritis (OA)	Adropin	ELISA	Clinical serum	57
Rheumatoid arthritis (RA)	Pentraxins C-Reactive protein	ELISA	Clinical serum and synovial fluid samples	58
Acute myeloid leukemia (AML)	Methylation-independent CHFR expression	PCR	Clinical bone marrow	59
Breast cancer	Human epidermal growth factor receptor 2 (HER2)	Sandwich type electrochemical immunosensor	Clinical serum	60
Prostate cancer	Prostate specific antigen (PSA) Prostate specific membrane antigen (PSMA) Platelet factor-4 (PF-4)	Electrochemiluminescence (ECL)	Clinical serum	61
Ovarian cancer	CA-125 EpCAM CD24	Immunomagnetic beads and an epifluorescence microscope	Clinical plasma	62
Non-small-cell lung cancer (NSCLC)	α-IGF-1R *p*-IGF-1R	Immunoaffinity isolation and protein analysis	Clinical plasma	63
Chronic lymphocytic leukemia	Cfc-DNA	Dielectrophoretic microarrays	Clinical whole blood	64
Hematopoietic malignancies	Cancer cells	Fluorescence *in situ* hybridization (FISH)	Whole blood, bone marrow, and cell lines (Jurkat)	65
Hepatotoxicity	Transferrin Albumin	Bead-based electrochemical immunosensor (LoC)	Liver-on-a-chip	66
	Albumin	Immunofluorescence staining		17
	Urea	ELISA		17

a variety of protein and cell-based biomarkers of cardiovascular disease and cancer,[81] thus revealing its benefits in disease diagnosis. The LoC platform is often coupled with highly sensitive biosensors to offer powerful functions in analytical applications. Apart from optical and fluorescence-based biosensors, enzyme-based and affinity-based electrochemical sensors on microfluidic devices have advanced to meet selectivity demands.[82] One group has developed an aptamer-based electrochemical microfluidic biosensor for the detection of creatine kinase, a cardiac biomarker. In this biosensor, a gold electrode surface is coated with a carboxy-terminal thiol, which is then functionalized with amine-linked aptamers *via* carbodiimide coupling. The impedance signal for creatine kinase is linear from 10 pg mL^{-1} to 100 ng mL^{-1} (clinically relevant concentrations) in both buffer and culture medium samples. A heart-on-a-chip cardiac bioreactor has been integrated with this device, and doxorubicin-induced cardiac damage has been assessed through changes in the creatine kinase concentration.[83] Another magnetic microbead-based electrochemical immunosensor has been integrated with a liver-on-a-chip for the in-line detection of transferrin (TF) and albumin, known as biomarkers of the normal liver. The results show a wide analytical range of TF concentrations (0–16 000 ng mL^{-1}) and a lower LOD (0.03 ng mL^{-1}) than those of ELISA (0.2

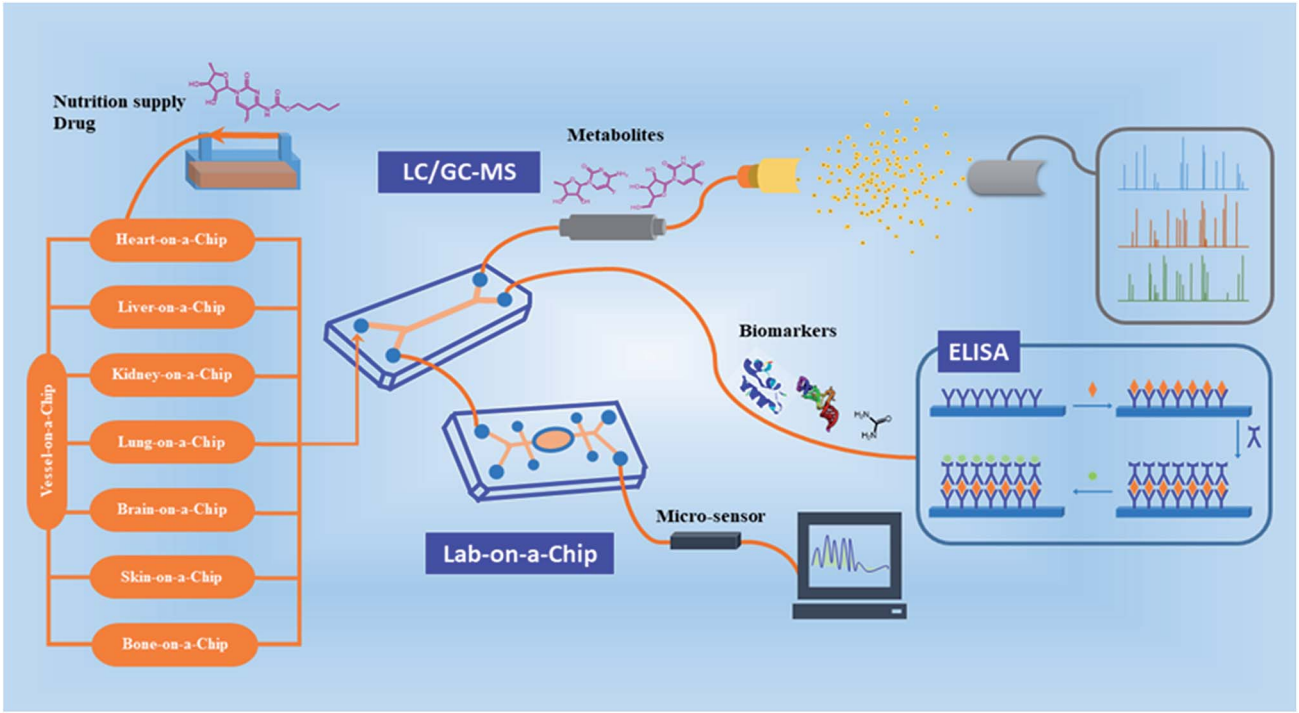

Fig. 2 The analytical methods for detection of biomarkers and metabolites on OoC.

ng mL^{-1}). In addition, hepatotoxicity of acetaminophen at different concentrations has been assessed with this integrated platform and evaluated through assessment of TF and albumin, thus yielding results consistent with those from ELISA kits and cytotoxicity analysis.[66]

In summary, because analytes can be detected from the continuous flow in OoC, *in vivo* information can be determined on the basis of changes in the analyte composition and concentration in the medium. Because of the small medium volumes in these OoC platforms, the sensing systems consume very little sample fluid, can be multiplexed, are noninvasive to organoids, and are amenable to microfluidic devices. A variety of analytical platforms have been reported recently for affinity capture of targeted biomarkers and pharmaceutical metabolites. A key area of improvement needed for many of these systems is the ability to maintain good detection limits with biological matrices. Multiplexing is another pursuit that may improve trace analysis in these microfluidic devices. Finally, efforts are needed to design detection assays that are well suited for point-of-care applications.

Application of OoC

Drug screening

Owing to inter-species and/or microenvironment differences, animal models and other *in vitro* models are not effective in selecting the best drug candidates to test in human clinical trials; hence, only a small fraction of drugs are able to enter the market during drug development.[17] To lower the cost of drug R&D and improve the efficiency of drug screening, OoC can serve as a superior platform in pharmacological and preclinical testing (Fig. 3).

Beyond the aforementioned hepatotoxicity, toxicity to other organs should be considered in drug screening. For instance, a co-culture microfluidic model has been developed to reconstitute the interactions between neurovascular endothelial cells and neuronal cells. This unique BBB-on-a-chip mimics the *in vivo* conditions and has been used in drug testing with histamine. The permeability of the BBB has been found to increase only in endothelial cells but not in co-cultured cells; thus, this model is a favorable platform to study neurological disorders.[85] Another group has developed a lung-on-a-chip to co-culture human non-small cell lung cancer cells (A549 cells) and human fetal lung fibroblasts (HFL1 cells). The authors have evaluated the EGFR-targeted anticancer drug gefitinib on this chip and have concluded that gefitinib induces apoptosis or death in A549 cells, and the addition of insulin-like growth factor-1 causes drug resistance to gefitinib in A549 cells.[86] In addition, a vessel-on-a-chip has been used in the first ultrasound-mediated drug delivery studies, which demonstrate the potential ability of OoC to model ultrasound-mediated drug delivery,[87] a novel OoC application. Moreover, a skin-on-a-chip can support the entire process of growth and differentiation of skin cells, thus providing a physiological platform for cosmetic and drug screening.[88] A platform of multiple vascularized micro-organs has been described to mimic vascularized micro tumors for drug screening at a larger scale, through time-lapse image sequences and time course images.[69]

Although traditional models are indispensable for preclinical drug screening, various disadvantages, such as species differences, exist and should be solved. Thus, OoC models have

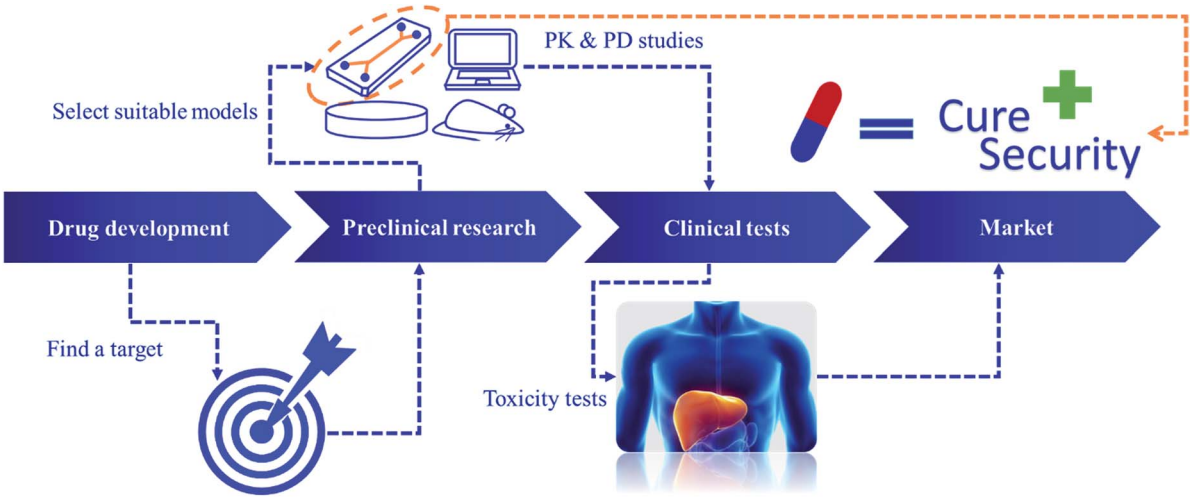

Fig. 3 Potential of OOCs to disrupt drug development.[42,84]

been widely used as a novel bio-mimic organ model, because they can maintain the cellular function and morphology and can be integrated to compose a basic system mimicking the human body.[89] OoC allows for predicting and testing physiological responses and provides scientists with an attractive approach to drug R&D that avoids the ethical constraints of animal experimentation.[42,90] With the development of analytical equipment, chemicals and their metabolites will be able to be detected in simpler and faster ways to achieve high-throughput drug screening.

Antibiotic development

With antimicrobial resistance becoming a major concern on a worldwide scale, it is urgent to discover another effective way to determine the antibiotic resistance profiles of bacteria and to find novel antibiotics. In recent years, in addition to the drug screening mentioned above, OoC platforms were applied to antibiotic testing as well.

As an essential procedure on the study of bacterial infections, antimicrobial susceptibility testing (AST) is mainly characterized by measuring bacterial growth in the presence of antibiotics.[91] Two widely used methods for AST are the microdilution method for liquid AST and the disk diffusion method for solid plate AST, generally running overnight for 16–24 h.[92] Recent studies have shown that we can undertake AST in a shorter time using microfluidic techniques.[93] Sunghoon Kwon *et al.*[103] had tested the MICs (minimal inhibitory concentrations) of several kinds of antibiotics on bacteria *via* a microfluidic agarose channel (MAC) system, shortening the AST time to 3–4 h; and Pak Kin Wong *et al.*[104] had developed AST on gas permeable micro-channels and could determine the antibiotic resistance profiles of bacteria in less than 1 h as well.

Furthermore, since a gut-on-a-chip was developed to mimic the complex structure and physiology of the living intestine, scientists were able to get novel intestinal disease models of transport, absorption, and toxicity studies.[35] In addition, normal intestinal microbes can be successfully co-cultured, so we can restructure the organ-on-a-chip and take intercrossed applications on microbiology. A previous study had presented a Host–Microbiota interaction module derived from gut-on-a-chip and showed a better understanding of adhering bacterial communities of the gastrointestinal tract.[94] Besides, Donald E. Ingber *et al.* had successfully cultured intestinal bacteria on the *in vitro* chip for more than one week to get further investigation of bacterial overgrowth and inflammation.[95] What's more, Paul Wilmes *et al.* had studied the molecular interactions at the host–microbe interface *via* the human-microbial cross talk (HuMiX) platform, which reproduced the dynamic microenvironment and anaerobic conditions. The work gives us a novel perspective to understand the microbial environment and new insights into the microbial ecology.[96] By using this co-cultivation chip, we could not only take the further investigation of the impact of antibiotics on human intestinal flora and understand their metabolic mechanism, but also get a new perspective to understand the problem of bacterial resistance, by mimicking the actual human intestine microenvironment. Thus, OoC models make superior contributions to microbiological research and express advantages that are not possible for the existing animal models.

Conclusion and prospects

With the advances in bioengineering and analytical methods, organ-on-a-chip has already been used as an attractive approach to predict human physiological responses to drug screening and disease diagnosis.[97] Providing typical advantages, such as low fluid volume consumption and high-throughput analysis, it has the potential to provide an inexpensive platform in comparison to traditional 2D dishes and animal models in the future.[98] However, in order to achieve reliable results and higher reduction of human organs, it is necessary to understand natural reactions in the microenvironment and the interactions on cell–cell, tissue–tissue and organ–organ systems, although multi-organ-on-a-chip or human-on-chip models could create a complex system to mimic a part of basic functions.

What's more, despite recent advances in organs-on-a-chip systems, it is also critical to obtain information about biomarkers that correlate with the behavior and status of these organoids during long-term culture. Surveillance of disease with only one biomarker is inaccurate. In fact, there are the same biomarkers existing in different types of cancer, such as VEGF.[99–101] In order to enhance the precision of drug screening processes, multi-biomarker analysis should be underlined in OoC models, which is bound to increase the difficulty of detection. The current gold standard for such measurements relies on ELISA,[102] which is typically insufficient in sensitivity in addition to the needs for off-chip operations that consume significant sample volumes. LC/MS is another main method to detect and identify the metabolites from chips, characterized by high-throughput and better efficiency. Using organ-on-a-chip coupled with LC/MS not only gives us acute information on metabolomics of organoids and drugs, but is also an efficient way to get a further insight into the mechanism of the organism. However, LC/MS couldn't be used to properly analyse immune responses of humans, which limits its scale of application. Therefore, a robust biosensor platform featuring high sensitivity and selectivity is an urgent need for seamless integration with organ constructs. Lab-on-a-chip was integrated with OoC models to give a more convenient and efficient platform for biomarker detection and drug screening. This integrated microfluidic platform with multiple functions may become a promising tool for drug screening within an engineered tumor microenvironment.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant no. 81501612).

References

1 J. W. Scannell, A. Blanckley and H. Boldon, *Nat. Rev. Drug Discovery*, 2012, **11**, 191.
2 M. L. Shuler, *Lab Chip*, 2017, **17**, 2345.
3 D. Caballero, S. Kaushik and V. M. Correlo, *Biomaterials*, 2017, **149**, 98.
4 H. A. B. Multhaupt, B. Leitinger and D. Gullberg, *Adv. Drug Delivery Rev.*, 2016, **97**, 28.
5 N. G. Frangogiannis, *J. Clin. Invest.*, 2017, **127**, 1600.
6 V. Gouirand and S. Vasseur, *Cell Death Discovery*, 2018, **4**, 1.
7 S. H. Lee and J. H. Sung, *Adv. Healthcare Mater.*, 2018, **7**, 1700419.
8 S. Sangiorgi, A. D. Benedictis and M. Reguzzoni, *J. Anat.*, 2018, **232**, 739.
9 C. Kang, L. Xia and Y. Chen, *Protein Cell*, 2018, **9**, 130.
10 H. Y. Lee, A. Marahatta and B. Bhandary, *Eur. J. Pharmacol.*, 2016, **777**, 104.
11 M. Xiong, X. Wu and X. Ye, *Front. Microbiol.*, 2016, **7**, 874.
12 G. R. Liguori, B. F. Jeronimus and T. T. de Aquinas Liguori, *Tissue Eng., Part C*, 2017, **23**, 850.
13 G. Francia, W. Cruz-Munoz and S. Man, *Nat. Rev. Cancer*, 2011, **11**, 135.
14 M. Ittmann, J. Huang and E. Radaelli, *Cancer Res.*, 2013, **73**, 2718.
15 S. Ahadian, R. Civitarese and D. Bannerman, *Adv. Healthcare Mater.*, 2018, **7**, 1700506.
16 J. Wu, Q. Chen and W. Liu, *Trends Anal. Chem.*, 2017, **87**, 19.
17 S. Mi, X. Yi and Z. Du, *Biofabrication*, 2018, **10**, 25010.
18 D. Huh, B. D. Matthews, A. Mammoto and D. E. Ingber, *Science*, 2010, **328**, 1662.
19 S. Kim, S. C. LesherPerez and B. C. Kim, *Biofabrication*, 2016, **8**, 15021.
20 S. Ishida, *Drug Metab. Pharmacokinet.*, 2018, **33**, 49.
21 B. I. Lee, M. Park and S. C. Heo, *Biomed. Chromatogr.*, 2018, **32**, e4107.
22 Y. K. Chan, K. H. Sy and C. Y. Wong, *Invest. Ophthalmol. Visual Sci.*, 2015, **56**, 3314.
23 N. V. Menon, H. M. Tay and K. T. Pang, *APL Bioeng.*, 2018, **2**, 16103.
24 H. E. Abaci, K. Gledhill and Z. Guo, *Lab Chip*, 2015, **15**, 882.
25 Y. I. Wang, H. E. Abaci and M. L. Shuler, *Biotechnol. Bioeng.*, 2017, **114**, 184.
26 Y. S. Zhang, A. Arneri and S. Bersini, *Biomaterials*, 2016, **110**, 45.
27 N. S. Bhise, V. Manoharan and S. Massa, *Biofabrication*, 2016, **8**, 14101.
28 P. Shah, J. V. Fritz and E. Glaab, *Nat. Commun.*, 2016, **7**, 11535.
29 S. Lee, D. Y. No and E. Kang, *Lab Chip*, 2013, **13**, 3529.
30 J. Park, B. K. Lee and G. S. Jeong, *Lab Chip*, 2015, **15**, 141.
31 Y. Fan, D. T. Nguyen and Y. Akay, *Sci. Rep.*, 2016, **6**, 25062.
32 E. Giacomelli, M. Bellin and L. Sala, *Development*, 2017, **6**, 1008.
33 G. Bergstrom and C. Mandenius, *Lab Chip*, 2015, **15**, 3234.
34 L. Li, X. Lv and S. Ostrovidov, *Mol. Pharm.*, 2014, **11**, 2009.
35 H. J. Kim, D. Huh and G. Hamilton, *Lab Chip*, 2012, **12**, 2165.
36 B. A. Hassell, G. Goyal, E. Lee and D. E. Ingber, *Cell Rep.*, 2017, **21**, 508.
37 K. Namdee, A. J. Thompson and P. Charoenphol, *Langmuir*, 2013, **29**, 2530.
38 S. Hao, L. Ha and G. Cheng, *Small*, 2018, **14**, 1702787.
39 Y. K. Chan, K. H. S. Sy and C. Y. Wong, *Invest. Ophthalmol. Visual Sci.*, 2015, **56**, 3314.
40 L. G. Rigat-Brugarolas, A. Elizalde-Torrent and M. Bernabeu, *Lab Chip*, 2014, **14**, 1715.
41 I. Maschmeyer, A. Lorenz and A. Ramme, *Toxicol. Lett.*, 2015, **238**, S176.
42 K. Ronaldson-Bouchard and G. Vunjak-Novakovic, *Cell Stem Cell*, 2018, **22**, 310.
43 L. Wang, T. Tao and W. Su, *Lab Chip*, 2017, **17**, 1749.
44 N. Tsamandouras, W. L. K. Chen and C. D. Edington, *AAPS J.*, 2017, **19**, 1499.
45 L. Vernetti, A. Gough and N. Baetz, *Sci. Rep.*, 2017, **7**, 42296.

46 S. Xiao, J. R. Coppeta and H. B. Rogers, *Nat. Commun.*, 2017, **8**, 14584.
47 O. Aydemir, B. Ozcan and H. Yucel, *J. Matern. Fetal Neonatal Med.*, 2014, **28**, 977.
48 E. Pérez-Ruiz, D. Decrop and K. Ven, *Anal. Chim. Acta*, 2018, **1015**, 74.
49 P. Yu, J. Zhao and H. Jiang, *Int. J. Cardiol.*, 2018, **257**, 238.
50 A. Zinellu, S. Sotgia and A. A. Mangoni, *Nutr. Metabol. Cardiovasc. Dis.*, 2015, **25**, 153.
51 S. Benito, A. S. Anchez-Ortega and N. Unceta, *J. Pharm. Biomed. Anal.*, 2018, **149**, 46.
52 M. M. van Timmeren, M. C. van den Heuvel and V. Bailly, *J. Pathol.*, 2007, **212**, 209.
53 S. M. Alharazy, N. Kong and R. Saidin, *Angiology*, 2014, **65**, 436.
54 C. A. Peralta, M. G. Shlipak and S. Judd, *J. Am. Med. Assoc.*, 2011, **305**, 1545.
55 J. Park, V. Sunkara and T. Kim, *Anal. Chem.*, 2012, **84**, 2133.
56 G. Hansmann, B. D. Plouffe and A. Hatch, *J. Mol. Med.*, 2011, **89**, 971.
57 G. Gundogdu and K. Gundogdu, *Clin. Rheumatol.*, 2018, DOI: 10.1007/s10067-018-4052-z.
58 A. Sharma, R. Khan and N. Gupta, *Clin. Chim. Acta*, 2018, **480**, 65.
59 J. Zhou, T. Zhang and X. Li, *J. Cell. Physiol.*, 2018, **233**, 4707.
60 M. Shamsipur, M. Emami and L. Farzin, *Biosens. Bioelectron.*, 2018, **103**, 54.
61 K. Kadimisetty, I. M. Mosa and S. Malla, *Biosens. Bioelectron.*, 2016, **77**, 188.
62 Z. Zhao, Y. Yang and Y. Zeng, *Lab Chip*, 2016, **16**, 489.
63 M. He, J. Crow and M. Roth, *Lab Chip*, 2014, **14**, 3773.
64 A. Sonnenberg, J. Y. Marciniak and J. McCanna, *Electrophoresis*, 2013, **34**, 1076.
65 F. Mughal, S. J. Baldock and E. G. Karimiani, *Gene Chromosome Canc.*, 2014, **53**, 255.
66 R. Riahi, S. A. M. Shaegh and M. Ghaderi, *Sci. Rep.*, 2016, **6**, 24598.
67 A. Skardal, S. V. Murphy and M. Devarasetty, *Sci. Rep.*, 2017, **7**, 8837.
68 S. Bauer, C. Wennberg Huldt and K. P. Kanebratt, *Sci. Rep.*, 2017, **7**, 14620.
69 D. Phan, X. Wang and B. M. Craver, *Lab Chip*, 2017, **17**, 511.
70 X. Wang, Y. Peng and M. Xie, *Proteonomics Clin. Appl.*, 2017, **11**, 1600163.
71 L. White, J. Ma and S. Liang, *J. Pharm. Biomed. Anal.*, 2017, **137**, 54.
72 G. Zhang, D. J. Sexton and R. R. Faucette, *Bioanalysis*, 2017, **9**, 1477.
73 C. S. Carroll, L. N. Amankwa and L. J. Pinto, *PLoS One*, 2016, **11**, e151260.
74 L. Lin, X. Lin and L. Lin, *Anal. Chem.*, 2017, **89**, 10037.
75 W. Liu and J. Lin, *ACS Sens.*, 2016, **1**, 344.
76 M. Jie, H. Li and L. Lin, *RSC Adv.*, 2016, **6**, 54564.
77 D. Gao, H. Wei and G. Guo, *Anal. Chem.*, 2010, **82**, 5679.
78 Z. Li, Y. Guo and Y. Yu, *Integr. Biol.*, 2016, **8**, 1022.
79 T. Satoh, S. Sugiura and K. Shin, *Lab Chip*, 2018, **18**, 115.
80 E. W. Esch, A. Bahinski and D. Huh, *Nat. Rev. Drug Discovery*, 2015, **14**, 248.
81 J. Wu, M. Dong and S. Santos, *Sensors*, 2017, **17**, 2934.
82 J. P. Lafleur, A. Jönsson and S. Senkbeil, *Biosens. Bioelectron.*, 2016, **76**, 213.
83 S. Shin, Y. S. Zhang and D. Kim, *Anal. Chem.*, 2016, **88**, 10019.
84 S. Ishida, *Drug Metab. Pharmacokinet.*, 2018, **33**, 49.
85 S. Jeong, S. Kim and J. Buonocore, *IEEE Trans. Biomed. Eng.*, 2018, **65**, 431.
86 X. Yang, K. Li and X. Zhang, *Lab Chip*, 2018, **18**, 486.
87 I. Beekers, T. van Rooij and M. D. Verweij, *IEEE Trans. Ultrason. Ferroelectrics Freq. Contr.*, 2018, **65**, 570.
88 S. Lee, S. Jin and Y. K. Kim, *Biomed. Microdevices*, 2017, **19**, 22.
89 H. Kimura, Y. Sakai and T. Fujii, *Drug Metab. Pharmacokinet.*, 2018, **33**, 43.
90 N. Alépée, A. Bahinski and M. Daneshian, *Altex-Altern. Anim. Exp.*, 2008, **31**, 441.
91 M. R. Pulido, M. Garcia-Quintanilla and R. Martin-Pena, *J. Antimicrob. Chemother.*, 2013, **68**, 2710.
92 J. Choi, Y. Jung and J. Kim, *Lab Chip*, 2013, **13**, 280.
93 J. Dai, M. Hamon and S. Jambovane, *Bioengineering*, 2016, **3**, 25.
94 M. Marzorati, B. Vanhoecke and T. De Ryck, *BMC Microbiol.*, 2014, **14**, 133.
95 H. J. Kim, H. Li, J. J. Collins and D. E. Ingber, *Proc. Natl. Acad. Sci. U. S. A*, 2016, **113**, 7.
96 M. M. G. Eain, J. Baginska and K. Greenhalgh, *Engineering*, 2017, **3**, 60.
97 F. Zheng, F. Fu and Y. Cheng, *Small*, 2016, **12**, 2253.
98 D. B. Go, M. Z. Atashbar and Z. Ramshani, *Anal. Methods*, 2017, **9**, 4112.
99 W. Ghazali, R. Iberahim and N. Ashari, *Malays. J. Med. Sci.*, 2017, **24**, 62.
100 Y. Zhao, B. Liang and Q. He, *OncoTargets Ther.*, 2015, **8**, 1075.
101 T. Sahutoglu, T. Sakaci and N. B. Hasbal, *Medicine*, 2017, **96**, e6543.
102 G. G. Nestorova, V. L. Kopparthy and N. D. Crews, *Anal. Methods*, 2015, **7**, 2055.
103 J. Choi, Y. Jung and S. Kwon, *Lab Chip*, 2012, **12**, 280.
104 Y. Lu, J. Gao and P. K. Wong, *Anal. Chem.*, 2013, **85**, 3971.

