Dynamic three-dimensional cell-culture systems for enhanced *in vitro* applications

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The advent of dynamic three-dimensional cell cultures has transformed the field of biological research as they bridge the gap between *in vitro* and *in vivo* systems. 3D cell-culture techniques can be categorized into two types: static and dynamic culture systems. Traditional cell-culture models are considered to be 'static' in nature, as the cells are grown on matrices or scaffolds with little focus given to the complexity of the growth conditions that exist in the *in vivo* tissue microenvironments (presence of continuous blood supply for the development of tumour vasculature). Thus, static 3D cultures do not accurately mimic *in vivo* cellular architecture and function. The development of a 'dynamic' culture environment has offered 3D culture models with the potential to improve the 'naturalness' of the cells being cultured and thereby have more *in vivo* relevance for translational research. This makes them relatively more superior than single cell-type static 3D cell cultures. Dynamic systems include magnetic- and acoustic-based assembly devices, micropocket cultures, dielectrophoretic and microfluidic platforms. Microfluidic devices might be the most versatile of these culture platforms, considering their engineering diversity, their potential to improve molecular crosstalk among culture elements and their prospective range of applications.

Keywords: *In vitro* applications, scaffold-based and scaffold-free systems, static and dynamic culture systems, three-dimensional cell culture.

IN VITRO cell and tissue culture studies have become an indispensable part of fundamental research and translational medicine over the past few decades. Such models offer an opportunity to replicate many characteristic features of *in vivo* environments. The earliest *in vitro* cultures are monolayers of cells cultured on rigid, flat surfaces. These two-dimensional (2D) cell cultures have various advantages associated with easier methodologies and low-cost maintenance, and their usage is also well established in cytotoxicity and drug-penetration studies[1,2]. However, such 2D cultures are minimalistic in their similarities to *in vivo* physiology as they are grown in simplistic, yet improbable conditions. The cells in 2D cultures appear more stretched out and flat than they are *in vivo*, thereby exhibiting a diverse cell morphology, according to the cell types being studied. They show a forced apical–basal polarity and have comparatively expeditious degrees of cell proliferation and poor levels of cell differentiation. They also show varied gene or protein expression levels, which cause a loss of diverse phenotypes. Therefore, cells in 2D cell cultures do not accurately mimic the natural tissue physiology and hence, they do not recapitulate the cell–cell and cell–extracellular matrix (ECM) interactions as observed *in vivo*[2,3].

Comparable replication of a true experimental *in vivo* environment is possible using appropriate animal models. However, the use of these models is mired in ethical controversy, especially concerning the pain and discomfort induced in the animals used; they are also cost-intensive. Furthermore, these animals do not have similar physiological, molecular and functional hallmarks as humans. Manipulated animal models, such as knock-outs or immunocompromised animals, do not replicate the same tumour-stroma physiological interactions (pertaining to drug susceptibility studies and cancer research) as naturally seen in humans. Animal models are therefore not considered to be efficient for accurate translation of laboratory research to clinical settings. For this reason, there arises a need for cellular models, such as the three-dimensional (3D) cell-culture models, that have the potential to better simulate the physiologically relevant complexities and physiognomies that are more close to the complex *in vivo* conditions compared to 2D cell-culture models[4,5]. Three-dimensional cell-culture systems emerged in the 1980s, with an aim to better understand the ECM and as an unconventional approach to better mimic the *in vivo* physiological conditions of tissues. Over the years, many studies have proven the superiority of 3D cultures over 2D cultures in terms of representing the key features of *in vivo* conditions[3,6,7].

A 3D cell culture model is considered to be 'ideal' if it has the ability to imitate a 3D cellular architecture that is biologically relevant to the specific tissue, and where cells are provided with the conditions to proliferate, differentiate and aggregate. These models have the capacity to display variable properties such as cell-to-cell and cell-to-ECM interactions, pH alterations, drug resistance, and fluctuating oxygen, waste and nutrient gradients required

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for the successful culture of cells[3,6]. Three-dimensional cell cultures are considered to be more advantageous than their 2D counterparts when it comes to maintaining cell junctions and allowing cell-to-cell communication. The 3D spheroids seem to have higher resistance to drugs. This gives a more accurate representation of the effects of the drug *in vivo*. For this reason, the 3D cell culture models are more promising when it comes to modelling and studying molecular mechanisms of a particular disease, identification of potential drug targets and also establishing prospective therapeutic strategies[7–11].

There are different types of 3D cell-culture techniques depending on the nature of the experiment being performed. These are mainly divided into either scaffold-based or scaffold-free techniques, or into static 3D and dynamic 3D cultures. The context of grouping 3D culture systems as static or dynamic is based on the absence or presence of a mechanism to constantly perfuse the cultured cells or tissues with the culture medium. For example, a culture model which utilizes only scaffolds or matrices to obtain 3D aggregates of cells is considered static, while an integrated microfluidic device which enables a constant flow-through of the culture medium and enables continuous collection of effluents is considered dynamic. We present here the major differences among scaffold-based and scaffold-free cell and tissue culture systems. Also, a comprehensive account on the various dynamic culture systems is presented.

Static versus dynamic 3D cell cultures

Traditional cell-culture models are considered to be static in nature, as the cells are grown on matrices or scaffolds with little focus given to the complexity of the growth conditions that actually exist in the *in vivo* tissue microenvironments (such as the presence of continuous blood supply for development of tumour vasculature). Contemporarily, there has been a surge in the research brought about by the advent of dynamic cell-culture systems such as microfluidic platforms, magnetic levitation, di-electrophoretic and acoustic-based approaches, that have the potential to improve molecular crosstalk among culture elements. This makes them relatively more superior than single cell-type 3D cell cultures. Due to this, a more dynamic environment is being offered to the 3D culture systems. The term 'dynamic' refers to the concept of inducing continuous motion, which stimulates cells to form aggregates or spheroids without depending on the scaffold. Dynamic culture systems make it possible to culture more than one type of cell, thereby enabling researchers to develop co-cultures, organotypic cultures and biological samples for tissue engineering. Pertaining to cancer research, this modality has made it possible to study the biological, biophysical and biochemical interactions within the tumour microenvironment (TME). Moreover, dynamic 3D cell cultures have shown significant differences in their morphology, proliferation rates and drug responses when compared to static 3D cell cultures[12], while their flow conditions, responses and functions have also enhanced to a greater extent[13]. Figure 1 shows the general scheme of a typical static 3D and dynamic 3D cell culture system; the fundamental difference between them being the continuous perfusion of the culture medium in the dynamic models.

Three-dimensional cell cultures can be obtained with either scaffold-based or with scaffold-free approaches. Scaffold-based techniques aid in providing support for the attachment of cells in a typical 3D cell culture, where the biomaterial used can be derived from either a naturally occurring or a synthetic biopolymer. Most cells residing in the ECM of human tissues are anchorage-dependent and so scaffold construction is based on the principle of recapitulating the ECM function (physiological architecture, biodegradability and mechanical properties compatible with the host tissue) *in vitro*, which has the potential to modify cellular organization and function in response to a particular therapy. With increase in the size and complexity of 3D culture systems, a requirement for the utilization of scaffold becomes more evident[14].

The involvement of scaffolds in 3D cell-culture systems enables spheroid generation in two ways, namely growth by embedding them within the matrix, or by growing them on the surface of the matrix. Three-dimensional spheroids could either be grown on static hydrogel scaffolds, which are considered to be passive in nature, or dynamic scaffolds which are vibratory (active) in nature. Static hydrogels are a suitable platform when it comes to cell behavioural studies

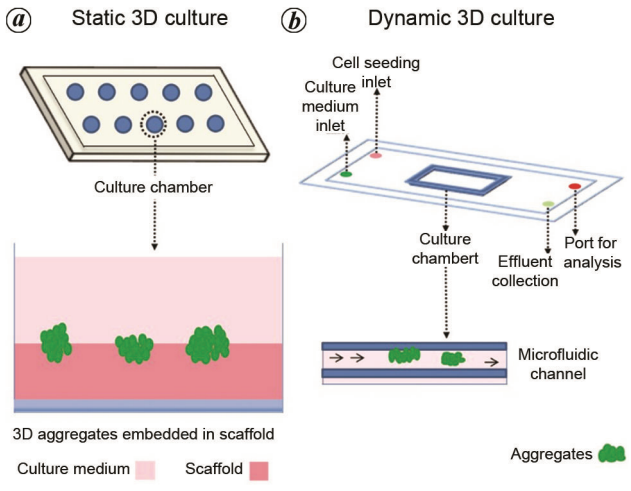

Figure 1. A generalized, typical scheme of (***a***) a static 3D culture and (***b***) a dynamic 3D culture. In static 3D culture systems which are scaffold-based, the cells and the medium do not experience much movement or circulation. However, in a dynamic 3D culture set-up, which may or may not be scaffold-based, the culture medium is always in perfusion, which resembles the natural circulatory system. In such dynamic culture systems, both the effluent and the cell aggregates or patient-derived biopsies can be studied for a variety of end-points.

relating to the physical, chemical and biological functions of the ECM. On the other hand, dynamic hydrogel scaffolds provide a mechanical stimulus to the cell-culture environment that allows for enhanced cell growth, adhesion and differentiation[15].

By displaying dynamic complexity, hydrogels assist in the formation of multicellular constructs through spatial patterning. This has enabled the use of high-throughput assays for studying cellular interactions and cellular behaviour pertaining to various complex physiological conditions such as wound healing[16].

Although the usage of scaffolds for obtaining 3D cell cultures has been shown to have high reproducibility, a natural disadvantage of these systems is that the construction of the scaffolds is arduous and challenging. There is also a variation in the composition between batches of ECM products and the costs associated with their purchase. Physiological ECMs are viscoelastic in nature (they equally display elastic and viscous behaviour), but the synthetic ECMs constructed *in vitro* are characteristically elastic in nature. Scaffolds have been applicable as delivery channels and reservoirs for proteins (growth factors) or drugs and also in tissue recovery experiments. However, for achieving functionally efficient scaffolds, the mechanical properties of the constructed scaffolds should be compatible with the properties of the host tissue, which is difficult to attain as the scaffolds have restricted intake of cells, nutrients and drugs[17]. Also, it is challenging to control and maintain the temperature and pH of the scaffolds, and also to efficiently separate and isolate all the cells for analysis. A consequence of this is decreased cell survival and proliferation[18]. Furthermore, spheroids within the ECM are of dissimilar size, unevenly (in homogeneously) distributed, tend to overlap and thus cannot be used for high-throughput screening or single spheroid analysis. To discourage potential overlap of the aggregates, microwells may be etched into the matrix using photolithography and soft lithography forming a hydrogel microarray[19–21].

This method of obtaining 3D aggregates has been extensively demonstrated in cancer cell lines. Many studies have shown that hydrogels simulate various features of biological tissues. However, they often lack structures that are responsible for stipulating cellular organization, cell–cell and cell–ECM communication, such as fibres. Due to this, these hydrogels cannot completely mimic the biological complexity pertaining to the dynamic constitution of the cellular microenvironments, as seen *in vivo*[16,17].

The motion of fluidic components has been emulated in 3D systems with the advent of many scaffold-free-techniques[10,15,22,23]. These techniques do not utilize any kind of scaffolds or matrices and allow the cells to assemble themselves in the form of free-floating spheroids within a cell suspension or as a cell sheet, wherein the cell components form the ECM in a 3D configuration without any exogenous support. Over the last decade, there has been a significant improvement in scaffold-free techniques. These techniques minimize the limitations of using matrices, associated with matrix-induced alterations in 3D cell culture. If the cells are confluent, then they have the potential to produce high amounts of ECM. Additionally, most of these techniques are generally dynamic in nature, which is an added advantage for understanding complex biological mechanisms. Such techniques are based on principles of electrical polarity, magnetic and acoustics resonance, rotary motion of the culture chamber itself and even microfluidics. Scaffold-free approaches are therefore dependent on the self-assembly of cells which generate biomimetic constructs that are utilized based upon the nature of the study, and the resources and expertise available[17,18,24,25]. Table 1 presents the types of scaffold-free techniques along with their advantages and limitations.

Dynamic 3D cell culture systems and devices

Magnetic levitation methods

Most cell-manipulation techniques are physical and make use of intrinsic cell properties such as density, magnetic susceptibility, electrical capacitance and resistance, or they are affinity-based (such as particle–antibody conjugates particular to a surface protein). Magnetic-assisted assembly depends on the strength and gradient of the magnetic field, and magnetic susceptibility of the cell and its microenvironment. When cells are moved under a magnetic field, the phenomenon is called magnetophoresis. It could either be positive (where the cells move towards a region of elevated magnetic field strength) or negative (where the cells move away from that region) in nature[26].

Since majority of the cells are non-magnetic in nature, researchers usually label cells with magnetic nanoparticles (MNPs), which are biocompatible in nature and have the ability to bind to a target cell selectively, with the help of specific recognition ligands present on their surfaces. The internalization of MNPs into individual cells causes their magnetization, and can influence the phenotype and differentiation ability of the cells. These cells are then assembled with the use of magnetic microtips into a configuration that fits the objectives of the study. In this way, with the help of positive magnetophoresis, cells can either be arranged into 3D spheroids where the size of the cell aggregates can be controlled[27], or they can be patterned into sheet-like structures with a suitable cellular microenvironment[28].

The drawbacks of labelling the cells with MNPs include cellular internalization problems, biological interference caused by the labels and the time taken in performing the steps that are required to inject MNPs into the cells. Thus, alternative label-free magnetic manipulation techniques have been developed over the last decade, which rely on

Table 1. Scaffold-free techniques for obtaining 3D cultures have the advantage of adaptability for scaling-up which is difficult using scaffold-based techniques. Thus large-scale cultures are possible using such scaffold-free approaches. However, as the cells are exposed to constant movement, sheer forces and similar physical disturbances might affect certain properties of the cultured cells

Technique	Method of spheroid/ aggregate generation	Advantages	Limitations	Reference
Hanging drop method	Aliquot of a single-cell suspension on an inverted plate (after seeding of cells) results in the localization of cells at the tip due to surface tension, in the form of compact spheroids	Simple technique Cost-effective High reproducibility Flexibility Generates 3D co-cultures	Difficulty in maintaining spheroids for long-term culture	74–77
Agitation-based techniques Spinner flask bioreactors Rotational flask bioreactors	Cell suspension in culture vessels will be kept in continuous motion by spinning action; promotes cell-to-cell contact and results in spheroid formation Culture flasks rotate horizontally Speed is increased once heavier spheroids are obtained	Encourages circulation of nutrients and removal of toxins Enhances long-term culture Sheer force acting on cells causes little impact Better than spinner flasks	Force of motion can affect cell physiology Spheroids vary greatly in size Non-uniformity in size of spheroids	78, 79
Forced floating technique	Coating of round-bottom plates with agar or pHEMA, post centrifugation, results in multi-cellular sphere formation	Easier for initiation Promotes cell-to-cell contact and adhesion of adjacent cells	Not all cells lines form compact spheroids Time-consuming during pre-coating of the plates Pre-coated plates are expensive	80, 81
Magnetic levitation method	Encapsulation of cells in 2D by magnetic nanoparticles renders them magnetic. External magnetic field exerted by the stirrer on cell suspension causes the cells to be concentrated and levitated at air–liquid interface as spheroids	Extracellular matrix generation more relevant to *in vivo* Shorter duration of initiation of spheroids Magnetic nanoparticles are non-toxic Fine special control with good scalability High-throughput screening	Higher magnetic field strength (800–4000 G) can influence cell behaviour Loss of cells due to poor encapsulation	82–84

the principle of negative magnetophoresis. In this technique, the cells were added to a medium containing a ferrofluid (fluid with magnetic suspension) or a paramagnetic salt solution. This caused the cells to focus within the region of lower magnetic field. In this way, the cells were successfully manipulated according to the magnetic field pattern of the medium[26]. This ferrohydrodynamic cell separation technique was used to enrich low-concentration circulating tumour cells (CTCs) from the blood of patients diagnosed with advanced non-small cell lung cancer (NSCLC), and also for the separation of HeLa (epithelial cervical cancer) cells from blood cells[29,30].

Di-electrophoresis

Particle separation technique is a purification process that works on the principle of extracting particles from a suspension or isolating them from other particles based on the differences in their chemical and physical properties. Di-electrophoresis is a type of particle separation technique that generates polarization forces to separate the suspended particles (which must be polarizable, but do not carry electrical charges) in the carrier fluid, which takes place within a non-uniform electric field (NUEF), after which the particles form electric dipoles. Nowadays this technique is commonly used with microfluidic bioparticle separation platform. It makes use of electricity which is supplied with the help of external electrodes (which produce the NEUF in the chip). The performance of di-electrophoresis (DEP) depends on the gradient of the electric field which thereby manipulates the spatial organization of cells. Due to its selectivity and accuracy, DEP is applicable in the fields of medicine and diagnostics. In DEP, the cultured cells do not sustain any harmful effects that may be a consequence of internalization of MNPs[31].

Acoustics

This has been widely used in biomedical research for droplet and bioparticle manipulation. Acoustics-based assembly efficiently makes use of non-invasive sound waves for the assembling of microgels (microscale hydrogels)

within seconds. Microgels can be used as carriers for cells or as drug-delivery systems and have often been labelled as the 'building blocks' of regenerative medicine and tissue engineering. For this, the microgels need to be manipulated via an assembly for fabricating larger constructs. Such an assembly in the microscale has been applicable for intracellular manipulation of particles, bioengineering, diagnostics and other biomedical applications. Acoustic-based approaches provide better flexibility and biocompatibility when it comes to spheroid fabrication, so as to carry out contact-free manipulation of cells while maintaining their native state[32].

Over the last decade, surface acoustic waves (SAWs; which are acoustic waves distributed along the surface of an elastic material) have been effectively used to control particles and fluids on microfluidic devices. Research has shown that with the help of a travelling SAW, the association between microfluidic devices and acoustic waves has the capacity to produce interference (standing wave) patterns within the microscale. The forces that are generated as a result of this interference can be used for the manipulation of particles and cells[33].

Fast fluid actuation, ease of fabrication, versatility and compatibility with other microfluidic devices are some of the advantages of SAW microfluidics. SAW-based microfluidics has been used for applications such as cell-focusing, manipulation, sorting, enrichment, biosensing, etc.[34].

MicroPocket Culture system

The MicroPocket Culture (MPoC) system is a biofabrication strategy that consists of valves prepared in a plate containing polyacrylamide hydrogels that aid in the robust development of spherical, uniformly sized aggregates or spheroids. Due to the geometry of this system, the spheroids formed can be maintained at precise positions in the plate, which makes it fairly easy to analyse them. It allows individual cells to enter one at a time, but prevents the escape of the aggregate once it is formed. These systems have therefore been developed to address issues, especially when it comes to rare samples involving washing, labelling, stimulating and imaging aggregates (which could make them susceptible to accidental sample loss and damage). MPoC has an open-faced-design that allows for the use of standard pipette-based handling techniques which are available in most wet laboratories, and fixes the aggregate spatially even during rigorous washing and liquid-handling steps. In the hydrogel-based pockets, aggregates can be easily transferred and embedded into an ECM on the device, enabling precise formation of tissues. The limitation of this technique is that it is only able to give rise to homogenous cell aggregates. Also, high seeding density is essential to obtain uniform-sized spheroids; hence, the cell count needs to be high. A template mould with overhanging structures was microfabricated using a 3D printer, which was later replica-moulded in a polyacrylamide hydrogel for long-term culture into the micropocket chamber[35].

Microfluidics

Microfluidics is a research area that involves the manipulation of fluids (which exhibit a laminar flow instead of a turbulent flow) in the range of microlitres and picolitres, through various microchannels that are tens to hundreds of micrometres in diameter. Depending on the substrate that is utilized for manufacturing devices, the devices are grouped into paper-based, polymer-based and glass- or silicon-based platforms. Microfluidic platforms enable growing of cells on a chip with continuously circulating media so as to mimic the *in vivo* microenvironments. This conserves the cell–cell and the cell–matrix interactions, taking into account cell signalling, proliferation, differentiation and cell death[36].

Working within the microscale level gives the opportunity to reduce the experiment time, boost the accuracy of the experiments while consuming fewer volumes of reagents and also enables researchers to run parallel experimental analyses[37].

There are various end-points that can be assessed with the use of microfluidics, such as examining the morphological aspects of different cancer cell lines, their protein and gene expressions and how susceptible they are to various cytotoxic and genotoxic agents. These devices allow the integration of various stimuli within the cellular microenvironment and aid in creating pertinent *in vitro* conditions for analysing the effects of certain chemotherapeutic agents on biopsy samples or on cancer cell lines grown *in vitro*, which have led to the development of approaches regarding personalized medicine[38]. The different types of microfluidic devices are listed in Table 2 along with a brief description of their design, principles, advantages and applications.

Polydimethyl-siloxane-based microfluidic devices: Since its introduction in 1998 by the White sides group[39], soft lithography has been used for most of the research work pertaining to microfluidics. Polymers are popularly used worldwide because they have a good biochemical performance and are less expensive compared to other materials. The polymer that is most utilized for fabrication of microfluidic devices is polydimethyl-siloxane (PDMS), which is also known as dimethicone; it is a part of the siloxane family. Soft lithography used as PDMS is originally a liquid before it is cured to form patterns by treatment with high temperature, after which it is etched (because of its softness) into patterns or multiple channels to build models for various biomedical applications. These can also easily integrate electronics[40].

Table 2. Microfluidic devices are probably the most versatile for obtaining 3D cell cultures. Such devices can be fabricated to meet specific experimental requirements for studies on samples ranging from simple single-cell type cultures to tissue biopsies. Also, these devices enable studying several parameters on the culture effluents and the cultured cell aggregates or tissue biopsies

Type of microfluidic device	Design	Principle	Benefits in 3D culture	Applications	Reference
Lab-on-a-chip	Microchip with intricate network of nanometre–micrometre-sized channels, electrodes, sensors and electrical currents	Miniaturized biomedical or chemistry laboratories built on a small chip	More efficiently recapitulates *in vivo* tissue microenvironment Minimal handling Decreases human error	DNA analysis Biomarker identification for disease diagnostics Study *in vivo* cellular physiology	85
Centrifugal microfluidic device (lab-on-a-CD)	Rotating device composed of a DC motor, rotating platform and speed controller	Cells arrayed in a circular fashion with identical hyper-gravity generated by centrifugal force	Significant maintenance of sphericity (cell shape and size) in spheroids	Mono and co-culture of 3D spheroids in various shapes	86
Droplet (digital) microfluidic device	Based on channel geometries, droplets are produced by co-flowing, T-junction and flow-focusing	Uses immiscible substances for the generation of droplets at the junction of the microchannels	Monodisperse droplets at the rate of 1000 droplets/sec Mass transport Scalability Individual control	Co-culture High-throughput screening in drug efficacy studies Biomimetics Organotypic culture	87–91
Insert-based microfluidic device	Laser-cut fibrous inserts assembled on 3D-printed microchips integrated with an analytical module	Fibrous scaffold-based microfluidic device	Understanding of cell-to-cell interactions Scalability Versatility	Co-culture in macrophage activation studies	12
Valve-enabled microfluidic device	Pressure-driven valve barrier separating two chambers	Passive pumping method combined with microfluidics	Study cell-to-cell interactions Cell migration	2D and 3D co-cultures. Real-time imaging of synapse in hippocampal neurons (neurobiology)	92
Organ-on-a-chip device	Ranges from a single perfused microchamber to complex designs with two or more channels	Cultures cells using continuous flow-delivery systems	More efficiently recapitulates tissue and organ *in vivo* characteristics Real-time imaging High resolution imaging	Organ-on-chips model High-throughput drug cytotoxicity Applicable in studying organ physiology and pathology	93, 94

It is a hydrophobic elastomer that is highly biocompatible, fairly inexpensive and easily mouldable. The several properties of PDMS such as its hydrophobicity, high elasticity, chemical inertness, low thermal conductivity, low toxicity, high optical transparency and convenience of handling make it favourable for use in cell and tissue culture. One of the major limitations of PDMS is that it undergoes ageing, and this restricts the performance of the device. Another drawback of PDMS is that it is gas-permeable and undergoes deformation while in the presence of certain chemicals such as strong organic solvents. Therefore, other materials such as glass and silicone are used for construction of the devices as they have significant solvent resistance[41].

Biomedical applications of microfluidic devices: Microfluidic technology offers the flexibility for carrying out different applications that extend to areas of life sciences, drug discovery, chemistry, personalized medicine, diagnosis, 3D printing, cell culture, agriculture and environmental sciences. Microfluidic chips enable considerable parameter regulation, which results in exceptional data quality. These devices require minimal sample handling and enable less consumption of the samples and reagents, thereby reducing the total cost of the experiment. Moreover, easier automation, portability and running parallel analyses are possible due to their compact size, which has allowed researchers to boost their analytical sensitivity, improved temperature/pH control and has condensed the overall experimental time.

Cancer: Lab-on-a-chip (LOC) devices usually focus on diseases diagnosis and DNA analysis. These devices have proven to be pertinent in identifying cancer biomarkers, in capturing and diagnosing circulating tumour cells (CTCs)[42–44], in recapitulating the *in vivo* microvasculature[45] and biomimicking carcinogenetic processes such as metastasis[46].

When testing for oral squamous cell carcinoma, the LOC that was used integrated saliva as the input sample through which the recognition of precancerous dysplastic and cancerous cells was carried out by cell-surface proteins which had distinctive gene transcription profiles[47].

Pauty *et al.*[48] established a vascular endothelial growth factor (VEGF)-dependent microvessel-on-a-chip model that mimicked an angiogenic sprouting from a primary blood vessel. This model allowed them to demonstrate the role of the NOTCH pathway in regulating the process of angiogenesis, to analyse the function of the endothelial barrier and study the effects of angiogenic inhibitors (sunitinib and sorafenib) on the VEGF-A/VEGFR-2 angiogenesis pathway.

Neurological diseases: Organ-on-chip (OOC) devices are used in translational research for comprehending the mechanisms of certain diseases and predicting the responses of humans to new therapies. By mimicking the native cellular microenvironment, these devices can reproduce the dynamic conditions of specific organs within the microfluidic chips *in vitro*. With respect to neurodegenerative disorders, OOC models can be used to study neural cell morphology, cell–cell interactions, establish neural networks[49], neuronal activity patterns[50] and to understand intraneuronal signalling by analysing the influence of biochemical (cytokines, chemokines and transcription factors) and mechanical factors (stiffness, sheer stress, confinement and interstitial flow) on neural and skeletal muscle cells in the central and peripheral nervous systems[51].

BBB (blood-brain barrier)-based microfluidic models enable researchers to understand the pathological mechanisms responsible for neurodegenerative disorders and also aid in developing drug-delivery systems[52,53]. A 3D human model of a neurovascular unit focusing on BBB was designed in 2019 by Brown *et al.*[54], which utilized human primary astrocytes and cerebral microvascular endothelial cells for real-time analysis of the pathological and physiological complexities of the human BBB.

Shin *et al.*[55] constructed a microfluidic platform for successfully evaluating the BBB function by *in vitro* modelling of the Alzheimer's disease (AD) system through the culture of brain endothelial cells. This model simulated the vascular remodelling that is observed AD patients; increased BBB permeability, accumulation of β-amyloid clumps, increased reactive oxygen species, matrix-metalloproteinase-2 (MMP2) and interferon-γ (INF-γ) expression, along with decreased expression of adherens junction and tight junction proteins[56].

The OOC models are also important for improving the drug screening for neurodegenerative disorders. Such brain-on-a-chip models using differentiated neuronal cells have proven useful for understanding disease development and progression apart from studying the effects of pharmacological interventions[56].

Cardiothoracic diseases: A reliable *in vitro* 3D cardiac tissue model should be able to mimic the electromechanical stimuli, in the presence of biochemical signals, corresponding to the native microenvironment of the myocardium. Marsano *et al.*[57] established a heart-on-a-chip platform containing a pneumatic actuation system, that applied uniaxial cyclic strain, on the cardiomyocytes derived from rat and human-induced pluripotent stem cells (iPSC), enabling them to produce efficient microengineered cardiac tissues (μECTs). Through biochemical and mechanical co-stimulation, this model aided in predicting cardiac hypertrophy[57]. Recently, another high-throughput model was developed by Parsa *et al.*[58] for the examination of pathological cardiac hypertrophy, that allows dynamic handling of cardiac microtissues.

Lee *et al.*[59] developed a microfluidic electrochemical biosensor for simultaneous biomarker analysis of multipulmonary hypertension disease. They constructed a microchip integrating five chambers equipped with in-built pneumatic microvalves for flow manipulation. Each chamber was separately connected to an electrochemical sensor for detecting four different biomarkers associated with pulmonary hypertension (low density lipoprotein (LDL), adiponectin, 8-isoprostane and fibrinogen) and a reference control. This approach could be utilized for rapid diagnosis of various human diseases through biomarker detection.

In 2019, with the intent to repair and regenerate ischaemic tissues, an endothelial biomimetic microvessel patch containing vascularized cardiac stem cells was constructed by Su *et al.*[60]. This patch mimicked the function and microarchitecture of venules and capillaries, and was transplanted into an immunodeficient nude rat model with acute myocardial infarction. Four weeks post treatment, it was observed that the patch promoted neovascularization and active proliferation of cardiomyocytes at the site of injury[60].

Renal diseases: The renal microenvironment plays a major role in the *in vitro* evaluation of pathophysiology and disease progression of renal diseases. Thus, a novel, reusable, proximal tubule microfluidic device that incorporated the function of the glomerulus was constructed to study the effects of sheer stress on the most standard renal disease states (kidney stones, hyperglycaemia, increased glomerular filtration rates (GFR) and drug-induced nephrotoxicity). This study allowed more physiologically relevant prediction of human renal responses to stress-related conditions[61].

The pathophysiology of acute kidney injury (AKI) is consistent with nephrotoxicity, sepsis and ischaemia. Among these, pharmaceutical nephrotoxicity is the main cause of AKI. In 2018, Qu *et al.*[62] developed a biomimetic, multilayer, dynamic nephron-on-a-chip to study the pathophysiology of AKI triggered by nephrotoxic drugs. This model helped in identifying altered states of pathogenesis

Table 3. A variety of microfluidic devices have been developed for the rapid and precise detection of bacteria and viruses. These can be utilized for not only human healthcare diagnostic applications, but also for testing samples such as milk for contamination

Target pathogen	Detection method	Technique used	Results	Reference
Salmonella typhimurium, *Vibrio parahaemolyticus*	Rotary microfluidic device incorporating LAMP and lateral flow strip-based detection	Centrifugal microfluidic technique applying microbead-based DNA extraction followed by LAMP and colorimetric lateral flow strip-based detection of pathogens	Multiplex analysis of *S. typhimurium* and *V. parahaemolyticus* in 80 min with an LOD of 50 CFU	95
S. typhimurium	Flow-focusing, droplet-based microfluidic device integrating LAMP assay	~10^6 simultaneous LAMP-assisted amplification reactions in millions of picolitre-sized water-in-oil droplets	Rapid detection of *S. typhimurium* from pure culture and contaminated milk samples	96
Hepatitis B virus	AuNP-PDMS amalgamated, LED-driven droplet microfluidic chip integrating digital LAMP	Au-NPs-PDMS-based LAMP chip that coalesced NIR-LED heating with fluorescent detection for absolute quantification of HBV	Detection of HBV DNA at 1×10^1 to 1×10^4 copies/µl concentration	97
Staphylococcus aureus	PDMS-based microfluidic chip integrating ~50–90 µm microspheres with coated *S. aureus* antibody	Semi-automatic microfluidic chip containing immune beads to capture and detect *S. aureus*	Detection of *S. aureus* at a detection limit of 1.5×10^1 CFU/µl and an injection rate of 5 µl/min reacted for 4 min	98
H1N1	PDMS-based preconcentration and nucleic acid amplification microfluidic device mounted on a heat block	Magnetic preconcentration of influenza A H1N1 virus in saliva samples with antibody-conjugated magnetic nanoparticles followed by on-chip RT-PCR	Detection of influenza A H1N1 virus in saliva samples within 2 h, with a LOD of 100 TCID50 (50% tissue culture infective dose)	99
SARS-CoV-2	Glass microfluidic CRISPR-based chip, integrating Isotachophoresis-purification of nucleic acids	Electrokinetic microfluidic technique implementing LAMP and CRISPR-Cas12 system for the detection of SARS-CoV-2	Detection of SARS-CoV-2 RNA from NP swab samples in 35 min post	100

LAMP, Loop-mediated isothermal amplification; ITP, Isotachophoresis; LOD, Limit of detection; CFU, Colony forming units; K562, Human CML cell line; THP-1, Human acute monocytic leukaemia cell line; Jurkat, Human acute T cell leukaemia cell line; AuNPs, Gold nanoparticles and NIR, Near infrared.

for cisplatin- and doxorubicin-induced nephrotoxicity, and it can be used as a reference for the assessment of drug toxicity and in clinical therapy[62].

A three-layer microfluidic kidney chip was also developed for nephrotoxicity evaluation, which consisted of a flow temperature-controlled platform and a drug concentration gradient generator for the efficient culturing of kidney cells[63].

Cell-behaviour studies: In 2018, Jastrzebska *et al.*[64] carried out the biological characterization of PDMS-based devices for studies aimed at comprehending cell behaviour and adhesion mechanisms. Toxicity assays of Balb 3T3/c, HMEC-1 and HT-29 cell lines were performed with celecoxib and oxaliplatin on PDMS devices refined with collagen, fibronectin and gelatin. They observed that normal cells are more sensitive to drugs than cancer cells. Also, the greatest cell viability, adhesion and proliferation were obtained by plasma activation. They also observed that a highly specific environment is not mandatory for the proliferation of carcinoma cells and how cell behaviour is influenced by the surface area to volume ratio (SA/V). These experiments are important for establishing distinct growth conditions for different cell types[64].

A novel microfluidic droplet-based system was fabricated for the maintenance of 3D cell cultures (post encapsulation) of human primary multiple myeloma (MM) stem cells and human mesenchymal stem cells (hMSCs), for generating a multicellular stem cell micro-niche within the microchip. This was done to observe the stem-cell behaviour, and test the therapeutic effects and possible toxicity of bortezomib and lenalidomide on the MM samples obtained from patients to develop a platform for *ex vivo* personalized drug screening[65].

Also, a centrifugal-based droplet microfluidic device was constructed for the generation of droplet-based 3D

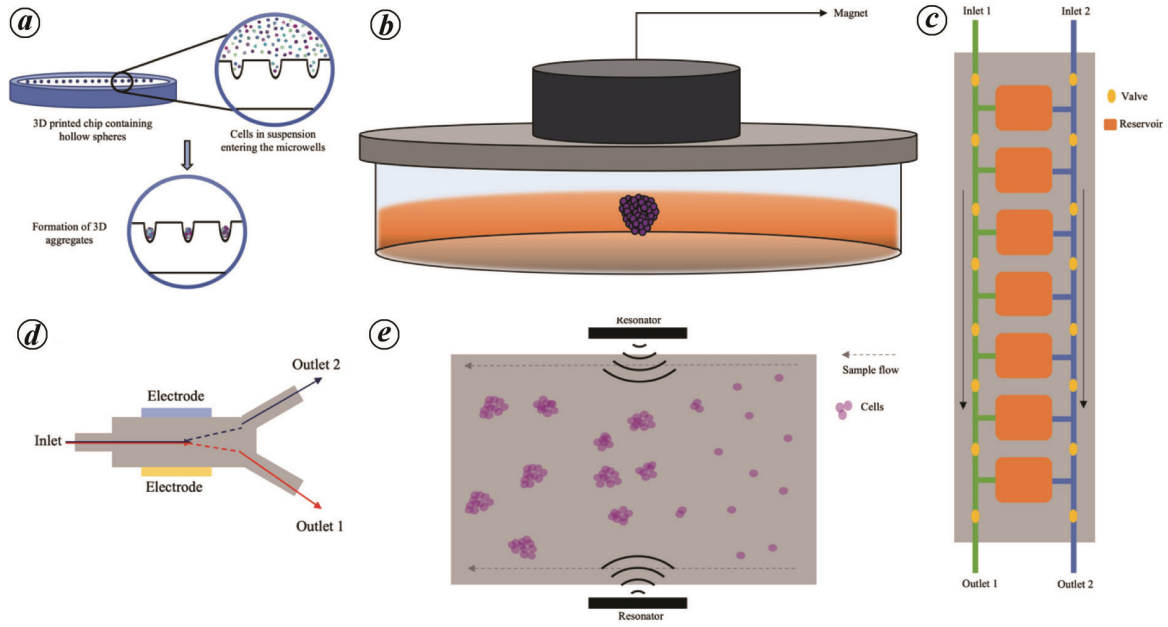

Figure 2. Due to advancements in culture devices, it is now possible to mimic dynamic *in vitro* cell and tissue culture conditions for a multitude of applications. Such devices integrate several biotechnological and biomedical applications for the purpose of mimicking *in vivo* physiological conditions to the best extent *in vitro*. Magnetic- and acoustic-based assembly devices, micropocket cultures, di-electrophoresis and microfluidic platforms are versatile culture platforms that display dynamic complexity. ***a***, Micropocket cultures allow the robust formation of hundreds of uniformly sized 3D aggregates inside the 3D printed microchannels. ***b***, Cells are labelled with magnetic nanoparticles, which upon exposure to a static magnet, can be assembled into a confirmation that fits the objective of the study being carried out. ***c***, In microfluidic devices, cells, media and any other bioparticles move in a laminar flow in microchannels to mimic cell–cell and cell–matrix interactions. ***d***, In di-electrophoretic devices, polarization forces are generated to separate suspended particles for the purpose of bioparticle separation. ***e***, In acoustics-based assembly, surface acoustic waves, generated by bulk resonators, produce standing-wave patterns along which bioparticles can be manipulated.

cell cultures, with each cell-encapsulated microsphere adjusted to encompass hundreds of cells. This device demonstrated week-long culture duration with controlled cell occupancy that was applicable for rapid 3D spheroid formation suitable for bioreactor studies of suspension cultures[66].

Pathogen identification: Pathogen identification in the early stages of a disease is significant as it aids in determining the choice of therapy. Microfluidic devices in the form of optical-based chips have proven to be effective in rendering rapid diagnosis for a variety of reasons. These microfluidic chips have high sensitivity for a specific pathogen, while relying on small sample volume requirements. Such devices are rapid and economical, as there is considerable reduction in the requirement of reagents as otherwise required for conventional diagnostic methods[67]. Microfluidic devices have been developed to detect airborne, foodborne and waterborne pathogens[68–70]. Integration of electronics and fluid dynamics concepts has enabled in the rapid detection of pathogens such as *Salmonella typhimurium*, *Vibrio parahaemolyticus*, hepatitis B virus, *Staphylococcus aureus*, H1NI and SARS-CoV-2 in the saliva, swab-obtained and milk samples (Table 3).

Microfluidic platforms as gene delivery systems: Chips developed on microfluidic device platforms have proven valuable for cell manipulation, exosome categorization and drug screening[71]. Microfluidic devices have aided in boosting the efficiency of gene transfection by delivering nucleic acids into hard-to-transfect cell lines such as embryonic stem cells and lymphoma cells. Also, such transfected cells showed higher viability compared to those transfected by traditional methods. Han *et al.*[72] introduced a microfluidic platform that achieved membrane deformation which made possible passive diffusion of Cas9 and sgRNA into cells, which are crucial for the activity of the CRISPR/Cas9 genome editing system. Li *et al.*[73] demonstrated a droplet microfluidics platform to transfect suspension cells which are considered to have a lower transfection efficiency otherwise (~5%). To accomplish successful gene delivery, this platform performed co-encapsulation of cationic lipids and plasmids in microdroplets. Upon undergoing chaotic advection, they gave rise to uniform lipoplexes containing enhanced green fluorescence protein (pcDNA3-EGFP) plasmids. These were then delivered to three suspension cell lines (Jurkat, THP-1 and K562) at an enhanced transfection efficiency (~50%) and lower transfection variability. Furthermore, they also demonstrated effectual *TP53BP1* gene knockout in K562 cells using the CRISPR-Cas9 mechanism. Figure 2 presents the principle, the general architecture and the mode of functioning of a few scaffold-free dynamic cell culture systems and devices that are discussed as follows.

Conclusion

It is increasingly apparent that 3D cell-culture systems are superior to conventional 2D cell-culture systems due to a variety of factors. Over the last decade, substantial progress has taken place to obtain active, dynamic cell-culture models in lieu of passive, static cell-culture models, which offer a way to obtain physiologically functional biological microenvironments *in vitro*. Scaffold-free culture systems such as microfluidics, micropocket cultures and cell-culture platforms integrating magnetic levitation, acoustic and di-electrophoretic assemblies have helped in obtaining dynamic 3D cell-culture models. These models have abundant prospects when it comes to fundamental, multidisciplinary and translational research. Fundamental research is aimed at gaining an insight into the actual biological process in the wake of certain diseases such as cancer. Therefore, dynamic cell-culture systems allow us to better mimic biological components, which in turn provide a more enriched functional niche for the 3D aggregates, spheroids, organoids or any other biological materials being studied.

Dynamic culture systems help in remodelling the ECM and allow better intracellular interactions (cell–cell and cell–ECM) in order for the cells to attach, proliferate and differentiate more accurately *in vitro*. Thus, with an improved nutrient supply and other pertinent physiochemical cues, dynamic cell-culture platforms offer effective growth conditions, better heterogeneity and improved cell-to-cell crosstalk for obtaining a tissue-specific dynamic environment that allows the cells to accurately recapitulate the tissue microenvironment.

The future research directions would be in developing point-of-care dynamic cell-culture systems, such as microfluidic platforms integrating microvasculatures, which allow for continuous perfusion, and enable efficient exploration of therapeutic possibilities. Devices which mimic human-like biological features by incorporating engineering designs inspired by human anatomy and tumour micro- and macro-environments will enable testing biological samples such as patient-derived tumour biopsies against chemotherapeutic and radiotherapeutic agents effectively. Such an advancement will be beneficial for personalized or individualized medicine, especially for conditions such as head and neck cancers where inter-individual differences pose a challenge in terms of therapeutic responses and outcomes.

1. Amelian, A., Wasilewska, K., Megias, D. and Winnicka, K., Application of standard cell cultures and 3D *in vitro* tissue models as an effective tool in drug design and development. *Pharmacol. Rep*., 2017, **69**(5), 861–870.
2. Edmondson, R., Broglie, J. J., Adcock, A. F. and Yang, L., Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. *Assay Drug Dev. Technol.*, 2014, **12**(4), 207–218.
3. Langhans, S. A., Three-dimensional *in vitro* cell culture models in drug discovery and drug repositioning. *Front. Pharmacol.*, 2018, **9**(6), 1–14.
4. Hoarau-Véchot, J., Rafii, A., Touboul, C. and Pasquier, J., Halfway between 2D and animal models: are 3D cultures the ideal tool to study cancer-microenvironment interactions? *Int. J. Mol. Sci.*, 2018, **19**(1), 181.
5. Mark, I. W., Evaniew, N. and Ghert, M., Lost in translation: animal models and clinical trials in cancer treatment. *Am. J. Transl. Res*., 2014, **6**, 114–118.
6. Souza, A. G. *et al.*, Comparative assay of 2D and 3D cell culture models: proliferation, gene expression and anticancer drug response. *Curr. Pharm. Des*., 2018, **24**(15), 1689–1694.
7. Maddaly, R., Paramesh, V., Kaviya, S. R. and Anuradha, E., Solomon FDP 3D cell culture systems: advantages and applications. *J. Cell. Physiol*., 2015, **230**(1), 16–26.
8. Imamura, Y. *et al*., Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer. *Oncol. Rep*., 2015, **33**(4), 1837–1843.
9. Costa, E. C., Moreira, A. F., de Melo-Diogo, D., Gaspar, V. M., Carvalho, M. P. and Correia, I. J., 3D tumor spheroids: an overview on the tools and techniques used for their analysis. *Biotechnol. Adv*., 2016, **34**(8), 1427–1441.
10. Chaicharoenaudomrung, N., Kunhorm, P. and Noisa, P., Three-dimensional cell culture systems as an *in vitro* platform for cancer and stem cell modelling. *World J. Stem Cells*, 2019, **11**(12), 1065–1083.
11. Kapałczyńska, M. *et al.*, 2D and 3D cell cultures – a comparison of different types of cancer cell cultures. *Arch. Med. Sci*., 2018, **14**(4), 910–919.
12. Chen, C., Townsend, A. D., Hayter, E. A., Birk, H. M., Sell, S. A. and Martin, R. S., Insert-based microfluidics for 3D cell culture with analysis. *Anal. Bioanal. Chem*., 2018, **410**(12), 3025–3035.
13. Wu, Q. *et al.*, Organ-on-a-chip: recent breakthroughs and future prospects. *BioMed. Eng. OnLine*, 2020, **19**, 9.
14. Jensen, C. and Teng, Y., Is it time to start transitioning from 2D to 3D cell culture? *Front. Mol. Biosci*., 2020, **7**, 33.
15. Yuan, H., Xing, K. and Hsu, H. Y., Trinity of three-dimensional (3D) scaffold, vibration and 3D printing on cell culture application: a systematic review and indicating future direction. *Bioengineering (Basel)*, 2018, **5**(3), 57.
16. Burdick, J. A. and Murphy, W. L., Moving from static to dynamic complexity in hydrogel design. *Nature Commun*., 2012, **3**(1269), 1–8.
17. Alghuwainem, A., Alshareeda, A. T. and Alsowayan, B., Scaffold-free 3-D cell sheet technique bridges the gap between 2D cell culture and animal models. *Int. J. Mol. Sci.*, 2019, **20**(19), 4926.
18. Antoni, D., Burckel, H., Ejosset, E. and Noel, G., Three-dimensional cell culture: a breakthrough *in vivo. Int. J. Mol. Sci.*, 2015, **16**(3), 5517–5527.
19. Haycock, J. W., 3D cell culture: a review of current approaches and techniques. *Methods Mol. Biol*., 2011, **695**, 1–15.
20. Chaudhuri, O. *et al.*, Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nature Mater*., 2016, **15**(3), 326–334.
21. Chan, B. P. and Leong, K. W., Scaffolding in tissue engineering: general approaches and tissue-specific considerations. *Eur. Spine J.*, 2008, **17** (*Suppl. 4*), 467–479.
22. Subramaniyan, A. and Maddaly, R., Agarose hydrogel induced MCF-7 and BMG-1 cell line progressive 3D and 3D revert cultures. *J. Cell. Physiol*., 2018, **233**(4), 2768–2772.
23. Lelièvre, S. A., Kwok, T. and Chittiboyina, S., Architecture in 3D cell culture: an essential feature for in vitro toxicology. *Toxicol. in Vitro*. 2017, **45**(Pt 3), 287–295.
24. Han, H. W., Hou, Y. T. and Hsu, S. H., Angiogenic potential of cospheroids of neural stem cells and endothelial cells in injectable

gelatin-based hydrogel. *Mater. Sci. Eng. C*, 2019, **99**, 140–149.
25. Lembong, J., Lerman, M. J., Kingsbury, T. J., Civin, C. I. and Fisher, J. P., A fluidic culture platform for spatially patterned cell growth, differentiation and cocultures. *Tissue Eng. – Part A*, 2018, **24**(23–24), 1715–1732.
26. Yaman, S., Anil-Inevi, M., Ozcivici, E. and Tekin, H. C., Magnetic force-based microfluidic techniques for cellular and tissue bioengineering. *Front. Bioeng. Biotechnol*., 2018, **6**, 192.
27. Parfenov, V. A. *et al.*, Scaffold-free, label-free and nozzle-free biofabrication technology using magnetic levitational assembly. *Biofabrication*, 2018, **10**(3), 034104.
28. Whatley, B. R., Li, X., Zhang, N. and Wen, X., Magnetic-directed patterning of cell spheroids. *J. Biomed. Mater. Res. Part A*, 2014, **102**(5), 1537–1547.
29. Zhao, W. *et al.*, Label-free and continuous-flow ferrohydrodynamic separation of HeLa cells and blood cells in biocompatible ferrofluids. *Adv. Funct. Mater*., 2016, **26**(22), 3990–3998.
30. Zhao, W. *et al.*, Label-free ferrohydrodynamic cell separation of circulating tumor cells. *Lab. Chip*., 2017, **17**(18), 3097–3111.
31. Zhang, H., Chang, H. and Neuzil, P., DEP-on-a-chip: dielectrophoresis applied to microfluidic platforms. *Micromachines (Basel)*, 2019, **10**(6), 423.
32. Xu, F. *et al.*, The assembly of cell-encapsulating microscale hydrogels using acoustic waves. *Biomaterials*, 2011, **32**(31), 7847–7855.
33. Collins, D. J., O'Rorke, R., Neild, A., Han, J. and Ai, Y., Acoustic fields and microfluidic patterning around embedded microstructures subject to surface acoustic waves. *Soft Matter*, 2019, **15**, 8691–8705.
34. Ding, X. *et al.*, Surface acoustic wave microfluidics. *Lab. Chip*., 2013, **13**, 3626–3649.
35. Zhao, L., Mok, S. and Moraes, C., Micropocket hydrogel devices for all-in-one formation, assembly, and analysis of aggregate-based tissues. *Biofabrication*, 2019, **11**(4), 045013.
36. Carr, S. D., Green, V. L., Stafford, N. D. and Greenman, J., Analysis of radiation-induced cell death in head and neck squamous cell carcinoma and rat liver maintained in microfluidic devices. *J. Otolaryngol. Head Neck Surg.*, 2014, **150**, 73.
37. Gale, B., Jafek, A., Lambert, C., Goenner, B., Moghimifam, H., Nze, U. and Kamarapu, S. A., Review of current methods in microfluidic device fabrication and future commercialization prospects. *Inventions*, 2018, **3**, 60.
38. Jodat, Y. A. *et al.*, Human-derived organ-on-a-chip for personalized drug development. *Curr. Pharm. Des.*, 2018, **24**(25), 5471–5486.
39. Duffy, D. C., McDonald, J. C., Schueller, O. J. A. and Whitesides, G. M., Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Anal. Chem.*, 1998, **70**, 4974–4984.
40. Ziolkowska, K., Jedrych, E., Kwapiszewski, R., Lopacinska, J., Skolimowski, M. and Chudy, M., PDMS/glass microfluidic cell culture systemfor cytotoxicity tests and cell passage. *Sensor. Actuat. B*, 2010, **145**(1), 533–542.
41. Friend, J. and Yeo, L., Fabrication of microfluidic devices using polydimethylsiloxane. *Biomicrofluidics*, 2010, **4**(2), 1–5.
42. Chen, W. *et al.*, Nanoroughened adhesion-based capture of circulating tumor cells with heterogenous expression and metastatic characteristics. *BMC Cancer*, 2016, **16**(614), 1–12.
43. Qian, C. *et al.*, Clinical significance of circulating tumor cells from lung cancer patients using microfluidic chip. *Clin. Exp. Med*., 2018, **18**, 191–202.
44. Hosseini, S. A. *et al.*, Cancer diagnosis: nanoelectromechanical chip (NELMEC) combination of nanoelectronics and microfluidics to diagnose epithelial and mesenchymal circulating tumor cells from leukocytes. *Small*, 2016, **12**, 883–891.
45. Pollet, A. M. A. O. and Toonder, J. M. J., Recapitulating the vasculature using organ-on-chip technology. *Bioengineering (Basel, Switzerland)*, 2020, **7**(1), 17.
46. Kong, J. *et al.*, A novel microfluidic model can mimic organ-specific metastasis of circulating tumor cells. *Oncotarget*, 2016, **7**(48), 78421–78432.
47. Tang, M., Wang, G., Kong, S. K. and Ho, H. P., A review of biomedical centrifugal microfluidic platforms. *Micromachines*, 2016, **7**(2), 1–29.
48. Pauty, J. *et al*., A vascular endothelia growth factor-dependent sprouting angiogenesis assay based on an *in vitro* human blood vessel model for the study of anti-angiogenic drugs. *EBioMedicine*, 2018, **27**, 225–236.
49. Wijdeven, R., Ramstad, O. H., Bauer, U. S., Halaas, O., Sandvig, A. and Sandvig, I., Structuring a multi-nodal neural network *in-vitro* within a novel design microfluidic chip. *Biomed. Microdevices*, 2018, **20**(1), 9.
50. Courte, R., Renault, A., Jan, J. L., Viovy, J. M., Peyrin, C. and Villard, C., Reconstruction of directed neuronal networks in a microfluidic device with asymmetric microchannels. *Methods Cell Biol.*, 2018, **148**, 71–95.
51. Osaki, T., Shin, Y., Sivathanu, V., Campisi, M. and Kamm, R. D., *In vitro* microfluidic models for neurodegenerative disorders. *Adv. Healthc. Mater*., 2018, **7**(2).
52. Cho, H. *et al.*, Three-dimensional blood-brain barrier model for *in vitro* studies of neurovascular. *Pathology. Sci. Rep.*, 2015, **5**(15222), 1–9.
53. Modarres, H. P. *et al.*, *In vitro* models and systems for evaluating the dynamics of drug delivery to the healthy and diseased brain. *J. Control Release*, 2018, **273**, 108–130.
54. Brown, T. D. *et al.*, A microfluidic model of human brain (μHuB) for assessment of blood brain barrier. *Bioeng. Transl. Med.*, 2019, **4**(2), 1–13.
55. Shin, Y. *et al.*, Blood-brain barrier dysfunction in a 3D *in vitro* model of Alzheimer's disease. *Adv. Sci. (Weinh)*, 2019, **6**(20), 1–10.
56. Miccoli, B., Braeken, D. and Li, Y. C. E., Barin-on-a-chip devices for drug screening and disease modeling applications. *Curr. Pharm. Des*., 2018, **24**(45), 5419–5436.
57. Marsano, A. *et al.*, Beating heart on a chip: a novel microfluidic platform to generate functional 3D cardiac microtissues. *Lab Chip*, 2016, **16**(3), 599–610.
58. Parsa, H., Wang, B. Z. and Vunjak-Novakovic, G., A microfluidic platform for the high-throughput study of pathological cardiac hypertrophy. *Lab Chip*, 2017, **17**(19), 3264–3271.
59. Lee, G., Lee, J., Kim, J., Choi, H. S., Kim, J., Lee, S. and Lee, H., Single microfluidic electrochemical sensor system for simultaneous multi-pulmonary hypertension biomarker analyses. *Sci. Rep*., 2017, **7**(1), 1–8.
60. Su, T. *et al.*, Cardiac stem cell patch integrated with microengineered blood vessels promotes cardiomyocyte proliferation and neovascularization after acute myocardial infarction. *ACS Appl. Mater. Interfaces*, 2018, **10**(39), 33088–33096
61. Sakolish, C. M., Philip, B. and Mahler, G. J., A human proximal tubule-on-a-chip to study renal disease and toxicity. *Biomicrofluidics*, 2019, **13**, 014107.
62. Qu, Y., An, F., Luo, Y., Lu, Y., Liu, T., Zhao, W. and Lin, B., A nephron model for study of drug-induced acute kidney injury and assessment of drug-induced nephrotoxicity. *Biomaterials*, 2018, **155**, 41–53.
63. Yin, L., Du, G., Zhang, B., Zhang, H., Yin, R., Zhang, W. and Yang, S. M., Efficient drug screening and nephrotoxicity assessment on co-culture microfluidic kidney chip. *Sci. Rep*., 2020, **10**(1), 1–11.
64. Jastrzebska, E., Zuchowska, A., Flis, S., Sokolowska, P., Bulka, M., Dybko, A. and Brzozka, Z., Biological characterization of the modified poly(dimethylsiloxane) surfaces based on cell attachment and toxicity assays. *Biomicrofluidics*, 2018, **12**(4), 044105.
65. Carreras, P., Gonzalez, I., Gallardo, M., Ortiz-Ruiz, A. and Martinez-Lopez, J., Droplet microfluidics for the *ex vivo* expansion of

human primary multiple myeloma cells. *Micromachines (Basel)*, 2020, **11**(3), 1–9.
66. De Lora, J. A., Velasquez, J. L., Carroll, N. J., Freyer, J. P. and Shreve, A. P., Centrifugal generation of droplet-based 3D cell cultures. *SLAS Technol*., 2020, **25**(5), 436–445.
67. Nasseri, B., Soleimani, N., Rabiee, N., Kalbasi, A., Karimi, M. and Hamblin, M. R., Point-of-care microfluidic devices for pathogen detection. *Biosens. Bioelectron*., 2018, **117**, 112–128.
68. Krokhine, S., Torabi, H., Doostmohammadi, A. and Rezai, P., Conventional and microfluidic methods for airborne virus isolation and detection. *Colloids Surf B*, 2021, **206**, 111962.
69. Han, X., Liu, Y., Yin, J., Yue, M. and Mu, Y., Microfluidic devices for multiplexed detection of foodborne pathogens. *Food Res. Int*., 2021, **143**, 110246.
70. Kumar, S., Nehra, M., Mehta, J., Dilbaghi, N., Marrazza, G. and Kaushik, A., Point-of-care strategies for detection of waterborne pathogens. *Sensors (Basel)*, 2019, **19**(20), 4476.
71. Peyravian, N., Malekzadeh Kebria, M., Kiani, J., Brouki Milan, P. and Mozafari, M., CRISPR-associated (CAS) effectors delivery via microfluidic cell-deformation chip. *Mater. (Basel, Switzerland)*, 2021, **14**(12), 3164.
72. Han, X. *et al.*, CRISPR-Cas9 delivery to hard-to-transfect cells via membrane deformation. *Sci. Adv.*, 2015, **1**(7), e1500454.
73. Li, X., Aghaamoo, M., Liu, S., Lee, D. H. and Lee, A. P., Lipoplex-mediated single-cell transfection via droplet microfluidics. *Small*, 2018, **14**(40), e1802055.
74. Ashraf, M. N., Asghar, M. W., Rong, Y., Doschak, M. R. and Kiang, T., Advanced *in vitro* HepaRG culture systems for xenobiotic metabolism and toxicity characterization. *Eur. J. Drug Metab. Pharmacokinet*., 2019, **44**, 437–458.
75. Salehi, H., Razavi, S., Esfandiari, E., Kazemi, M., Amini, S. and Amirpour, N., Application of hanging drop culture for retinal precursor-like cells differentiation of human adipose-derived stem cells using small molecules. *J. Mol. Neurosci*., 2019, **69**, 597–607.
76. Foty, R., A simple hanging drop cell culture protocol for generation of 3D spheroids. *J. Vis. Exp*., 2011, **51**, 1–4.
77. Tung, Y. C., Hsiao, A. Y., Allen, S. G., Torisawa, Y. S., Ho, M. and Takayama, S., High-throughput 3D spheroid culture and drug testing using a 384 hanging drop array. *Analyst*, 2011, **136**(3), 473–478.
78. Greuel, S. *et al.*, Effect of inoculum density on human-induced pluripotent stem cell expansion in 3D bioreactors. *Cell Prolif*., 2019, **53**(4), 1–12.
79. Phelan, M. A., Gianforcaro, A. L., Gerstenhaber, J. A. and Lelkes, P. I., An air-bubble isolating rotating wall vessel bioreactor for improved spheroid/organoid formation. *Tissue Eng. Part C*, 2019, **25**(8), 479–488.
80. Amaral, R. L. F., Miranda, M., Marcato, P. D. and Swiech, K., Comparative analysis of 3D bladder tumor spheroids obtained by forced floating and hanging drop methods for drug screening. *Front. Physiol*., 2017, **89**(605), 1–15.
81. Breslin, S. and O'Driscoll, L., Three-dimensional cell culture: the missing link in drug discovery. *Drug Discov. Today*, 2013, **18**(5–6), 240–249.
82. Souza, G. R. *et al.*, Three-dimensional tissue culture based on magnetic cell levitation. *Nat. Nanotechnol*., 2010, **5**(4), 291–296.
83. Haisler, W., Timm, D., Gage, J., Tseng, H., Killian, T. C. and Souza, G. R., Three-dimensional cell culturing by magnetic levitation. *Nature Protoc*., 2013, **8**, 1940–1949.
84. Tseng, H., Gage, J. A., Raphael, R. M., Moore, R. H., Killian, T. C., Grande-Allen, K. J. and Souza, G. R., Assembly of a three-dimensional multitype bronchiole co-culture model using magnetic levitation. *Tissue Eng. Part C*, 2013, **19**(9), 665.
85. Conde, J. P., Madaboosi, N., Soares, R. R., Fernandes, J. T., Novo, P., Moulas, G. and Chu, V., Lab-on-chip systems for integrated bioanalyses. *Essays Biochem*., 2016, **60**(1), 121–131.
86. Park, J., Lee, G. H., Yull, P. J., Lee, J. C. and Kim, H. C., Hypergravity-induced multicellular spheroid generation with different morphological patterns precisely controlled on a centrifugal microfluidic platform. *Biofabrication*, 2017, **9**, 045006.
87. Christopher, G. F. and Anna, S. L., Microfluidic methods for generating continuous droplet streams. *J. Phys. D: Appl. Phys*., 2007, **40**(19), R319.
88. Alessandri, K. *et al.*, Cellular capsules as a tool for multicellular spheroid production and for investigating the mechanics of tumor progression *in vitro*. *Proc. Natl. Acad. Sci. USA*, 2013, **110**(37), 14843–14848.
89. Sabhachandani, P., Motwani, V., Cohen, N., Sarkar, S., Torchilin, V. and Konry, T., Generation and functional assessment of 3D multicellular spheroids in droplet based microfluidics platform. *Lab Chip*., 2016, **16**(3), 497–505.
90. Yu, Y., Wen, H., Ma, J., Lykkemark, S., Xu, H. and Qin, J., Flexible fabrication of biomimetic bamboo-like hybrid microfibers. *Adv. Mater*., 2014, **26**(16), 2494–2499.
91. Bawazer, L. A. *et al.*, Combinatorial microfluidic droplet engineering for biomimetic material synthesis. *Sci. Adv*., 2016, **2**(10), e1600567.
92. Gao, Y. *et al.*, A versatile valve-enabled microfluidic cell co-culture platform and demonstration of its applications to neurobiology and cancer biology. *Biomed. Microdevices*, 2011, **13**(3), 539–548.
93. Bhatia, S. N. and Ingber, D. E., Microfluidic organ-on-chips. *Nature Biotechnol*., 2014, **32**(8), 760–772.
94. Huh, D. *et al.*, A human disease model of drug toxicity-induced pulmonary edema in a lung-on-a-chip microdevice. *Sci. Transl. Med*., 2012, **4**(159), 159ra147.
95. Park, B. H. *et al.*, An integrated rotary microfluidic system with DNA extraction, loop-mediated isothermal amplification, and lateral flow strip based detection for point-of-care pathogen diagnostics. *Biosens. Bioelectron*., 2017, **91**, 334–340.
96. Azizi, M., Zaferani, M., Cheong, S. H. and Abbaspourrad, A., Pathogenic bacteria detection using RNA-based loop-mediated isothermal–amplification–assisted nucleic acid amplification via droplet microfluidics. *ACS Sens*., 2019, **4**(4), 841–848.
97. Zhang, Z., Zhao, S., Hu, F., Yang, G., Li, J., Tian, H. and Peng, N., An LED-driven AuNPs-PDMS microfluidic chip and integrated device for the detection of digital loop-mediated isothermal DNA amplification. *Micromachines (Basel)*, 2020, **11**(2), 177.
98. Song, B., Wang, J., Yan, Z., Liu, Z., Pan, X., Zhang, Y. and Zhang, X., Microfluidics for the rapid detection of *Staphylococcus aureus* using antibody-coated microspheres. *Bioengineered*, 2020, **11**(1), 1137–1145.
99. Kim, Y. *et al.*, Integrated microfluidic preconcentration and nucleic amplification system for detection of influenza A virus H1N1 in saliva. *Micromachines (Basel)*, 2020, **11**(2), 203.
100. Ramachandran, A. *et al.*, Electric field-driven microfluidics for rapid CRISPR-based diagnostics and its application to detection of SARS-CoV-2. *Proc. Natl. Acad. Sci. USA*, 2020, **117**(47), 29518–29525.

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