Paper-based cell culture platform and its emerging biomedical applications

Kelvin Ng[1,3,4,7], Bin Gao[2,3,4,7,*], Kar Wey Yong[1,3,4], Yuhui Li[3,4], Meng Shi[4,5], Xin Zhao[3,4], Zedong Li[3,4], XiaoHui Zhang[3,4], Belinda Pingguan-Murphy[1], Hui Yang[6] and Feng Xu[3,4,*]

[1] Department of Biomedical Engineering, Faculty of Engineering, University of Malaya, Lembah Pantai, 50603 Kuala Lumpur, Malaysia
[2] Department of Endocrinology and Metabolism, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, PR China
[3] The Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, PR China
[4] Bioinspired Engineering and Biomechanics Center (BEBC), Xi'an Jiaotong University, Xi'an 710049, PR China
[5] School of Energy and Power Engineering, Xi'an Jiaotong University, Xi'an 710049, PR China
[6] School of Life Sciences, Northwestern Polytechnical University, Xi'an 710072, PR China

Paper has recently attracted increasing attention as a substrate for various biomedical applications. By modifying its physical and chemical properties, paper can be used as an alternative to conventional cell culture substrates. Because it can be stacked into a three-dimensional (3D) structure, which can better mimic the *in vivo* cell microenvironment. Paper has shown great potential as a 3D cell culture platform for developing normal and diseased models. This platform gives precise control over extracellular matrix (ECM) composition as well as cell distribution and precise analysis of the interactions between cells. Paper-based platforms have been applied for pathophysiological studies and therapeutic intervention studies. In this paper, we first discuss the modifications of the physical and chemical properties of paper to develop various 2D and 3D cell culture platforms. We then review the applications of paper-based cell culture platforms for the construction of *in vitro* disease models, drug screening, and cell cryopreservation applications. Because of its advantages such as biocompatibility, eco-friendliness, cost efficiency, and ease of large-scale production, we believe that paper-based cell culture platforms would play an important role in the fields of biomedicine.

Introduction

Paper, as one of the most ancient inventions, has led to tremendous changes to human beings along the history. In ancient Egyptians, Greek, and Romans, 'papyruses' (paper-like materials) were used to record information, while the modern paper that is composed of cellulose fibers held by hydrogen bonds was invented and used in ancient China. Ever since its invention, paper has been used in a wide range of fields, from the original writing substrates to sanitary products, packaging, and even bank notes (cash). Recently, with the significant advances in biotechnology, paper has been applied as substrates to a variety of biomedical applications, such as the production of low-cost and disposable analytical test papers in healthcare applications [1–3], flexible electronics [4,5], paper-based biosensors [6–8], and most recently, cell culture platforms [9–14]. This is mostly attributed to its attractive intrinsic properties, such as biocompatibility, ease of chemical and physical modifications, cost efficiency, eco-friendliness, and ease of large-scale manufacturing.

Selection of materials/substrates for cell culture *in vitro* has evolved from the original cell expansions to disease models, tissue regeneration, as well as *in vitro* diagnostics along the emerging

*Corresponding authors:. Gao, B. (bingao0726@163.com), Xu, F. (fengxu@mail.xjtu.edu.cn)
[7] These authors contributed equally to this work.

1369-7021/© 2016 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). http://dx.doi.org/10.1016/j.mattod.2016.07.001

Please cite this article in press as: K. Ng, et al., Mater. Today (2016), http://dx.doi.org/10.1016/j.mattod.2016.07.001

technologies in biomedical fields [15]. Therefore, the concept of an ideal material for cell culture has shifted from a bio-inert material to a biocompatible material that can fulfill the necessary criteria such as cell inductivity and biodegradability [15]. Paper, given its fibrous, porous, and flexible properties, has shown great potential as an alternative platform for cell culture, particularly with the advancements in micro/nanotechnologies that allow more effective modifications of its physical and chemical properties [6,16]. Cell culture systems developed using nanocellulose-based paper have shown improved cell viability, adhesion, migration, and proliferation compared with tissue culture plates (TCPs) [17–19]. Moreover, paper can also withstand various chemical, thermal, and ultraviolet (UV) sterilization processes without suffering drastic changes in its properties [20].

Paper is a thin material produced by pressing together the moist fibers of cellulose pulp derived from wood, rags, or grasses, and later drying into flexible sheets. Therefore, it offers the advantages of nanofibrous materials like electrospun fibrous mats, such as the fibrillar structure that mimic the extracellular matrix (ECM) [17–19,21,22]. However, the electrospinning technique has a few limitations, such as limited extensibility in the fabricated fiber [21] and lack of spatial control over pore size [23]. These drawbacks decrease the pore sizes of electrospun fibrous mats when stacked into multilayer three-dimensional (3D) scaffolds, which may limit the ability of the cells to penetrate deep into the core of the scaffold and result in nonhomogenous cell distribution [24]. The manufacturing method of paper provides a better control over the paper dimensions and porosity. As a result, when paper is stacked into multilayer 3D scaffolds, the cells can reach greater depths, which better simulates the *in vivo* environment [9]. These make paper preferable over electrospun fibrous mats in the construction of ischemic disease models, where cell migration toward ischemic stress is a major aspect [12,25]. Moreover, patterns can be easily created on paper using wax printing, and microchannels can easily be fabricated on paper [2,9,26]. On the basis of these characteristics and advantages, paper can be used in many bioassays, including molecular assays, paper-based enzyme-linked immunoassay (ELISA), and cell culture studies, whereas electrospun sheets are mainly used for cell culture, implant material, drug delivery, and wound dressing [26–28].

The major advantage of using paper as a cell culture platform is its intrinsic three-dimensional (3D) configuration, which mimics the native cell microenvironment [11,29–31]. Such 3D cell culture systems can create physiologically relevant fluid flow, and oxygen and nutrient gradients, which better reflect the native microenvironment than the conventional 2D *in vitro* systems [10–12,30–33]. The construction of functional *in vitro* 3D models requires systematic control and optimization of their biophysical and biochemical characteristics. Paper as an alternative to traditional 3D cell culture platforms, such as hydrogels and porous scaffolds, has been shown with a great diversity of surface topography and internal porous microstructure, enabling the use of paper-based systems to manipulate cell behaviors [10,12,13,34]. Moreover, paper has an inherent ability to absorb fluids through capillary action attributed to its porous structure and large void volume ratio [1,35], making it possible for cell migration inside the scaffolds [9,10]. In some studies, paper was mixed with hydrogel to construct 3D *in vitro* culture platform [9,12,13,25], where the paper fibers provide strong mechanical support, acting as a supporting frame to maintain the configuration of the thin, mechanically fragile soft hydrogel [19,36], allowing repeat handling of the cell-laden paper system without significant disruption of cell behaviors [10,12,34]. Furthermore, thickness of the paper can be tailored to $\leq$200 μm to ensure delivery of sufficient oxygen to all the residing cells. Finally, multiple paper units containing different cell types can be stacked up with a defined spatial distribution to recapitulate native 3D architecture *in vivo* [10,12,37]. Together, paper has become an interesting and unique substrate for creating 3D cell culture systems [12].

The unique features of paper-based system in supporting 3D cell culture enables it to be used for a range of studies, such as the development of normal or disease models *in vitro* by manipulating the physical or chemical properties of the paper to control nutrient and oxygen diffusion (Fig. 1) [9,10]. Such 3D cell culture models can also be used to study cell–drug interactions in a high-throughput manner, thus facilitating the understanding of diseases mechanism and drug metabolism [9,12]. Further, the absorbing ability of paper makes it a carrier for small-volume vitrification as it can reduce the vitrification solution surrounding the cells [38,39]. A variety of commercially available papers are currently used in cell culture and biomedical applications. A detailed comparison of different types of papers is listed in Table 1.

To date, there has been no comprehensive study on the applications of paper-based cell culture platforms. In this study, we discuss the modification of the physical and chemical properties of paper in detail, to develop various 2D and 3D cell culture platforms. We also provide an in-depth review of the applications of such paper-based cell culture platforms for the construction of *in vitro* disease models and drug-screening models and for cell cryopreservation.

Modification of paper for cell culture

Paper is an interesting alternative to the conventional cell culture materials (e.g., ceramic, glass, and polymer) because of its dimensional versatility and adjustable porosity [14,40,41]. The mechanical properties of strong cellulose fibers present in paper facilitate its reshaping and stacking into structures that favor cell growth, with no detrimental effect on the bulk properties of paper [10–12]. Furthermore, the high porosity of paper possesses fluid wicking capabilities, which allows the flow of cell culture medium across the paper and is thus beneficial for transportation of nutrients (e.g., oxygen) and waste product. By tuning the porosity of paper, the amount of nutrient and oxygen reaching the targeted tissue can be precisely controlled, thus creating an accurate normal or ischemic diseased tissue model [9,11,12,32,34].

However, paper itself is not a suitable material for cell culture because of the absence of cell adhesion moieties [34]. Furthermore, paper is produced from cellulose pulps that are bound together through mechanical interlocking and intermolecular hydrogen bonds, and thus long-term exposure of paper to the cell culture medium might destroy the bonding between the cellulose fibers and cause cell loss because of the loss of their anchoring sites, thereby disrupting cell–matrix adhesion [34,42]. A few recent studies have shown that native nanocellulose fibers (e.g., bacterial nanocellulose and nanofibrillar cellulose) have good biocompatibility to human cells in 3D cell culture systems because of their native ECM-mimicking structure and dimension [17–19].

Please cite this article in press as: K. Ng, et al., Mater. Today (2016), http://dx.doi.org/10.1016/j.mattod.2016.07.001

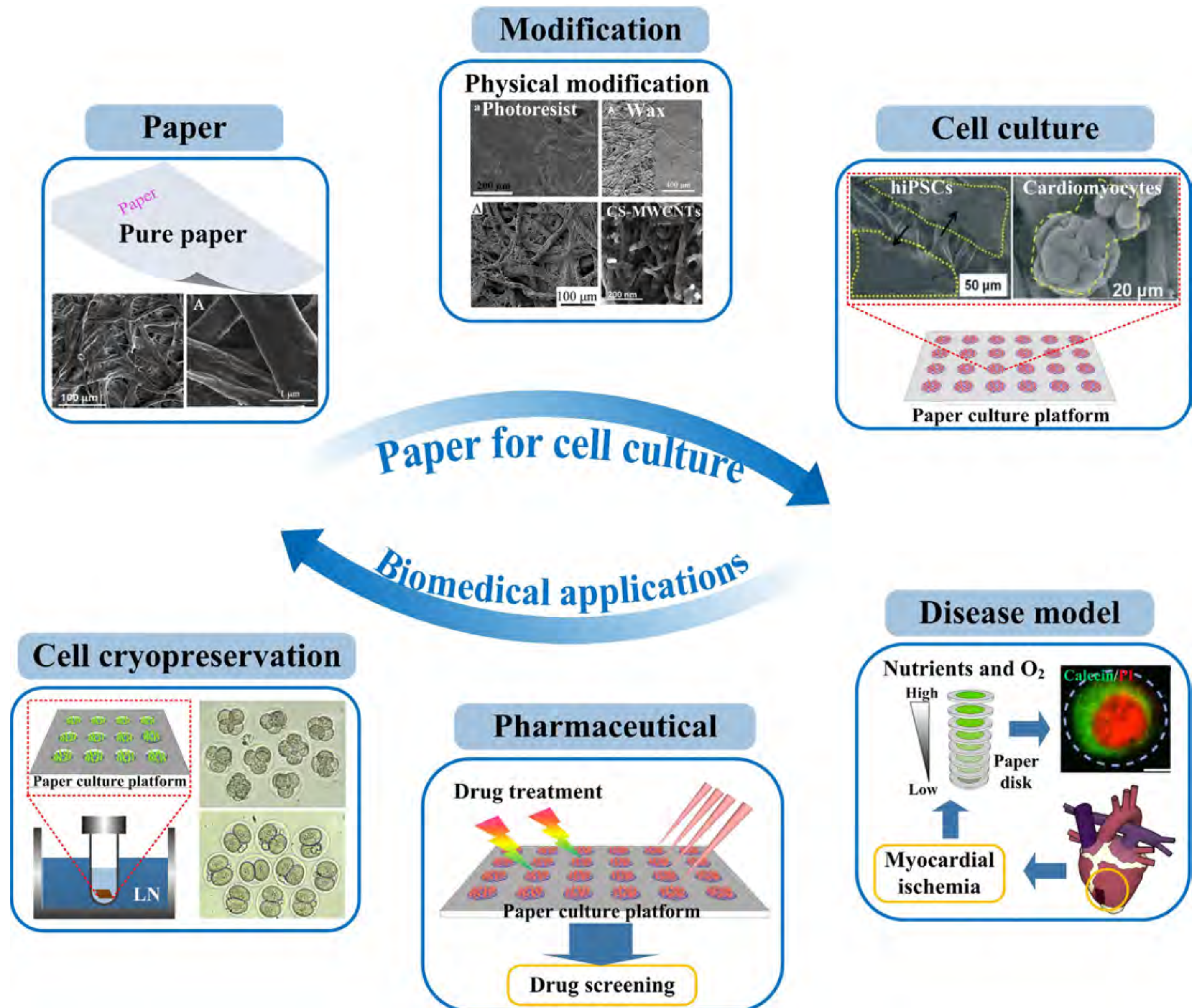

FIGURE 1

Schematic of outline for paper-based cell culture and its emerging biomedical applications. Modification of physical and chemical properties of paper enables it to be used in cell culture and to control nutrient and oxygen diffusion mimicking the healthy and diseased environment *in vivo* (disease model). The cell culture models can also be used to study the cell–drug interactions in a high-throughput manner for drug screening. The absorbing ability of paper renders it to become a holder that reduces the vitrification solution around cells, and can be for cell cryopreservation. Images reproduced from [9,12,25,89,95].

Both the physical (e.g., topography and mechanical properties) and chemical properties (e.g., adhesive ligands) of ECM play vital roles in guiding cell fates during development, healing, tissue remodeling, and disease genesis. In this sense, paper made by micro- and nanocellulose fibers offers several advantages as cell culture platform because of its relatively better physical properties (e.g., topography, permeability, and mechanical properties), and micro- and nanostructure networks similar to native ECM (e.g., protein and polysaccharide network) [18,43,44]. The porous structures of paper not only provide the required space for cell growth but also facilitate modification of paper with biological ligand (e.g., peptides or nucleotides), as they can affect cell adhesion and proliferation. The physical properties can also be regulated to enhance the cell functions. For instance, both topography and stiffness of the paper could activate cellular mechanotransduction through focal adhesion formation and intracellular cytoskeleton to enhance cell contractility, spreading, migration, and differentiation [18,44–48]. Furthermore, paper with high stiffness could induce cell–matrix traction, resulting in the secretion of ECM that may enhance the mechanical properties of paper. Besides, the physical and chemical properties of hydrogels integrated with papers, including porosity, stiffness, viscoelasticity, and ligand density, may regulate cell adhesion, proliferation, and differentiation. Cells will degrade the hydrogels by secreting various enzymes (e.g., matrix metalloproteinase and chymotrypsin) and subsequently remodeling the local microenvironment by secreting their own ECM.

Please cite this article in press as: K. Ng, et al., Mater. Today (2016), http://dx.doi.org/10.1016/j.mattod.2016.07.001

TABLE 1

Paper types and their characteristics in biomedical applications.

Paper types	Pore size (μm)	Roughness	Thickness (μm)	Cell types	Mechanical strength	Applications	Advantages	Disadvantages	Refs
Whatman filter paper #114	25	Rough	190	Cardiomyocyte/Aortic valvular interstitial cell/Human acute promyelocytic leukemia cell	2.8 MPa (Ref. http://www.gelifesciences.com.cn/CNLS/indexAction.action)	Cell culture/Disease model/Drug screening	1. Sufficient diffusion property for 3D cell culture 2. Suitable pore size for cell migration inside a paper 3. Less expensive and easy to obtain	1. Hard to fix cells inside a paper Low mechanical strength	[9–12,84]
Whatman® Protran® nitrocellulose membrane	1.0	Smooth	130–160	Human breast cancer cell	1.8–2.6 MPa (Ref. http://www.gelifesciences.com.cn/CNLS/indexAction.action)	Cell culture/Disease model	1. Uniform pore size 2. Sufficient diffusion property for 2D cell culture. 3. Easy to stack and destack in cell co-culture systems 4. Good mechanical strength	1. Limited pore size for 3D cell culture 2. Expensive	[32]
Janus paper	–	Smooth	–	Human lung fibroblast	392 kPa	Disease model	1. Hydrophobic and suitable for cancer cell aggregates fabrication 2. Good biocompatibility and low cell toxicity 3. Less expensive and widely accessible	Hard to stack for cell co-culture systems due to hydrophobicity	[50]
Kimwipes Kimberly Clark 34155	–	Smooth	100–160	Mouse embryonic fibroblast	–	Cell cryopreservation	1. Good mechanical strength 2. Less expensive and easy to obtain	Low cooling rate	[89]
Weighing paper	–	Smooth	100–200	Bovine blastocyst/Human adipose-derived stem cell	150–650 kPa (Ref. http://www.paperonweb.com/paperpro.htm)	Cell culture/Disease model	1. Suitable stiffness for osteogenic differentiation 2. Less expensive and easy to obtain	Low biodegradation rate *in vivo*	[13,95]
Print paper	–	Rough	100–200	Human-induced pluripotent stem cell	–	Cell culture	1. Suitable stiffness for myogenic differentiation 2. Less expensive and easy to obtain	1. High impurities with potential cytocompatibility issue 2. Poor porosity	[66]

Please cite this article in press as: K. Ng, et al., Mater. Today (2016), http://dx.doi.org/10.1016/j.mattod.2016.07.001

Therefore, the modification of paper's chemical and physical properties has become an inevitable and essential part in paper-based cell culture systems to create a more feasible environment for cell growth. To date, various modification methods have been developed to manipulate the chemical and physical properties of paper to fit different cell culture applications.

Modification of chemical properties of paper for cell culture

Paper has various chemical compositions, which can be classified into organic and inorganic materials. The organic materials for most paper consist of cellulose, hemicellulose, and lignin. The hemicellulose is a group of compounds composed of different sugars and the lignin is formed by phenylpropanes. The inorganic materials comprise clay, alum, calcium carbonate, titanium oxide, and rosin, which are mostly filled and loaded during paper manufacturing. By adding these chemical materials, the desired performance of paper can be obtained. For instance, clay and calcium carbonate are added to obtain better brightness and printability of paper, while the rosin and alum increase its water resistance. Hence, it is important to regulate the chemical composition of paper for specific paper-based applications.

It is important to chemically modify the paper surface to improve cell attachment and proliferation [14,49]. This can be done by conjugating with various biomolecules, such as peptides and nucleotides [1]. The chemical properties of the paper surface can also be modified by various surface treatment methods, such as initiator chemical vapor deposition (iCVD) [13], corona discharge surface treatment [50], and printing [14] (Fig. 2a). iCVD is a solvent-free vapor-phase polymer-coating method, which can systematically modify the paper surface into a favorable biochemical surface that exhibits desirable water resistance and adhesiveness [13]. The iCVD approach polymerizes a functional polymer coating onto the surface of paper through a free-radical polymerization process, which can reduce the undesirable side chain through polymerization reactions, which may destroy the functional groups of the polymer coating [51]. A recent study used iCVD for the deposition of glycidyl methacrylate polymer (pGMA) on perfluorodecyl acrylate polymer (pPFDA)-coated paper, and showed increase of cell adhesion. (Fig. 2a(ii–iv)) This is attributed to the epoxy group of the bioreactive polymer – pGMA, which facilitates the binding of serum protein in the medium onto the paper substrate [13]. Corona discharge surface treatment has also been used in paper modification to increase the surface energy, thus leading to increased wettability for cell adhesion. For instance, a hybrid cell culture platform for air–liquid interface (ALI) made of janus paper and polydimethylsiloxane (PDMS) was treated with corona discharge to enhance the flow in the microchannels embedded in the platform, which resulted in enhanced cell viability (>95%) [50]. Furthermore, the printing method can also be used to modify the chemical properties of paper by printing various chemical substances onto the paper [14,52]. For instance, ECM proteins and cell adhesion proteins, such as vitronectin and fibronectin, have been printed onto the paper to enhance cell adhesion [53–56].

In addition to the aforementioned chemical modification, the surface properties and bulk properties of paper can also be altered by chemically modifying the cellulose fibers. For example, hydroxylpropyl cellulose (HPC) is a derivative of cellulose, which is soluble in both water and organic solvents. Once hydrated with water, the HPC forms a soft elastomer with a Young's modulus similar to that of soft tissues [57–59]. Cross-linking of HPC grafted with methylacryclic anhydride by UV irradiation can form a hydrophilic network, which provides anchoring sites for cell–matrix interaction. In order to further enhance cell attachment, CDI activation of the hydroxyl groups of HPC chain with 1,1′-carbonyldiimidazole has been used to introduce biochemical cues to HPC, which enables it to conjugate with matrix protein (acting as active cell-binding sites). This hydrophilic network degrades gradually along with cell proliferation, allowing the cells to slowly replace the materials and regenerate the tissues [34].

Modification of physical properties of paper for cell culture

The physical cell microenvironment (e.g., topography, stiffness, roughness, and water permeability) has significant impact on cellular behavior, such as cell proliferation, migration, alignment, and differentiation [33,60–63]. Materials with smoother surfaces have shown improved growth, spreading, and attachment of epithelial cells, whereas those with a rougher surface are more favorable for the growth, spreading, and attachment of osteoblast cells [14,15,62,64]. Hence, we can precisely manipulate the cell behavior by modifying the physical properties of paper. In addition, it was observed that the paper stiffness can significantly affect anchorage-dependent cells in terms of their cellular behavior, including stem cell proliferation, adhesion, locomotion, spreading, morphology, striation, and even differentiation [65]. In addition, the surface of the paper can also be modified to be hydrophobic or hydrophilic to inhibit or facilitate cell attachment, thus allowing to create a cell culture platform integrated with both 'cell zone' and 'cell-free zone' [10–12,66].

The physical properties of paper can also be modified through printing or coating of different substances. For instance, paper has been coated with four types of substrates (calcium carbonate/latex binder, kaolin pigments/latex binder, duo kaolin pigments/latex binder, and styrene butadiene/polystyrene) to modify its physiochemical properties (e.g., topography, roughness and surface energy), which was later used to study cell attachment and growth [14]. In addition, common laboratory filter paper has also been modified with plasmonic gold nanorods as a three-dimensional scaffold for cancer detection [67]. These coatings altered the surface properties of paper, including surface roughness. It was found that paper with low roughness promotes cell growth of human arising retinal pigment epithelia cells (ARPE-19) [9,10]. Hydrophobic materials (e.g., PDMS, wax, and Teflon) have low surface energy and inhibit cell attachment, thus acting as barriers to direct and constrain cell growth in the desired area of the paper [14,68–70] (Fig. 2b(ii)). For instance, PDMS was printed on the paper to form 96 individual thin circular well slabs (hydrophilic zones) that contain hydrogels encapsulating cells, where the PDMS functions as a hydrophobic barrier to isolate the cell-containing zones from each other and prevent the lateral flow of aqueous medium and cell growth across each zone (Fig. 2b(iii,iv) [10,12,71]. This cell culture platform is called the cell-in-gel-in-paper (CiGiP) culture system [9,24]. Paper has also been coated with a thin layer of highly hydrophobic pPFDA to increase its mechanical durability and long-term stability in an aqueous environment [13].

Please cite this article in press as: K. Ng, et al., Mater. Today (2016), http://dx.doi.org/10.1016/j.mattod.2016.07.001

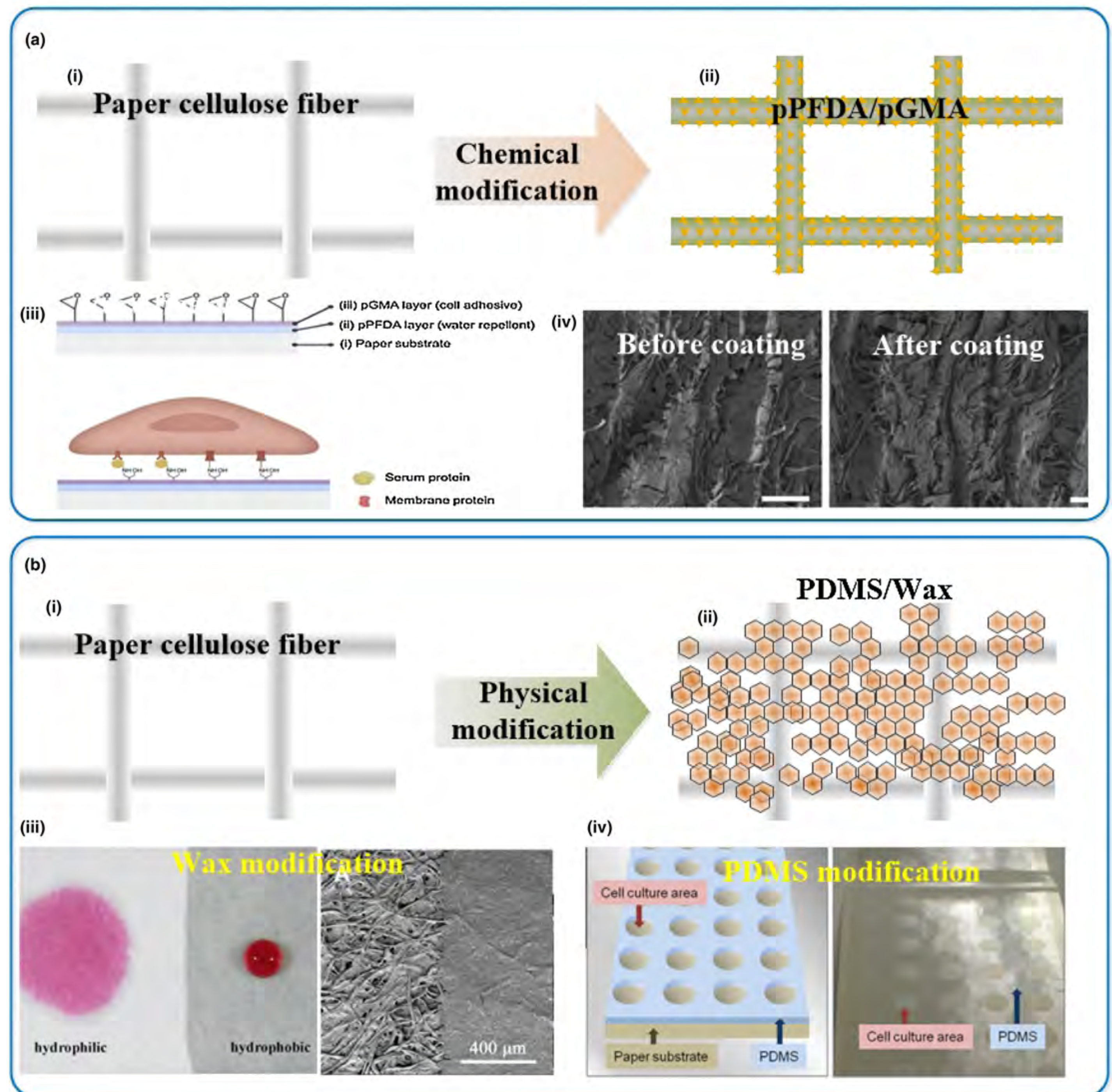

FIGURE 2

Modification of paper properties for cell culture. (a) Chemical modification through iCVD coating of various components on paper scaffold for enhanced cell attachment [13]. (b) Physical modification of paper using wax and polydimethylsiloxane to modify the hydrophobicity and hydrophilicity of the paper surface. Images reproduced from [10,14,96].

Existing paper-based cell culture platform

With the advances in the micro/nanotechnologies, modification of the physical and chemical properties of paper for various 2D and 3D cell culture platforms has been developed. For instance, a weighing paper was coated with a thin polymer (e.g., pPFDA) using iCVD, which increases its hydrophobicity and long-term dimensional stability in the cell culture medium. The paper was also coated with pGMA to induce cell attachment and proliferation on the paper. Moreover, both polymer coatings can provide structural and mechanical support to facilitate the osteogenic differentiation of human adipose-derived stem cells [13].

Although 2D cell culture platforms have provided valuable insights into cell–microenvironment interactions, they could not closely represent the native 3D cell microenvironment with complex cell–cell and cell–ECM interactions and mass transfer barriers [29]. In addition, the effect of mechanical properties of the

Please cite this article in press as: K. Ng, et al., Mater. Today (2016), http://dx.doi.org/10.1016/j.mattod.2016.07.001

2D environment on cells is irrelevant to that of cells *in vivo*, where cells are surrounded by a 3D microenvironment [72]. Therefore, it is difficult to predict the actual response of cells toward external stimulant (e.g., toxic effect and mechanical strain). Compared with a 2D platform, 3D cell culture platform can better mimic the *in vivo* microenvironment with microscale tissue geometry, ECM-like stiffness, and complex organization of ECM. The paper-based 3D cell culture system (e.g., CiGiP) is unique with the ability to stack and destack. The durability of paper sheets is sufficient for stacking into a multilayer 3D structure that could resemble *in vivo* microenvironment. Layers of paper impregnated with suspension of different cells in hydrogel can be stacked to form multilayer 3D cell culture models, with easy handling and precise control on the different parameters in each layer. Before stacking, hydrophobic and cell-culturing zones are printed on papers using a wax printer, followed by stacking of these papers held together with mechanical clamps [10,12]. These models can be easily destacked by simply using tweezers for further culture or analysis [9]. This stacking technology is a key feature of CiGiP and enables researchers to design customized cell culture platform that could suite their experimental needs [9]. Another advantage of the CiGiP 3D culture platform is the detachability of the platform, which enables the examination of cells at a specific layer and area [10].

Recently, paper-based platforms have also been used for the proliferation and direct differentiation of human-induced pluripotent stem cells (hiPSCs) into functional beating cardiac tissues, and to create 'a beating heart' on paper [66]. Three types of paper (print paper, chromatography paper, and nitrocellulose (NC) membrane) were coated with PDMS and tested for the growth and differentiation of human-derived iPSCs. hiPSCs grew into a 3D-like morphology on these paper substrates maintaining pluripotent properties in 5 days of culture. The hiPSCs on papers lost their pluripotency after 5 days and were fully induced to cardiac cells, which retained their long-term stable contractile frequency of 40–70 beats per minute for 3 months on the print paper and chromatography paper. The human iPSCs grown on the NC membrane differentiated into retinal pigment epithelium even under cardiac-specific induction, indicating that the properties of the paper and the mechanical cues regulate stem cell differentiation. There is also another study, where single-layered papers cultured with osteogenically differentiated human adipose stem cells (hASCs) were stacked with those cultured with human endothelial cells to promote the formation of vascularized bone *in vivo*, which was then implanted into mice with calvarial bone defect resulting in significantly enhanced bone regeneration *in vivo* [13]. This shows that a paper-based cell culture platform is a promising scaffold for the support of various cell functions, including cell attachment, proliferation, and differentiation.

Applications of paper-based cell culture platform

The paper-based 3D cell culture system also exhibits several advantages from a material standpoint. First, paper is a porous scaffold composed of micro- and nanofibrous structures, which are similar to native ECM. The mechanical and chemical properties can be tuned by changing the structures (e.g., fiber diameter and porosity) and material composition (e.g., bacterial nanocellulose and nanofibrillar cellulose). Second, porous structures can enhance the diffusion of nutrients, paracrine molecules, and oxygen, which are important for cell culture. In addition, paper is a flexible and easily foldable material, which holds high potential as a 3D complex scaffold for cell culture. This is important because engineering of complex tissue constructs for tissue engineering applications remains a challenging task to date. Taken together, paper-based system can provide a useful tool for establishing *in vitro* 3D cell culture systems.

Paper-based cell culture as disease models

In vitro 3D cellular models that mimic a normal or diseased tissue structure *in vivo* are important to understand the cellular physiological or pathological behavior [11,12,29,73]. Many studies have proved that a paper-based cell culture platform can be used to mimic various native pathophysiological microenvironments [11,12,32,66]. Functional changes in the microenvironment, such as inadequate oxygen supply, adaptive changes in metabolism, and changes in the pH in microenvironment, can be achieved with a paper-based culture platform. For example, CiGiP is a simple method for culture of thin planar sections of tissue on a printed paper array, either alone or stacked to create a complex 3D tissue construct [10]. The CiGiP platform provides better control over the parameters dictating the cellular microenvironment, which enables the manipulation of the concentration gradient of soluble molecules such as oxygen and glucose to create a native-tissue-mimicking hyperoxic and hypoxic microenvironment [9,10,74].

For instance, the CiGiP platform was successfully used to fabricate a 3D cell culture platform, which well mimics low-, medium-, and high-ischemic stress conditions to study the response and interaction of cardiomyocytes and cardiac fibroblasts (Fig. 3a) [12]. Two experimental systems used the CiGiP platform to mimic ischemic conditions in occluded blood vessels and studied the effect of ischemic conditions on cardiac fibroblasts. One system mimicked the region of cardiomyocytes between a healthy blood vessel in the top of a large stack and an occluded blood vessel at the bottom of a large stack. For this, six mini stacks consisting of 20 pieces of paper were stacked together, where openings are present on the top plate that allow the medium to diffuse in. As the medium diffused from the top to the bottom block, the nutrient was metabolized by cardiomyocytes, leaving an ischemic environment at the bottom. The other system forms a co-culture by stacking neonatal rat cardiomyocytes and cardiac fibroblasts to study the migration of cardiac fibroblasts toward the cardiomyocytes under ischemic pressure. For this, two mini stacks of cardiomyocytes were placed at the bottom, while different numbers of mini stacks containing fibroblasts were stacked on top to create cellular environment of low, medium, and high ischemia. With this model, it was found that the metabolically stressed cardiomyocytes could produce cytokines in accordance with the level of ischemia, which will induce the fibroblast to migrate to the high concentration of cytokines (injured area) to repair the damaged area. Then, the addition of transforming growth factor beta (TGF-β) neutralizing antibody could suppress the migration of fibroblasts to the cardiomyocyte-containing layer, further confirming that the chemotaxis of fibroblast is triggered by the cytokine signal generated by cardiomyocytes.

This paper-based ischemic disease model was further used to study the progression of calcific aortic disease using aortic valvular interstitial cells (VICs) under ischemic stress [75]. In this study,

Please cite this article in press as: K. Ng, et al., Mater. Today (2016), http://dx.doi.org/10.1016/j.mattod.2016.07.001

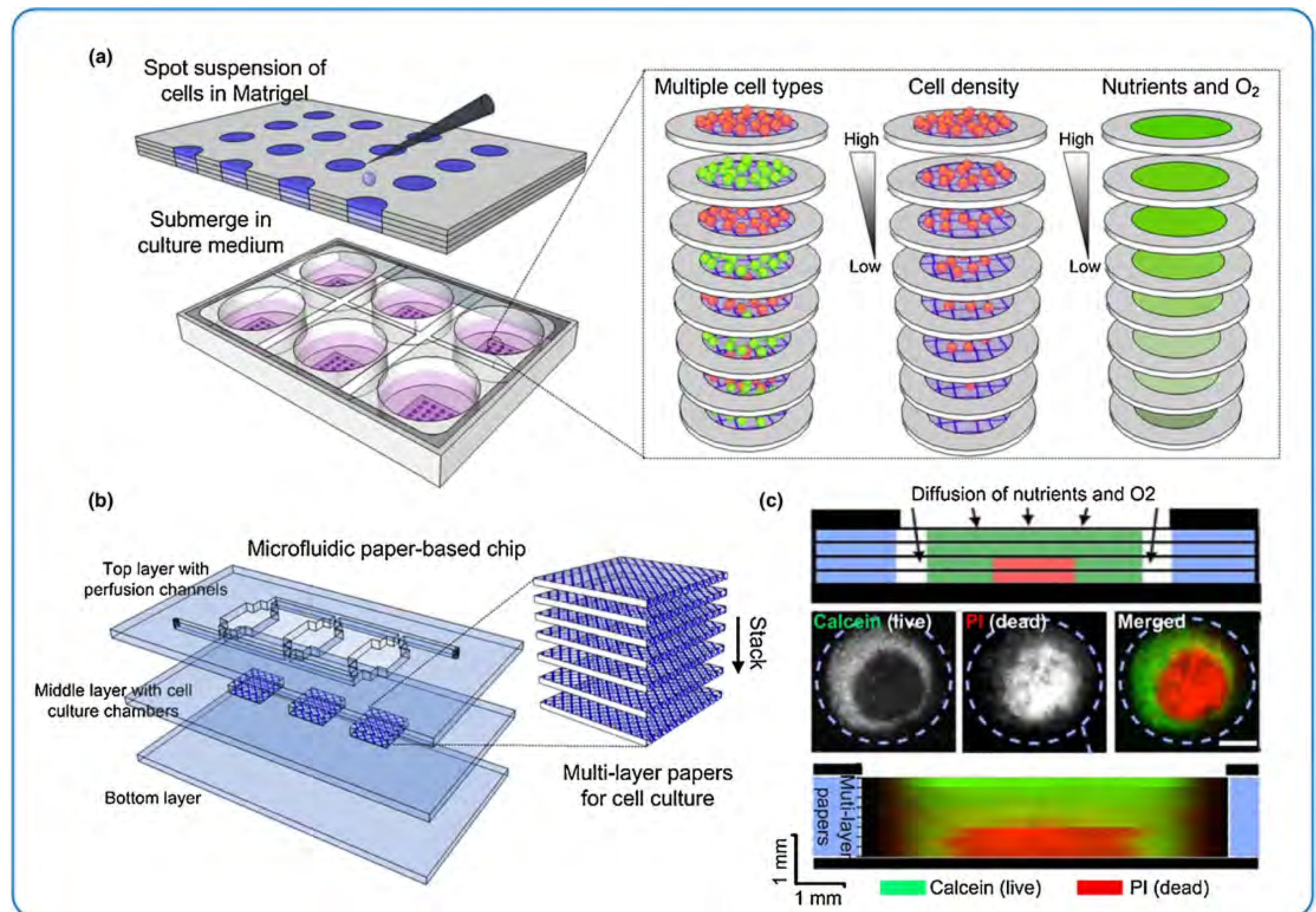

FIGURE 3

Disease model using paper-based cell culture platform. (a) Construction of cell-in-gel-in-paper (CiGiP) model [12]; (b) 3D breast cancer model [32]; (c) fluorescent images showing the distributions of living (green) and dead (red) cells in a multilayered paper-based cell culture model. Images reproduced from [10].

paper sheets were stacked and compressed by top and bottom solid plates to ensure that the paper layers were in contact to allow migration. Manipulating the position of solid plate gives control on the medium flow, which will lead to the formation of oxygen and nutrient gradient along the thickness of the paper layers. With this model, it was found that expression of α-smooth muscle actin (α-SMA), a defining marker of myofibroblasts and an indicator of VIC activation, was increased in VICs located in the areas of higher hypoxic stress at the bottom of stack (Fig. 3a). It was also found that the addition of collagen type I to this model was able to reduce the expression of α-SMA, thus reducing myofibroblast differentiation and calcification. In addition, a stacked paper invasion assay based on CiGiP has been developed recently, which provides a cancer cell model to study the effect of the gradients of oxygen tension on the cancer cell migration within a 3D culture environment [25]. In this model, the gradients of oxygen are easily controlled with the aid of a designed holder, which generates monotonically decreasing gradients of oxygen through the stack. And the migratory response of the cells is easily measured by peeling stacked layers apart. Through fluorescence microscopy, the movement of cells in the paper-based cell culture system can be monitored and tracked in real time, which facilitates the investigation of the chemotaxis behavior of cancer cells [76].

Cancer cells with powerful proliferation ability trigger an excessive accumulation of lactic acids, resulting in acidic extracellular microenvironment [32,77,78]. CiGiP platform has been used to establish an acidified microenvironment model to study the tumor metabolic activity of the cells. The multilayers of paper in CiGiP platform can mimic the pH gradient near tumor cells, where the lactic acid that accumulates at the middle paper layer can hardly be flushed away by active perfusion. CiGiP is also flexible to dimensional changes through simple stacking and destacking of the paper stack, allowing the analysis of lactic acid accumulation at different layers. For instance, CiGiP has been used to develop a breast cancer model (Fig. 3b) [32]. Human breast cancer cells (MCF-7 cells) were encapsulated in a hydrogel and then seeded on a layer of NC membrane (NC). Eight layers of NC membranes treated with oxygen plasma were stacked up and packed together into a microfluidic chip to mimic the *in vivo* microenvironment of breast cancer cells. This breast cancer tissue was cultured in a perfusion manner at varying flow rate to investigate the tumor acidification of the microenvironment. It was found that the

Please cite this article in press as: K. Ng, et al., Mater. Today (2016), http://dx.doi.org/10.1016/j.mattod.2016.07.001

degree of microenvironment acidification in the breast cancer tissue depended on the perfusion flow rate. Establishing such an acidified tumor microenvironment model plays an important role in studying tumor metabolisms, including invasiveness, metastatic behavior, and resistance to cytotoxic agents. In addition, CiGiP was also used to evaluate the sensitivity of A549 cells (lung cancer cells) to ionizing radiation in a gradient of oxygen, a condition which is similar to *in vivo* microenvironment of a solid tumor. It has been found that radiosensitivity of lung cancer cells subjected to hypoxia is lower than those subjected to high oxygen tension. Reduced radiosensitivity of lung cancer cells could be associated with the lack of oxygen as a radiosensitizer, which produces free-radical species to kill the cells. This indicates that oxygen plays an important role in radiation therapy of cancer. The findings of this study would aid to discover novel and effective therapies to improve outcome of cancer patients [79]. The disease models made of paper-based cell culture platform are favorable because of their unique advantages compared with the conventional *in vitro* 3D model (e.g., hydrogel and bio-elastomers). For example, spatial control over cell distribution is challenging for hydrogel and bio-elastomers, but it can be achieved by patterning cells in single-layered papers and stacking these papers into a 3D model [10,12]. Besides, the cell culture microenvironment can also be controlled by placing different reagents and ECM proteins into papers before stacking up the papers into a 3D model [11]. Furthermore, the wicking ability of paper allows the exchange and transport of nutrients in the paper-based cell culture platform (Fig. 3c) [10,12,32]. In addition, the hydrophobic or hydrophilic properties of paper can be altered by adding wax or PDMS, which is essential in limiting the transfer of nutrient, thus leading to an efficient ischemic model [9–12]. With the advancement of technology, imaging and quantification of VIC in the CiGiP platform can be performed easily using confocal microscope and gel scanner after destacking the paper layers [11].

Paper-based cell culture platform for drug screening

Cell-based drug screening through monitoring of cell responses (e.g., cell apoptosis and cell metabolic products) to the drug treatment is very important for development of new drugs [80,81]. Therefore, significant effort has been made on the development of various methods for sensitive and specific detection of drug-stimulated cell responses. Compared with conventional plastic based platforms, paper provides distinct advantages, including low cost, eco-friendliness, short time of testing, and low consumption of reagent volume [82]. Furthermore, paper can provide a 3D microstructure for *in vitro* cell culture mimicking native 3D microenvironment [12,9], thus holding great promise for drug screening.

Monitoring cell responses to drug treatment has been developed recently for drug screening. First, the cells are captured in a modified paper detection zone and then the captured cells are subsequently treated with drugs. The drug screening is completed through detecting cell responses induced by drug treatment to evaluate the performance of the drugs (Fig. 4a). Recently, Yu and coworkers have created electrochemical detection of cancer cell responses in paper for anticancer drug screening (Fig. 4b). The paper culture platform was fabricated by stacking multilayers of punched paper forming four wells to store culture medium containing anticancer drugs (Fig. 4b(i)). The human acute promyelocytic leukemia cells (HL-60) were captured through aptamer-modified paper-based electrode, which was fabricated by the growth of gold nanoparticles (AuNPs) on the surfaces of cellulose fibers in paper. Sequentially, bovine serum albumin (BSA) was loaded for blocking the nonspecific binding sites. For anticancer drug screening, the paper tab containing captured cells was gripped by the paper culture dish containing anticancer drugs. The paper cell zones were aligned with the corresponding wells of the paper culture dish to make the cells cultured in culture medium with the drugs. After the drug treatment, the cell apoptosis was obtained, which was successfully shown by the translocation of the membrane phosphatidylserine (PS) to the extracellular environment of the apoptotic cells [83]. Furthermore, the PS molecules can be specifically recognized and bound by annexin-V. Therefore, drug-induced cancer cell apoptosis can be monitored by the interaction of PS and horseradish peroxidase-labeled annexin-V (HRP-annexin-V) bioprobe (Fig. 4b(ii)), and the electrochemical response of HL-60 cell apoptosis was obtained (Fig. 4b(iii–v)), which was induced by cycloheximide, etoposide, and camptothecin, respectively. The camptothecin drug was shown to be the best one for the HL-60 cell apoptosis. In addition to detection of drug-induced cell apoptosis, monitoring specific biomarkers released from drug stimulated cells is another effective approach for drug screening [84].

The monitoring of cell response to drug treatment is an effective way for drug screening. Paper-based platforms are capable of performing high-throughput cell culture by physical modification (e.g., wax printing), forming multiple cell zones defined by barriers in paper. The paper-based platform has shown promising ability for multiple detection of cell responses to various drugs. Considering that the cell responses are normally at an extremely low level, the paper-based electrochemical detection methods are widely used for the detection of cell responses because of their high sensitivity, selectivity, and quantitative nature [85]. In addition, high-throughput electrochemical detection of cell responses can be achieved by using the separated electrodes on paper cell zone to achieve high-throughput drug screening applications.

Paper-based platform for cell cryopreservation

Cryopreservation has been widely used for long-term storage of biospecimens, such as cells, cell aggregates, and tissues. Among various cryopreservation methods, vitrification holds the advantages of maintaining viability, genetic profiles, and cytoskeletal structure of cells [86]. For instance, various vitrification methods, such as open pulled straw, quartz micro-capillary, cryotop, cryoloop [38], and electron microscope grid methods [87], have been adopted to vitrify mammalian embryos and successfully maintain the functional properties of post-thawing embryos. However, these methods require special devices to acquire a small volume for achieving high cooling rate for vitrification, which may be expensive and not suitable for large-scale cryopreservation of embryos [88]. In this sense, paper-based cell culture platform can serve as a more convenient and inexpensive device to achieve minimized droplet and efficient embryo vitrification through its favorable absorbing property (Fig. 5a) [39,89]. In this case, embryos were cultured on paper substrates, which act as an absorbent to reduce the vitrification solution around embryos to achieve small-volume vitrification. For instance, approximately 10 bovine embryos in a 5-μL droplet located on the surface of a weighing paper were directly vitrified

Please cite this article in press as: K. Ng, et al., Mater. Today (2016), http://dx.doi.org/10.1016/j.mattod.2016.07.001

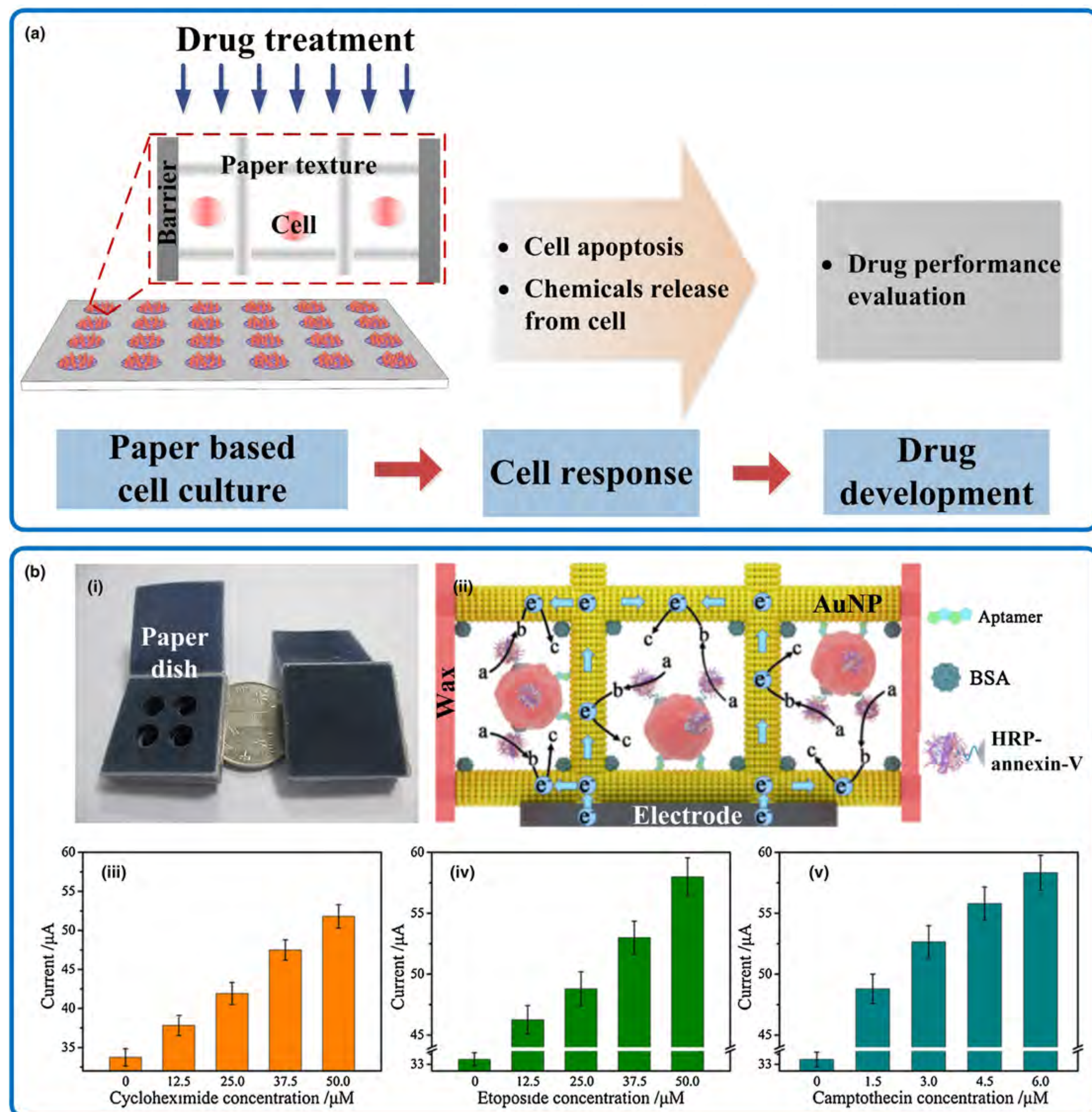

FIGURE 4

Application of paper-based cell culture platform for drug screening. (a) Schematic diagram of the working principle of cell-based drug screening on paper; (b) paper-based platform was used for anticancer drug screening. (i) Paper culture dish for cell culture. (ii) Schematic detection of cell apoptosis. (iii)–(v) Fluorescent evaluation of HL-60 cell apoptosis after treatment with cycloheximide (iii), etoposide (iv), and camptothecin (v). Images reproduced from [97].

in liquid nitrogen, and the results show that the viability of the embryos was comparable to those vitrified on an electron microscope grid [39]. In order to further increase the vitrification efficiency, Kimwipes tissue with high absorbing ability was used together with a cryotube to vitrify almost 20 mouse embryos in a 1- to 2-μL droplet on paper (Fig. 5b). The cell viability and birth rate of mouse embryos vitrified on Kimwipes tissue were comparable to those vitrified using cryoloop (a standard vitrification method for embryos) [89] (Fig. 5c). Moreover, Kimwipes tissue is inexpensive with large porosity to arrange several embryos on its surface, which enables large-scale vitrification. Taken together, a paper-based platform offers a promising cryopreservation method, which is convenient, inexpensive, and holds a high potential to preserve biospecimens such as oocytes and embryos in a large scale.

Please cite this article in press as: K. Ng, et al., Mater. Today (2016), http://dx.doi.org/10.1016/j.mattod.2016.07.001

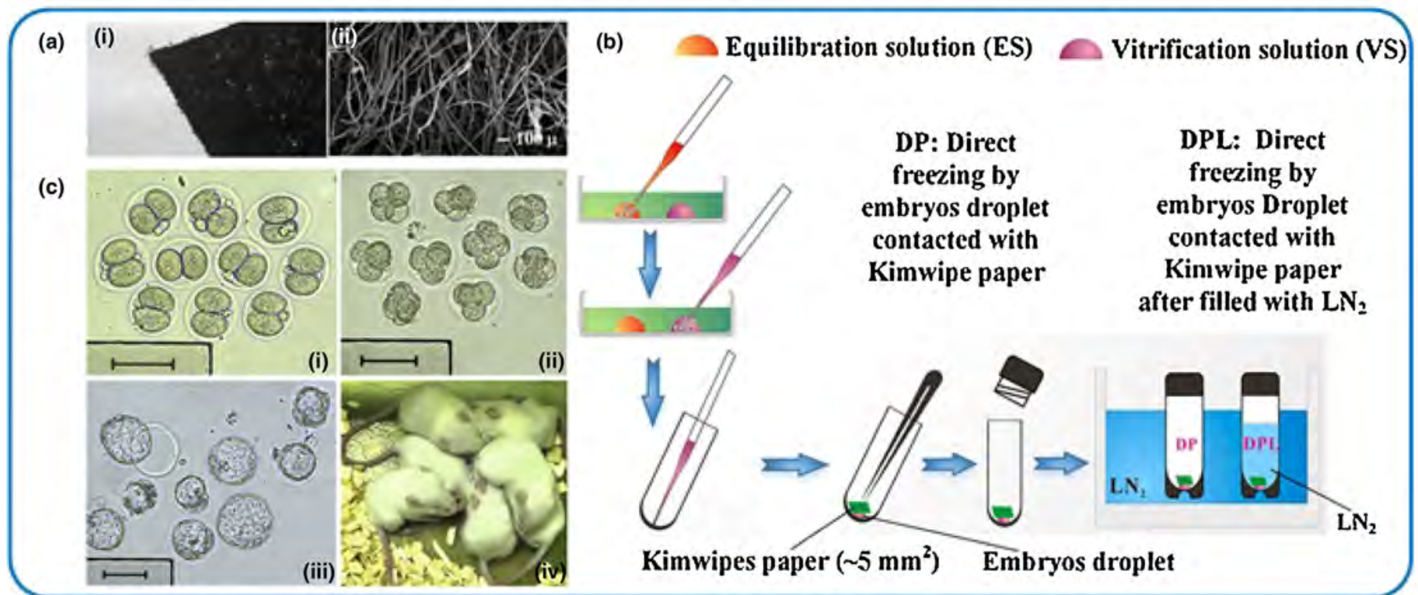

FIGURE 5

Paper-based cryopreservation. (a) Microscope observation of the paper used for bovine blastocyst vitrification: (i) inverted microscope, (ii) scanning electron microscope [39]; (b) Vitrification of mouse embryo in cryotube using Kimwipes paper (KP) as cell holder; (c) Representative embryos and living pups born from mouse embryos vitrified in paper-based cryotubes. (i) Two-celled embryos before vitrification, (ii) four-celled embryos after warming and culturing, (iii) blastocysts after warming and culturing, (iv) Living pups were born from vitrified embryos. Images reproduced from [89].

Conclusion and future perspectives

Paper has emerged as a promising cell culture substrate that could offer high potentials in developing 2D and 3D cell culture platforms, although modifications of its chemical and physical properties are required. For instance, paper needs to be coated with some beneficial chemical groups to provide binding sites for cell attachment or its surface roughness and stiffness can be adjusted to allow a better cell growth. Existing 3D cell cultures based on hydrogel and porous scaffold are mostly nonuniform in dimension, failing to provide information about cells in different areas within a single 3D construct, and this physical isolation of cells from different regions of the 3D construct are needed before staining and biochemical characterization [10,90]. Meanwhile, paper-based cell culture platforms that have been used in many biomedical applications show better results. For example, the CiGiP system can better mimic condition of *in vivo* microenvironment to create 3D normal or diseased tissue models, such as ischemic diseased models[9–12]. By using such system, accurate cell behavior in response to chemical or physical stimulation can be studied. These models can be used for pathophysiological studies and therapeutic intervention studies (e.g., drug screening or cell-based therapy), leading to the development of pharmaceutical and regenerative medicine. However, paper-based cell culture platforms are still associated with some limitations. Similarly, culture medium was laterally wicked to the hydrophobic zone of CiCiP [9,10,12] and matrigel used in CiGiP was found to increase the risk of tumorigenesis in the cells [91]. Further, paper substrate is difficult to stretch, making evaluation of mechanically induced cell response difficult. Mechanical properties of paper decline drastically when immersed in cell culture medium because of the breakage of hydrogen bonds that hold the cellulose together. The insufficiency of 'wet' mechanical properties affects the paper's efficiency as a scaffold, which is required as anchoring sites for cells. The slow degradation of paper may also limit its application in transplantation, where the transplant for some tissues (e.g., cardiac muscle and skin) needs to degrade in a certain period of time to allow the growth of tissue to replace the implant, so that a secondary surgical procedure to remove the implant is unnecessary. The commercially available paper contains additives (e.g., clay, calcium carbonate, and titanium oxide) that are added during the manufacturing processes, which may be released with the degradation of paper and induce adverse effect to the cells. In addition, paper-based cell culture platforms have relied on fluorescence imaging for absolute quantification of cells [92], which is inaccurate due to limited background fluorescence arising from the paper fibers.

In order to enhance the properties of paper material and extend its applications, the structural design and manufacture process need to be further optimized. For instance, mechanical properties of paper can be enhanced by increasing the fiber diameter, porosity, and porous orderliness. In addition, the formation of paper-based composites by incorporating with other strengthened functional materials (e.g., carbon fiber and graphene) can also reinforce the mechanical property of paper materials. For increasing biocompatibility of paper materials, it is necessary to modify the biological molecules (e.g., peptide and adhesive proteins) in the fibrous networks of paper-based materials, which can promote the cell activities including adhesion, proliferation, and differentiation. Another method is regulating the topographical properties of paper fibers by optimizing the processing characters, which can also enhance the cell adhesive behavior. In addition, to address the limitation of insufficiency of 'wet' mechanical properties, it is necessary to use the hydrophobic bonding agent (e.g., alum) that can further promote the interaction between paper fibers while the paper is immersed in the liquid, especially for cell culturing system. Furthermore, existing advanced micro/nanotechnologies

Please cite this article in press as: K. Ng, et al., Mater. Today (2016), http://dx.doi.org/10.1016/j.mattod.2016.07.001

(e.g., bioprinting, biopen, microfluidics, and electrochemistry) can be integrated into the paper-based cell culture platform. For example, bioprinting would allow cells encapsulated in ECM to be ejected onto the desired position on paper-based cell culture platform accurately with a well-controlled spatial distribution of cells throughout the printed area. This purpose can also be achieved by using a more cost-effective biopen, which allows cells to be written onto a paper-based cell culture platform [93]. Moreover, microfluidic networks can be integrated into paper-based cell culture platforms as blood vessels and capillaries, enabling studies on the interaction among various cells or tissues. With such integration, a paper-based cell culture platform that better mimics the characteristics of native tissues can be developed efficiently [30,31]. Following the development of a tissue model or disease model, electrochemical device can be integrated by writing electrodes using a ball pen onto the model, to assess the cell responses (e.g., secretion of soluble molecules such as neurotransmitter) and metabolic activities (e.g., pH and oxygen consumption) upon experimental intervention (e.g., drug screening), which may be beneficial in pharmaceutical development [85]. Furthermore, the integration of paper substrate with various detection modalities (e.g., optical and electrical sensing platforms) enables broad biomedical applications. For instance, a cellulose paper substrate integrated with a mobile phone system was successfully developed to detect *Escherichia coli* in plasma [94]. Particularly, it will be beneficial to integrate paper-based cell culture platforms with these detection modalities for cell-based diagnostics because of the advantages including low cost, high sensitivity, and high selectivity. In addition, to accurately quantify cells in the paper-based cell culture platforms, a polymerase chain reaction (PCR)-based method with a DNA barcoding strategy was developed, enabling a small population of cells, which is distinguished from other cell types to be detected in the paper-based cell culture platforms [92]. We believe that due to its unique advantages, paper will find a wide spectrum of application in the field of biomedicine.

Acknowledgments

This study was financially supported by the National Natural Science Foundation of China (11372243, 11522219, 11532009, 81300696), the International Science & Technology Cooperation Program of China (2013DFG02930), China Postdoctoral Science Foundation (2014M552463), Natural Science Foundation of Shaanxi Province of China (2015JM8471), Fundamental Research Funds for the Central Universities, Key Program for International S&T Cooperation Projects of Shaanxi (2013KW33-01), the Fundamental Research Funds for the Central Universities (xjj2014059), High Impact Research Grant (UM.C/HIR/MOHE/ENG/44) and Fundamental Research Grant Scheme (FP054-2015A) from the Ministry of Higher Education Malaysia.

References

[1] Y.-H. Chen, et al. Trends Biotechnol. 33 (1) (2015) 4.
[2] A.W. Martinez, et al. Angew. Chem. Int. Ed. 46 (8) (2007) 1318.
[3] N.R. Pollock, et al. Sci. Transl. Med. 4 (152) (2012) 152ra129.
[4] D. Tobjörk, R. Österbacka, Adv. Mater. 23 (17) (2011) 1935.
[5] A. Russo, et al. Adv. Mater. 23 (30) (2011) 3426.
[6] C. Parolo, A. Merkoçi, Chem. Soc. Rev. 42 (2) (2013) 450.
[7] L. Ge, et al. Biomaterials 33 (4) (2012) 1024.
[8] G.G. Lewis, et al. Angew. Chem. 124 (51) (2012) 12879.
[9] R. Derda, et al. Proc. Natl. Acad. Sci. U. S. A. 106 (44) (2009) 18457.
[10] R. Derda, et al. PLoS ONE 6 (5) (2011) e18940.
[11] M.C. Sapp, et al. Acta Biomater. 13 (0) (2015) 199.
[12] B. Mosadegh, et al. Adv. Healthc. Mater. 3 (7) (2014) 1036.
[13] H.-J. Park, et al. Biomaterials 35 (37) (2014) 9811.
[14] H. Juvonen, et al. Acta Biomater. 9 (5) (2013) 6704.
[15] M.M. Stevens, Mater. Today 11 (5) (2008) 18.
[16] A. Struss, et al. Anal. Chem. 82 (11) (2010) 4457.
[17] H. Bäckdahl, et al. J. Tissue Eng. Regen. Med. 2 (6) (2008) 320.
[18] D. Klemm, et al. Angew. Chem. Int. Ed. 50 (24) (2011) 5438.
[19] M. Bhattacharya, et al. J. Control. Release 164 (3) (2012) 291.
[20] K.A. Simon, et al. Biomaterials 35 (1) (2014), http://dx.doi.org/10.1016/j.biomaterials.2013.09.049.
[21] X. Zong, et al. Biomaterials 26 (26) (2005) 5330.
[22] R. Ravichandran, et al. Macromol. Biosci. 13 (3) (2013) 366.
[23] G. Luo, et al. ACS Appl. Mater. Interfaces 7 (50) (2015).
[24] S. Fleischer, et al. Biomaterials 34 (34) (2013) 8599.
[25] B. Mosadegh, et al. Biomaterials 52 (2015) 262.
[26] S. Wang, et al. Biosens. Bioelectron. 31 (1) (2012) 212.
[27] W.-J. Li, R.S. Tuan, Current Protocols in Cell Biology, John Wiley & Sons Inc., 2001.
[28] X.Y. Liu, et al. 2011 IEEE 24th International Conference on Micro Electro Mechanical Systems (MEMS), 2011, . p. 75.
[29] A. Abbott, Nature 424 (6951) (2003) 870.
[30] O. Frey, et al. Nat. Commun. (2014) 5.
[31] S. Rismani Yazdi, et al. Lab Chip 15 (21) (2015) 4138.
[32] W. Yan, et al. Chin. J. Anal. Chem. 41 (6) (2013) 822.
[33] G. Huang, et al. Biofabrication 4 (4) (2012) 042001.
[34] A. Qi, et al. Adv. Healthc. Mater. 3 (4) (2014) 543.
[35] Z. Liu, et al. Appl. Therm. Eng. 88 (2015) 280.
[36] G. Huang, et al. Soft Matter 8 (41) (2012) 10687.
[37] F. Xu, et al. Adv. Mater. 23 (37) (2011) 4254.
[38] X. Zhang, et al. Nanomedicine 6 (6) (2011) 1115.
[39] Y. Kim, et al. Theriogenology 78 (5) (2012) 1085.
[40] J. Hu, et al. Biosens. Bioelectron. 54 (0) (2014) 585.
[41] J.R. Choi, et al. Biosens. Bioelectron. 74 (2015) 427.
[42] H. Holik, Handbook of Paper and Board, Wiley, 2013.
[43] S. Kalia, et al. Int. J. Polym. Sci. 2011 (2011).
[44] R. Mormino, H. Bungay, Appl. Microbiol. Biotechnol. 62 (5) (2003) 503.
[45] A. Engler, et al. Biophys. J. 86 (1) (2004) 617.
[46] D. Mitrossilis, et al. Proc. Natl. Acad. Sci. U. S. A. 106 (43) (2009) 1824.
[47] C.M. Lo, et al. Biophys. J. 79 (1) (2000) 144.
[48] A.J. Engler, et al. Cell 126 (4) (2006) 677.
[49] A.W. Feinberg, et al. J. Biomed. Mater. Res. A 86 (2) (2008) 522.
[50] R. Rahimi, et al. J. Micromech. Microeng. 25 (5) (2015) 055015.
[51] W.E. Tenhaeff, K.K. Gleason, Adv. Funct. Mater. 18 (7) (2008) 979.
[52] J. Sarfraz, et al. Sens. Actuators B: Chem. 173 (2012) 868.
[53] E.A. Vogler, Adv. Colloid Interface Sci. 74 (1) (1998) 69.
[54] Y. Arima, H. Iwata, Biomaterials 28 (20) (2007) 3074.
[55] P. Silva-Bermudez, et al. Appl. Surf. Sci. 258 (5) (2011) 1711.
[56] C.A. Haynes, W. Norde, J. Colloid Interface Sci. 169 (2) (1995) 313.
[57] S.P. Hoo, et al. J. Mater. Chem. B 1 (24) (2013) 3107.
[58] S. Sen, et al. Cell. Mol. Bioeng. 2 (1) (2009) 39.
[59] I. Levental, et al. Soft Matter 3 (3) (2007) 299.
[60] C. Min Jin, et al. Biofabrication 4 (4) (2012) 045006.
[61] D.E. Discher, et al. Science 310 (5751) (2005) 1139.
[62] B. Baharloo, et al. J. Biomed. Mater. Res. A 74 (1) (2005) 12.
[63] S. Chung, M.W. King, Biotechnol. Appl. Biochem. 58 (6) (2011) 423.
[64] L. Wang, R.L. Carrier, Biomimetic Topography: Bioinspired Cell Culture Substrates and Scaffolds, INTECH Open Access Publisher, 2011.
[65] J.R. Tse, A.J. Engler, Current Protocols in Cell Biology, John Wiley & Sons, Inc., 2001.
[66] L. Wang, et al. Lab Chip (2015).
[67] L. Tian, et al. Anal. Chem. 84 (22) (2012) 9928.
[68] F. Deiss, et al. Angew. Chem. Int. Ed. 53 (25) (2014) 6374.
[69] J.-P. Frimat, et al. Anal. Bioanal. Chem. 395 (3) (2009) 601.
[70] M. de Silva, et al. Biomed. Microdevices 6 (3) (2004) 219.
[71] F. Deiss, et al. Anal. Chem. 85 (17) (2013) 8085.
[72] Y. Li, et al. Adv. Funct. Mater. 25 (37) (2015) 5999.
[73] L. Wang, et al. Curr. Med. Chem. 21 (22) (2014) 2497.
[74] G.M. Whitesides, Nature 442 (7101) (2006) 368.
[75] K.J. Rodriguez, K.S. Masters, J. Biomed. Mater. Res. A 90 (4) (2009) 1043.

[76] R.M. Kenney, et al. Analyst 141 (2) (2016) 661.
[77] P.A. Schornack, R.J. Gillies, Neoplasia 5 (2) (2003) 135.
[78] S.K. Parks, et al. J. Cell. Physiol. 226 (2) (2011) 299.
[79] K.A. Simon, et al. Biomaterials (2016).
[80] R. Pérez-Tomás, Curr. Med. Chem. 13 (16) (2006) 1859.
[81] R.W. Johnstone, Nat. Rev. Drug Discov. 1 (4) (2002) 287.
[82] Y.H. Chen, et al. Trends Biotechnol. 33 (1) (2015) 4.
[83] T. Liu, et al. Anal. Chem. 81 (6) (2009) 2410.
[84] F. Liu, et al. Chem. Commun. 50 (71) (2014) 10315.
[85] Z. Li, et al. Analyst (2015).
[86] M. Shi, et al. Sci. Rep. (2015) 5.
[87] A. Martino, et al. Biol. Reprod. 54 (5) (1996) 1059.
[88] H. Matsunari, et al. J. Reprod. Dev. 58 (5) (2012) 599.
[89] K.-H. Lee, et al. Cryobiology 66 (3) (2013) 311.
[90] Y. Xiao, et al. Methods 84 (2015) 44.
[91] R. Fodde, Cancer Cell 15 (2) (2009) 87.
[92] A.S. Truong, et al. Anal. Chem. 87 (22) (2015) 11263.
[93] Y.L. Han, et al. Sci. Rep. (2014) 4.
[94] H. Shafiee, et al. Sci. Rep. (2015) 5.
[95] Y. Kim, et al. Reprod. Fertil. Dev. 16 (2) (2003) 173.
[96] T. Songjaroen, et al. Talanta 85 (5) (2011) 2587.
[97] S. Min, et al. Anal. Chim. Acta 847 (2014) 1.

Please cite this article in press as: K. Ng, et al., Mater. Today (2016), http://dx.doi.org/10.1016/j.mattod.2016.07.001