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REVIEW

Fabrication of paper-based devices for in vitro tissue modeling

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Abstract
Paper devices have recently attracted considerable attention as a class of cost-effective cell culture substrates for various biomedical applications. The paper biomaterial can be used to partially mimic the in vivo cell microenvironments mainly due to its natural three-dimensional characteristic. The paper-based devices provide precise control over their structures as well as cell distributions, allowing recapitulation of certain interactions between the cells and the extracellular matrix. These features have shown great potential for the development of normal and diseased human tissue models. In this review, we discuss the fabrication of paper-based devices for in vitro tissue modeling, as well as the applications of these devices toward drug screening and personalized medicine. It is believed that paper as a biomaterial will play an essential role in the field of tissue model engineering due to its unique performances, such as good biocompatibility, eco-friendliness, cost-effectiveness, and amenability to various biodesign and manufacturing needs.

Keywords Paper-based devices · In vitro · Tissue modeling · Disease modeling · Drug screening · Personalized medicine

Introduction

Paper, known as one of the most ancient inventions, has entailed a massive change in human life throughout our history, enabling a wide variety of applications across different disciplines such as literature, art, science, and engineering [1, 2]. Paper also is a flexible and porous material, which can be used as a potential substitute to the traditional substrates, such as plastics [3], glass [4], and elastomers [5] that are commonly adopted for cell culture. The paper material consists primarily of a network of cellulose micro- and/or nanofibers that essentially form a three-dimensional (3D) multi-porous structure [6], providing a variety of controllable surface morphologies, internal microstructures, and physicochemical properties [7–9]. In addition, paper-based materials possess good biocompatibility, mainly attributed to their natural origin and the fact that they can be maintained with a sufficient supply chain at low production costs using mature processing procedures [10, 11]. Thus, this class of materials have been utilized in a variety of biomedical and healthcare applications, such as disposable analytical devices [12, 13], flexible electronics devices [14, 15], biosensor devices [16, 17], and, most recently, cell culture devices for tissue modeling [18–20] and drug screening [21, 22]. The paper-based devices offer a variety of advantages, for instance flexibility, ability to be shaped into 3D structures, ease to be modified, cost-effectiveness, environmental friendliness, and large-scale production capacity [2, 23, 24].

As discussed, a key feature of the paper biomaterial is its unique 3D fibrous network feature that enables physiologically relevant gas and liquid transport for the cells, simulating the local microenvironments better than the traditional two-dimensional (2D) in vitro cultures [18, 25–27]. In addition, the microporous structures and large void volume ratios of the paper-based devices can quickly absorb fluids through the capillary action and enhance the wicking process [28], thus increasing the capacity of cell proliferation and migration inside the paper-based scaffolds [29, 30].

To this end, 3D paper-based devices have been applied to fabricating in vitro tissue models by stacking multilayered

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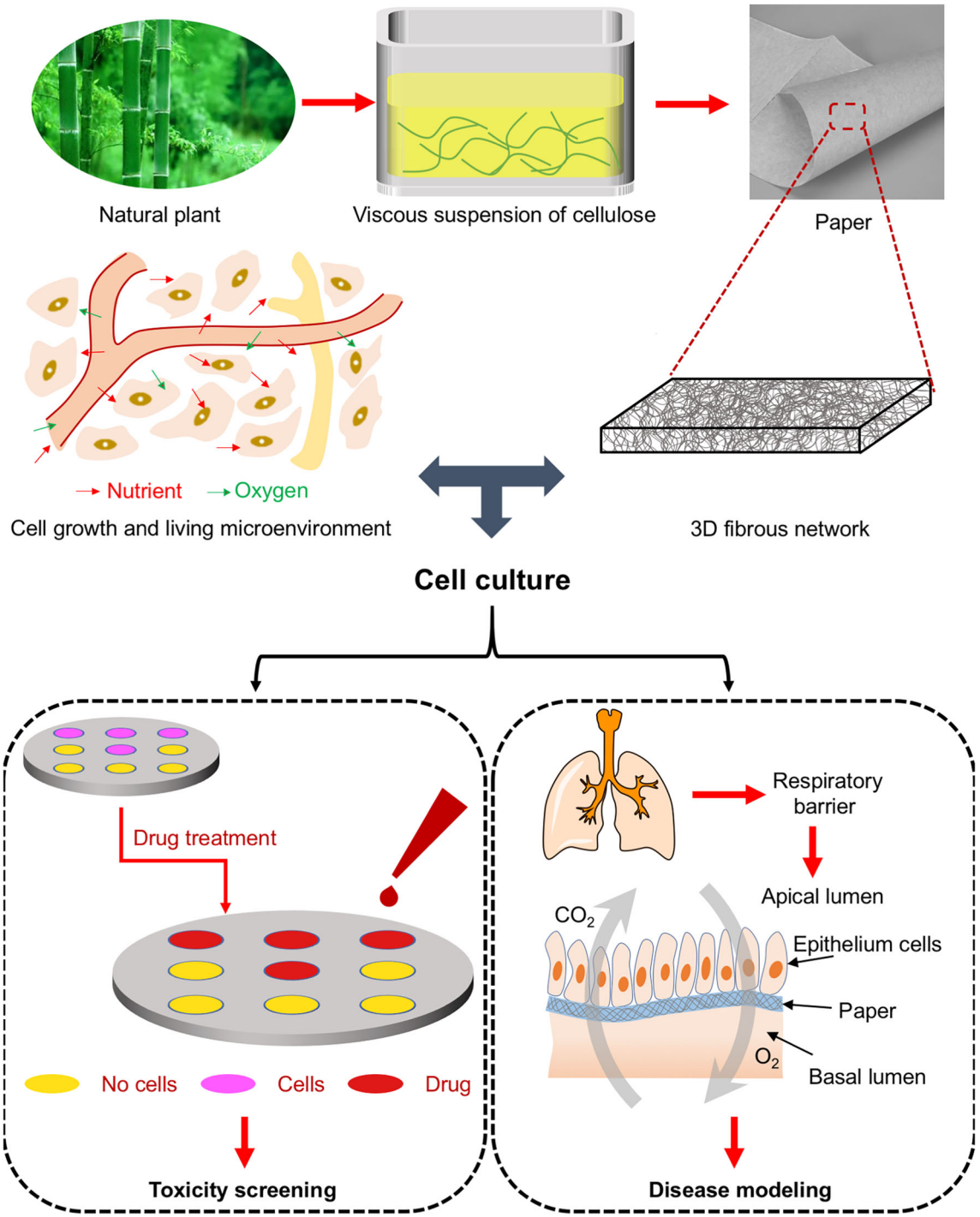

Fig. 1 Natural plants are used as raw materials for paper production at low costs. The 3D fibrous networks of the paper biomaterials can partially mimic the ECM microenvironments for cell growth. As such, paper-based human tissue/disease models can be used to study the cell—drug interactions and to screen therapeutics. As an example, the porous nature of paper renders it possible to create an in vitro lung model for studies of the human respiratory system and disorders. Images reproduced with permissions [32, 33, 72, 91]. *3D* three-dimensional, *ECM* extracellular matrix

paper substrates to support and maintain the 3D soft hydrogel structures containing cells [18, 19, 31]. It was also demonstrated that these 3D tissue structures might be processed to enable desired cellular spatial distributions and that the stacked paper substrates could be easily disassembled into individual layers for investigations into cell morphologies

Table 1 Paper types, their characteristics, and representative utility cases

Paper type	Thickness (μm)	Mechanical strength	Cell type	Application	References
Whatman® filter paper #114	190	2.8 MPa	Cardiomyocytes; aortic VICs; human acute promyelocytic leukemia cells	Cell culture/disease modeling/drug screening	[25, 30, 34]
Whatman® Protran® nitrocellulose membrane	130–160	1.8–2.6 MPa	Human breast cancer cells	Cell culture/disease modeling	[27]
Janus paper	–	392 kPa	Human lung fibroblasts	Disease modeling	[35]
Weighing paper	100–200	150–650 kPa	Bovine blastocysts; hADSCs	Cell culture/disease modeling	[36]
Printing paper	100–200	–	hiPSCs	Cell culture	[23]

hADSCs human adipose-derived stem cells, *hiPSCs* human-induced pluripotent stem cells, *VICs* valvular interstitial cells

and functions without needing any optical or histological sectioning [29]. For example, an in vitro model of the human lung tissue based on a paper device was fabricated by the direct-patterned laser-treated hydrophobic paper technology [32]. Furthermore, the 3D printing technology has been implemented in the creation of microchannels in a volumetric manner to obtain a cost-effective paper-based vascularized tissue model [33]. With these technological advancements in biodesign and manufacturing, paper-based devices have become a class of ideal substrates for generating 3D in vitro tissue models [2, 23]. Such low-cost, disposable paper-based devices, therefore, have facilitated our understanding of the disease mechanisms and drug responses (Fig. 1) [25, 30, 33]. A variety of commercially available paper-based materials have been selected for the purpose. Table 1 summarizes the main characteristics of the different types of paper-based materials that have been applied to tissue model engineering.

In this review, we discuss the design and manufacturing of paper-based in vitro human tissue models toward applications in drug screening and therapeutics development.

Fabrication of the paper-based devices

Paper-based devices can be fabricated with different methods such as wax printing [37], inkjet printing [38], flexographic printing [39], flash foam stamp lithography (FFSL) [40], origami [29], and 3D printing [33, 41] (Fig. 2). The above-mentioned techniques further enable the possibility to create a hydrophobic–hydrophilic microfluidic channel contrast in additional to the intrinsic capillarity of the paper biomaterial itself, making the devices suitable for handling small volumes of fluids for quantitative analysis in many potential applications related to health care and medicine.

To obtain low-cost paper-based devices for cell culture, wax printing, a type of fused deposition modeling (FDM), has been developed based on the combination of a commercial printer with a heating plate [42, 43]. The melted wax ink loaded into the printhead is used to print a pattern on the surface of a paper, which experiences instantaneous cooling and solidification without further lateral diffusion. Then, the wax pattern can be melted and allowed to penetrate inside of the paper matrix by the underlying high-temperature heating plate. This technology can be adopted to build integrated and continuous hydrophobic barriers inside and outside of the paper substrates [42]. As such, printing with wax makes it easy to create microfluidic patterns on paper substrates (Fig. 2A) [44]. Like wax printing, wax screen-printing is also regarded as a low-cost strategy for producing paper-based microfluidic systems [45, 46]. The process is similar to wax printing, with the main difference being that the patterns are built by pressing solid wax into a screen stencil rather than printing on a paper surface [47]. In addition, wax as a hydrophobic material that also exhibits no cell-binding moieties can inhibit cell adhesion as a barrier material that directs and restricts cell presence in the specific areas of the paper-based devices [25, 48].

Inkjet or flexographic printing is another method for producing paper-based devices [49, 50]. For example, arrays used for 2D cell culture can be created by printing patterns on a paper matrix using a hydrophobic polydimethylsiloxane (PDMS) ink [51]. Like wax, PDMS is also a biocompatible material that hinders the attachment of most cell types [52]. Similarly, the PDMS ink can be selected to obtain array structures through flexographic printing with a patterned printing plate for cell culture (Fig. 2B) [53].

Unlike flexographic printing, FFSL is a stamping-based fabrication method for rapid prototyping of various types of microscale and nanoscale structures [40, 54]. A typical FFSL process is shown in Fig. 2C [55]. First, a flash foam stamp (FFS) with designed channels is fabricated and then immersed in the hydrophobic ink for absorption. When the FFS is stamped onto the paper, the hydrophobic ink is transferred to the paper. The paper-based devices with hydrophobic barriers are consequently obtained after the ink has solidified. It was demonstrated that 3D cell cul-

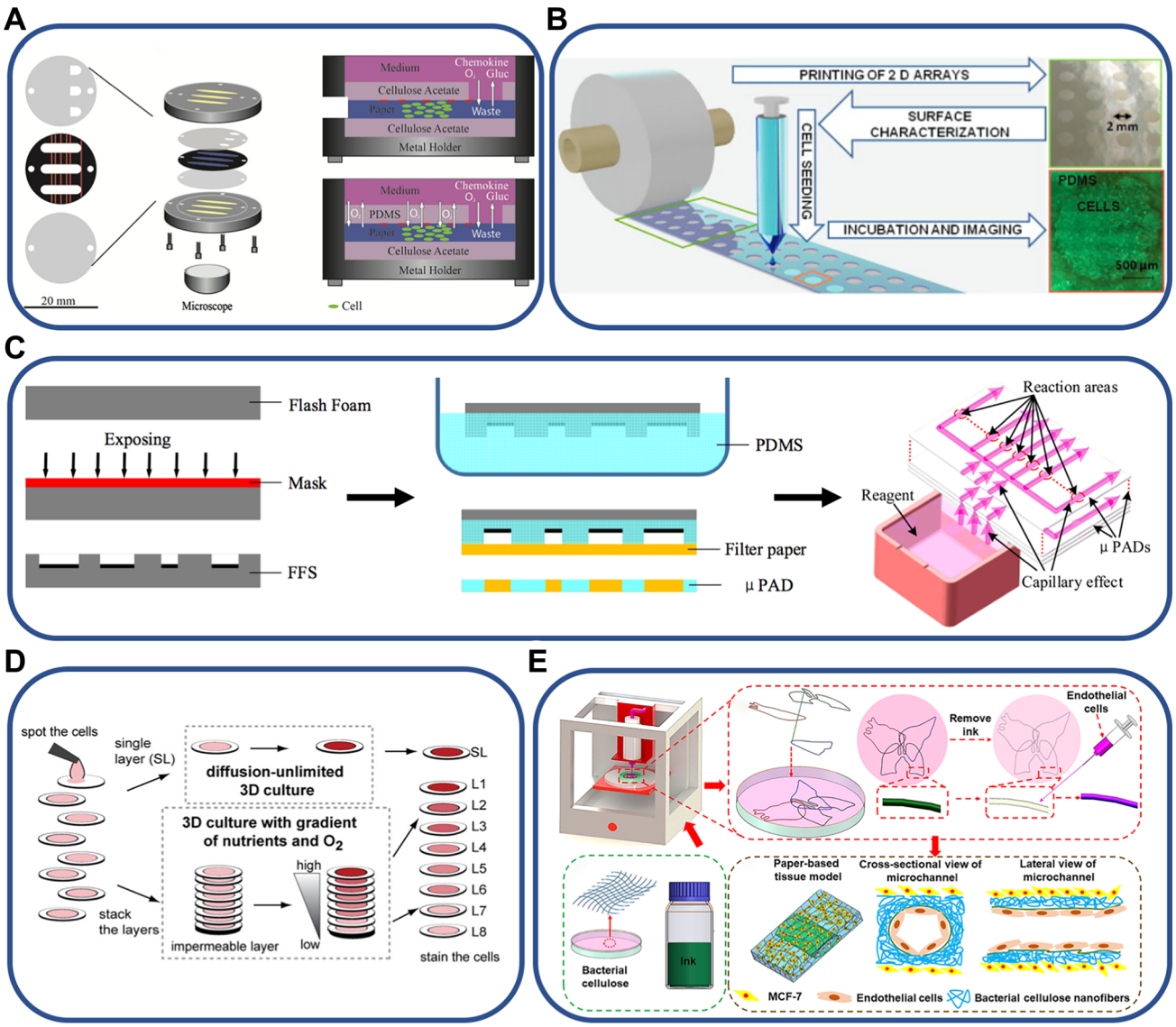

Fig. 2 Fabrication of paper-based devices. **A** Wax printing. Images reproduced with permission [44]. **B** Inkjet and flexographic printing. Images reproduced with permission [53]. **C** FFSL. Images reproduced with permission [55]. **D** Multilayer stacks with hydrogel. Images reproduced with permission [29]. **E** 3D printing. Images reproduced with permission [33]. *FDM* fused deposition modeling, *FFS* flash foam stamp, *FFSL* flash foam stamp lithography, *PDMS* polydimethylsiloxane, *µPAD* microfluidic paper-based analytical device

turing could be performed using these paper-based devices with continuous perfusion, which may serve as a promising paper-based biofluidic platform for tissue modeling and drug screening.

In addition, origami-inspired paper-based in vitro cell culture devices have been further developed for the construction of multiform scaffolds [31, 56]. They allow to simulate the characteristics of the in vivo tissue microenvironments through porosity, stiffness, and flexibility, which are critical in obtaining physiologically relevant 3D tissue models. For example, to mimic the tumor microenvironment, a method of fabricating multilayered paper devices with cell-laden hydrogels was proposed (Fig. 2D) [29]. In their study, Whitesides and colleagues suggested that scaffolds with multiple layers of stacked paper substrates could serve as a model of breast cancer, which was used to analyze metastasis. In another work, the formation of oxygen gradient similar to that of the natural tissue was shown to be inducible by the multilayered paper-based devices [25].

More recently, 3D printing has drawn increasing attention in medical and biomedical applications as it allows the generation of structurally relevant scaffolds to support the attachment, growth, migration, and functions of cells through its ability to pattern biomaterials in an automated and spa-

tially defined manner [31, 57]. For example, paper-based devices containing hollow microchannels were successfully fabricated by extruding a 3D sacrificial ink based on wax into an aqueous suspension of bacterial cellulose, where the printed fibrous templates could maintain their integrity during the drying process and be subsequently removed (Fig. 2E) [33]. The results indicated that both endothelial cells and breast tumor cells had good proliferation when they were seeded into the microchannels and the surrounding paper matrices, respectively. This work may present a new method for creating volumetric in vitro tissue models due to its automation nature comparing to other fabrication methods such as origami.

Paper-based devices for in vitro tissue modeling

Paper-based devices exhibit significant advantages for in vitro tissue modeling. First, the micro- and/or nanofibrous structures of the paper-based devices are close to those of the native extracellular matrix (ECM). Moreover, the paper-based devices with varied structures can be easily accessed by altering the fiber and multicomponent properties. Second, the porous structures of the paper-based devices can enable the diffusion of paracrine molecules, oxygen, and nutrients, which are crucial in maintaining the viability and functions of cells grown on/in these devices. Third, paper-based materials are flexible substrates, which can be easily folded or bended for emulating the necessary macroscopic structures or shapes. Thanks to all these favorable features, paper-based devices have been increasingly considered to serve as a potentially powerful platform for establishing in vitro 3D human tissue and disease models.

Paper-based devices for human lung research

The lung is one of the body's vital organs, which performs the exchange of gases between the body and the external environment [58]. Under normal functional conditions, the lung acts as a physical barrier that controls gas exchange rates and maintains life activities of the human body [59]. However, the factors that cause life-threatening respiratory diseases coming from external stimuli such as harmful substances, allergens, pathogens, and smoking may damage the integrity of epithelial cells and other relevant cell types [60, 61] and even lead to severely impaired respiratory functions [62, 63].

To this end, researchers have analyzed the structures and functions of the pulmonary epithelial tissues by the use of different animal models in vivo or the static planar culture systems in vitro [64–66]. Among the different in vitro human-based models, the paper-based lung tissue models have been considered as an enabling tool for mimicking the lung diseases. For instance, a study reported that the surface properties of a commercial hydrophobic paper (parchment paper) coated with silicon were altered by CO_2 laser-assisted processing, creating a unique porous matrix that formed a hydrophilic region as an effective semipermeable membrane, which could be used to study the functions of the lung tissue (Fig. 3A-i) [32]. The formed hydrophilic regions could improve cell attachment and control fluid diffusion, which was similar to the semipermeable properties in the basement membrane of the human respiratory system. Furthermore, the barrier formation of the airway epithelium was compared between the air–liquid interface (ALI) cultures under static (transwell) and flow (paper-based model) conditions. The tight junctions could be observed to form much better under the flow culture (Fig. 3A-ii, iii). This paper-based in vitro lung tissue model generated the monolayer of lung epithelial cells, which could be applied further to disease modeling and in vitro drug screening.

In another study, researchers proposed a 3D paper-based system for cell culture that could evaluate the metabolic responses of lung cancer cells to ionizing radiation (Fig. 3B) [67]. The device was prepared by stacking multiple layers of paper substrates containing cell-embedded hydrogels. The hydrogels could reduce the concentrations of oxygen and nutrients in the stack forming a gradient from top to bottom (Fig. 3B-ii). As such, the metabolic sensitivity of the cells to ionizing radiation could be effectively investigated in this paper-based 3D lung cancer model for rapid assessment of radiation resistance of the cells (Fig. 3B-iii, iv).

Paper-based devices for human cardiovascular research

Cardiovascular diseases remain a major cause of death in developed countries despite the various clinical therapies that are performed to treat patients with this illness [68]. For instance, morbidity and mortality rates of ischemic heart disease and heart failure are increasing worldwide [69]. To this end, numerous microengineering strategies such as soft lithography, microcontact printing, and photopatterning have been selected to create 2D and 3D in vitro tissue models for mimicking cardiovascular diseases [70, 71]. However, these strategies are applicable to a limited number of materials and often involve sophisticated fabricating processes. To address these problems, low-cost and easy-to-mass producing processes have been investigated by the use of paper-based devices.

Wang and collaborators developed a method to obtain a simple array of circular zones made of commonly available paper biomaterials (nitrocellulose membrane, print paper, filter paper) for the culture and differentiation of human induced pluripotent stem cells (hiPSCs) into cardiac-specific tissue

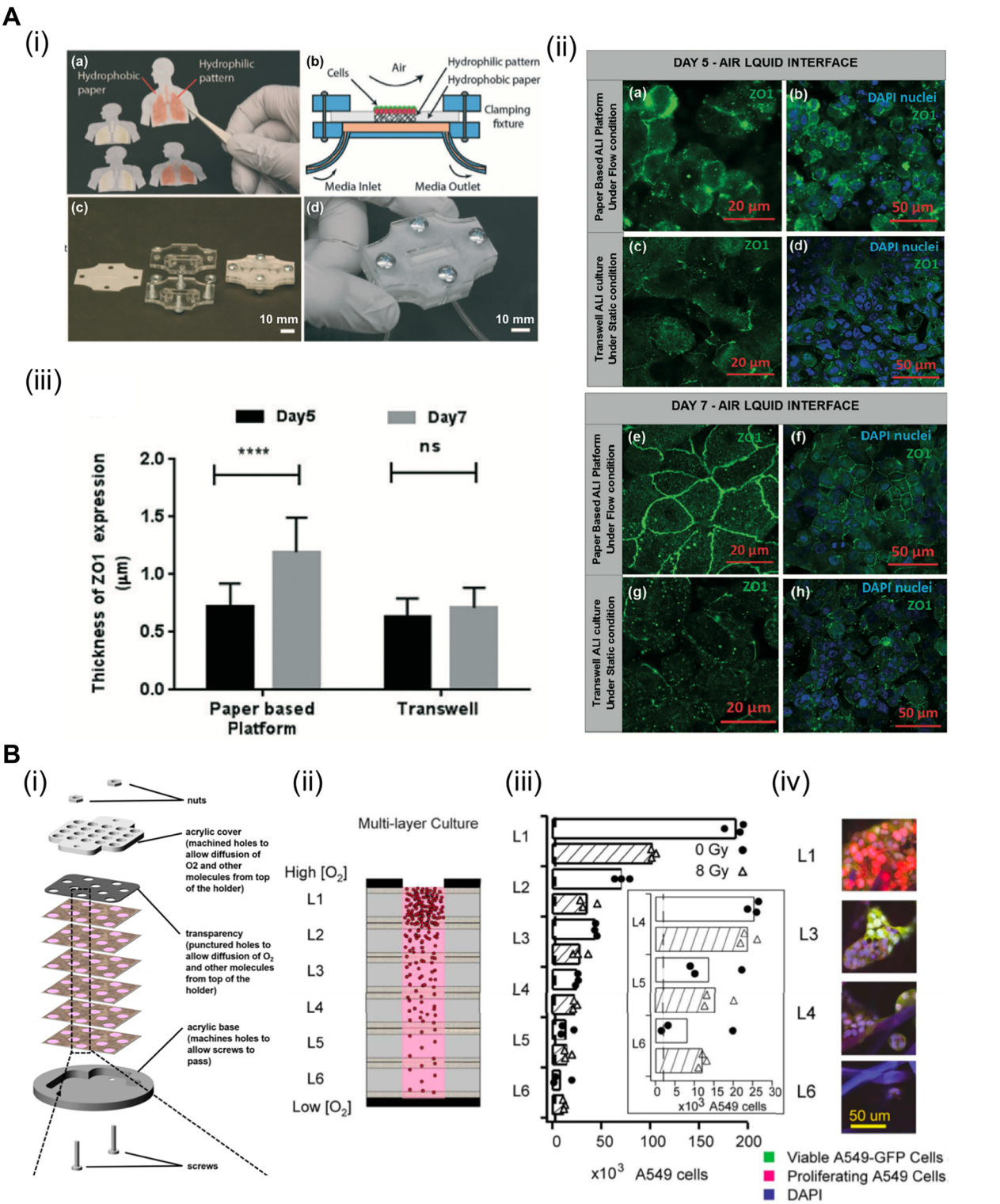

Fig. 3 Paper-based devices for in vitro lung tissue modeling. **A** Preparation of paper-based ALI platform (i), comparison of airway epithelium integrity (ii), and thickness of ZO1 expression (iii) between the paper-based ALI and conventional transwell ALI cultures at day 5 and day 7. Images reproduced with permission [32]. **B** Fabrication of the paper-based multilayered culture device (**i**); schematic of a vertical column of cells in the multilayered culture stack (ii); graph summarizing the number of metabolically active cells in layers L1 to L6 in the absence and presence of ionizing radiation (iii); and confocal fluorescence images of A549 cells in a non-irradiated, multilayered culture showing decrease in the density of cells and decrease in the incorporation of the proliferation stain, EdU, from L1 to L6 (iv). Images reproduced with permission [67]. *ALI* air–liquid interface

[72]. The structure of the print paper was similar to that of the filter paper (pore sizes of 15–25 μm), which was woven from a bundle of microfibers but had more compact arrangement of these microfibers. The nitrocellulose membrane was a cavernous structure with a pore size of 0.4–0.5 μm. The paper-based devices were created by duplicating a layer of multi-well PDMS mold from a multi-column poly(methyl methacrylate) (PMMA) or SU-8 mold and then bonding together the PDMS structure and the selected paper substrate via a thin layer of pre-cured PDMS glue. It was shown that the protein-based precoating of the bare paper effectively enhanced cell attachment to the various paper substrates (Fig. 4A-i). Finally, the fabricated paper-based devices were used to culture hiPSCs-derived cardiomyocytes to generate the "beating heart-on-a-paper" platform (Fig. 4A-ii), which could maintain long-term spontaneous beating and steady rate of contraction for the cells over 3 months on the print paper. These established paper-based devices may provide great opportunities for predicting cardiac toxicity of drugs.

Relating to the heart disease, paper substrates and cells suspended in the hydrogels were stacked to form a layered 3D model to mimic the pathology of the cardiac tissue undergoing an ischemic event [25]. Twenty circular hydrophilic zones of each layers were fabricated on a piece of Whatman filter paper by a solid ink printer printing wax. Then, the circular zones were seeded with primary rat neonatal cardiomyocytes and co-cultured with cardiac fibroblasts. Moreover, the six-layered stack (approximately 1.2 mm in total thickness) was placed in a custom-made Delrin holder to limit the mass transport of nutrients entering the stack. Due to that nutrients were metabolized by the cardiomyocytes and gradually depleted, ischemia could be obtained in the bottom layers. This ischemic stress induced the cardiomyocytes at the bottom of the stack to secrete chemokines, which subsequently triggered fibroblasts residing in adjacent layers to migrate toward the ischemic region. This study demonstrated the usefulness of stackable paper as a model for in vitro evaluation of cellular motility and viability in the laminar ventricle tissue of the heart.

In another study, Sapp and colleagues developed a multilayer 3D construct made of porous filter paper to analyze and culture valvular interstitial cells (VICs) based on the cells-in-gels-in-a-filter-paper system (Fig. 4B) [18]. To fabricate the construct, printing and fusion techniques were employed to draw wax barriers onto the porous filter paper substrate. Subsequently, the 3D culture devices were created by stacking the filter paper, where each piece was modeled against the spatial dimensions of a 96-well plate. This model of the paper-based system was adapted to culture VICs in thick collagen gels. Confocal images of the wells in the filter paper suggested an elongation in the morphology of VICs following seeding and successful culture for 14 days. Therefore, the filter paper-based platform can be considered as a new direction for analyzing VICs in normal as well as disease models of the aortic valve.

Paper-based devices for human liver research

The liver is the most important organ for drug metabolism [73]. Unfortunately, drug-induced hepatoxicity is responsible for producing acute liver failure in 50% of the cases, causing the limitation or withdrawal of approved medications [74, 75]. Nonetheless, the development of advanced in vitro models has improved the quality of information obtained from drug metabolism and hepatoxicity evaluations, since systems based on human cells in proper architectures enhance the recognition of potential toxic drugs comparing to conventional simplified cell culture models [76, 77].

The filter paper has been used as the scaffold array to create a 3D liver tissue model that enabled the assessment of drug-induced hepatotoxicity (Fig. 5A-i, ii) [78]. The study showed that the 3D morphology could be generated by co-culturing human-induced hepatocytes (hiHeps) and human umbilical vein endothelial cells (HUVECs), where liver functions indicated by production of albumin and urea were maintained for over 2 months. As shown in Fig. 5A-iii a, when the traditional 2D culture was compared to the 3D paper-based platform, an improvement in cell proliferation and migration was observed in the paper device featuring microporous structures, showing an adequate hiHeps distribution when cultured on the 3D paper-based devices. As further revealed in Fig. 5A-iii b, c, larger aggregates of the co-culture than single culture of hiHeps with a spheroid-like appearance were found on the paper-based platform.

In addition, dysregulation of cellular phosphorylation is one of the main causes of tumor growth and progression in the liver [79, 80]. To address this challenge, a microreactor device made of filter paper was selected to evaluate the effect of cytokine on the cellular phosphorylation of human liver cancer cells (Fig. 5B-i) [81]. In this work, an array of circular zones was processed into the paper-based device for carrying a variety of cell culture and subsequent immunoassays. These circular detection areas were directly used to culture the liver cancer cells. Cells with a spherical morphology cultured on the paper-based device were observed after 1 day of culturing, and after 5 days, the cells were observed to proliferate and aggregate on the microfibers of the device (Fig. 5B-ii). Interleukin (IL)-6 treatment was carried out in the circular detection/cell culture areas. The higher level of IL-6 treatment led to the higher activation level of phosphorylated Stat3 (P-Sat3) (Fig. 5B-iii). Thus, under a given stimulated condition, the cellular phosphorylation and signaling pathway may be analyzed on such a device for providing insights on the pathogenesis of cancer.

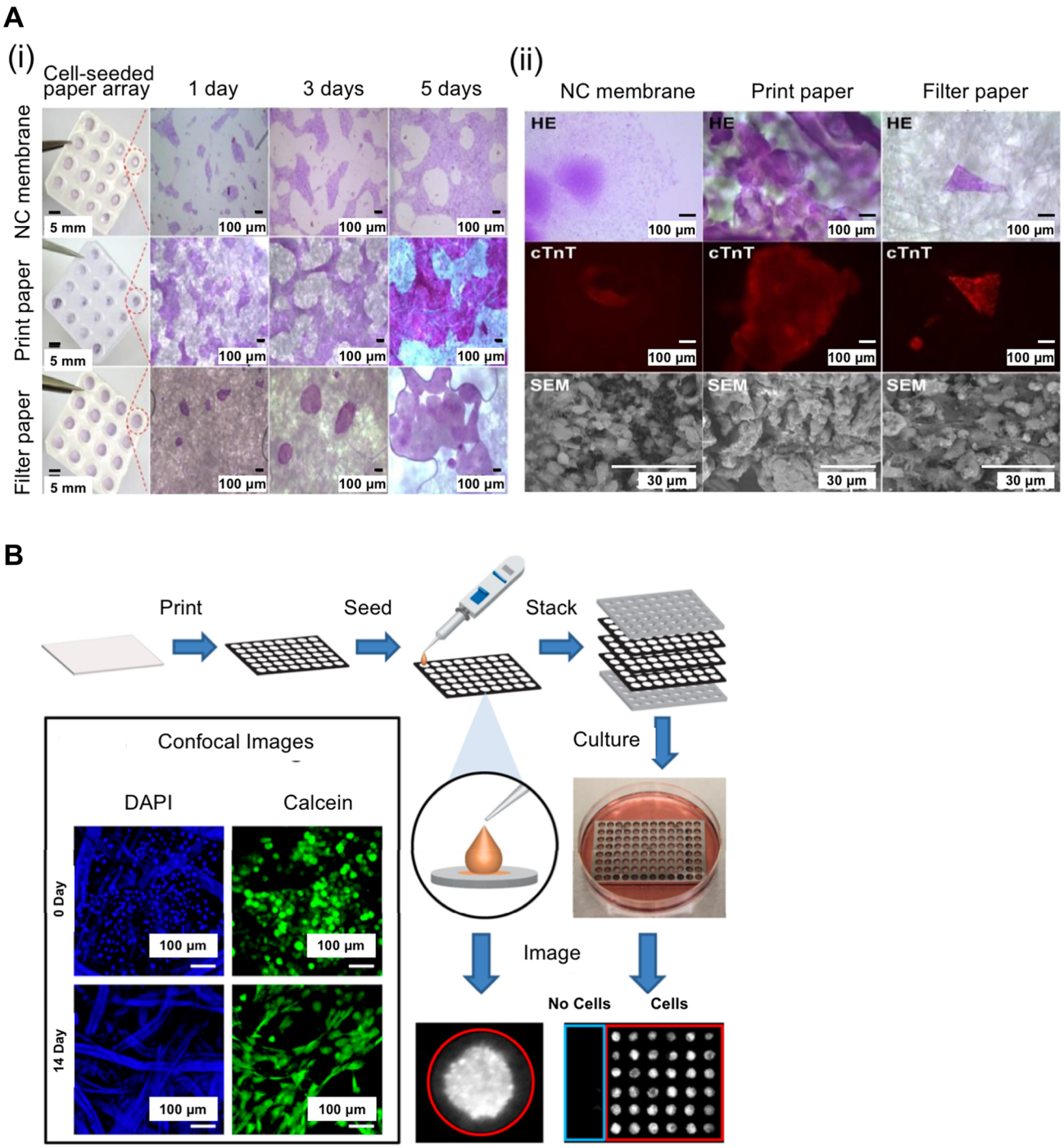

Fig. 4 Paper-based devices for in vitro cardiac tissue modeling. **A** The proliferation of hiPSCs on the different papers with different lengths of time in mTeSR1 medium (i) and the H&E staining, immunofluorescent imaging with a cardiac-specific marker (cTnT) and SEM images of hiPSCs-derived cardiac tissues on the three types of paper were stained with after 3 months (ii). Images reproduced with permission [72]. **B** The filter paper-based culture sheets were fabricated by wax printing for culturing VICs. Images reproduced with permission [18]. *H&E* hematoxylin–eosin, *hiPSCs* human-induced pluripotent stem cells, *NC* nitrocellulose, *PMMA* poly(methyl methacrylate), *SEM* scanning electron microscopy, *VICs* valvular interstitial cells

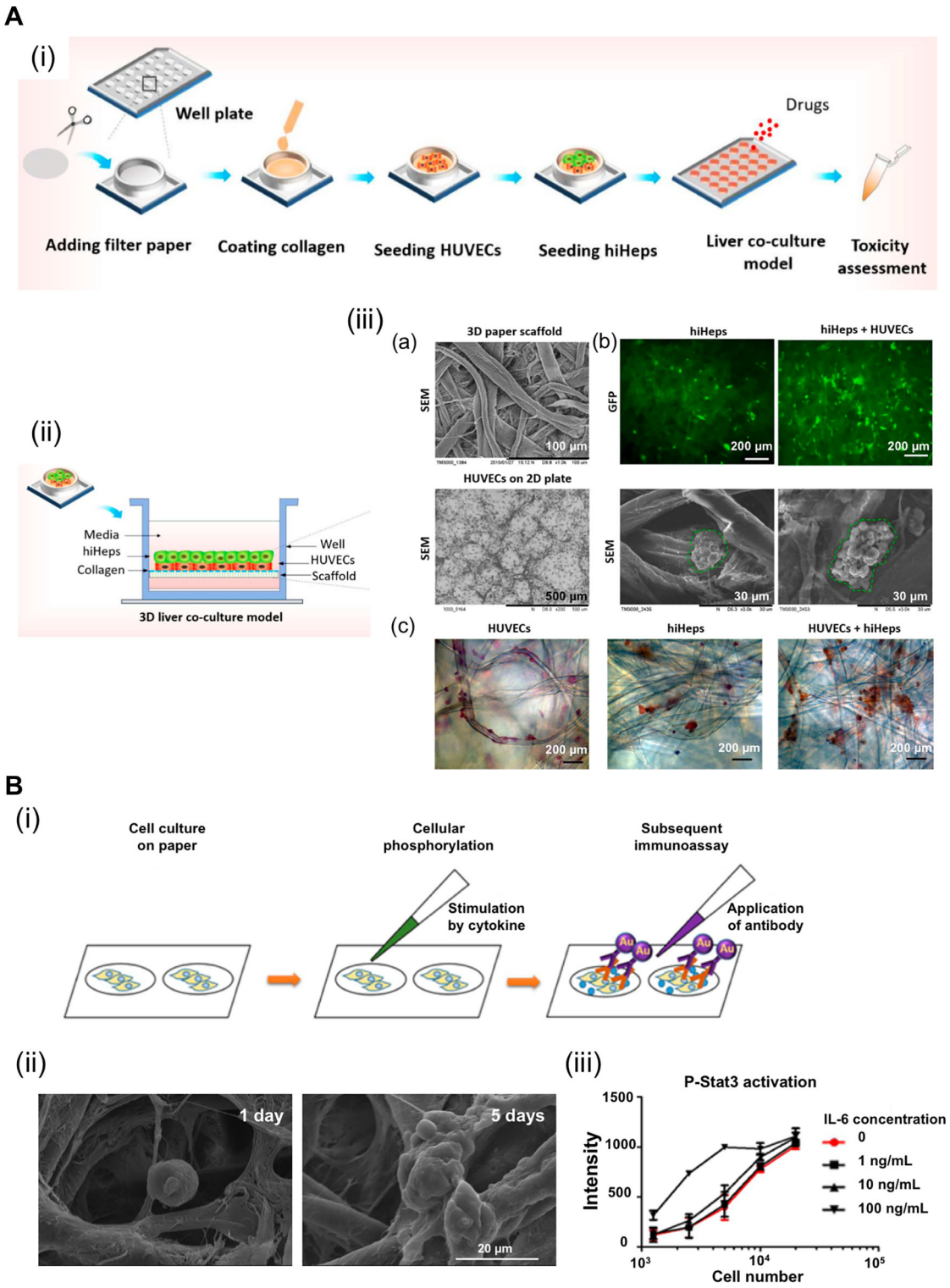

Fig. 5 Paper-based devices for in vitro liver tissue modeling. **A** The construction of the 3D liver co-culture model on the filter paper-based scaffold (i), schematic illustration of the co-culture of hiHeps/HUVECs on the scaffold (ii), and the formation of cell aggregates identified by SEM (a), fluorescence images and SEM (b), and hematoxylin/eosin staining (c) on day 7 (iii). Images reproduced with permission [78]. **B** Methodology of the paper-based microreactor integrating cell culture (**i**), SEM image of cell morphologies after (left) 1 day and (right) 5 days of culturing in the paper-based microenvironment (ii), and the activation level of P-Sat3 activation for the study of cellular phosphorylation of liver cancer cells stimulated by cytokine (iii). Images reproduced with permission [81]. *hiHeps* human-induced hepatocytes, *HUVECs* human umbilical vein endothelial cells, *P-Sat3* phosphorylated Stat3; *SEM* scanning electron microscopy

Paper-based devices for human bone research

Scaffolds play a pivotal role in the bone tissue engineering and modeling [82, 83]. Generally, these platforms are composed of materials with porous 3D microstructures and functionalized polymers that can be used for the proliferation, migration, recruitment, and differentiation of the osteoprogenitor cells [84]. Moreover, the mechanical properties of the bone scaffold play an important role in bone regeneration and remodeling [85]. The paper biomaterial can thus be used as an alternative to meet many of these performance requirements.

To demonstrate the advantages of the paper-based devices in engineering the bone tissue, equine adipose-derived stem cells (EADSCs) were seeded on a paper-based scaffold, based on Grade 1 cellulose-based qualitative filter paper (thickness 180 μm; maximum pore size 11 μm), for 3D culture, and their growth and osteogenic differentiation were studied [86]. By comparing with the EADSCs cultured on traditional 2D polystyrene surfaces, it was found that the 3D paper-based culture was better in terms of osteogenic differentiation of the cells. Furthermore, it was found that the spherical morphology of the cells could be formed in 3D culture, rather than the flat and elongated morphology present in the 2D culture.

Additionally, Park and colleagues created a surface-engineered paper-based device and used it as the scaffold for stem cell culture and bone tissue reconstruction in a mouse model (Fig. 6A-i) [36]. By comparing three types of commercially available papers (weighing paper, chromatography paper, and wiping tissue, and polystyrene plate as a control substrate) modified by the initiated chemical vapor deposition (iCVD) process, it was demonstrated that filter paper had lower attachment of the human adipose-derived stem cells (hADSCs), and filter paper and wiping tissue had lower viability of the cells than weighing paper (Fig. 6A-ii a-c). However, the attached hADSCs on the weighing paper showed highly extended cellular morphology than those on filter paper (Fig. 6A-ii a) and were able to maintain their proliferation ability for up to 2 weeks for in vitro culture (Fig. 6A-ii d). This result confirmed the biocompatibility of iCVD polymer-treated weighing paper scaffold for hADSC culture (Fig. 6A-ii e). In addition, hADSCs grown on weighing paper substrates were successfully transfected with enhanced green fluorescence protein (EGFP)-encoding plasmid DNA by using the poly(b-amino ester) (Fig. 6A-ii f). Further, the promoted osteogenic differentiation of hADSCs on the weighing paper scaffolds was demonstrated through physical and mechanical stimulation. In the in vivo study, the stacked hADSCs-loaded weighing paper scaffolds were seeded with HUVECs before implantation and demonstrated an enhanced vascularized, volumetric bone formation.

In another study, Camci-Unal and co-workers demonstrated an origami approach for the fabrication of paper (Whatman 114 filter paper)-based scaffolds for bone tissue engineering. The partially mineralized scaffolds of the centimeter-scale 3D structures in numerous shapes (circular, triangular, rectangular, pentagonal, hexagonal; Fig. 6B-i) were prepared by a cells-in-gels-in-a-paper method, for which calcium phosphate deposition by osteoblasts was observed [87]. The osteoblasts were cultured for 21 days, and micro-computed tomography (micro-CT) scans were utilized to examine the distribution of mineralized areas in the paper-based origami scaffolds. They observed in the micro-CT images that the origami scaffolds had relatively uniform mineralization with some patches located in different areas (Fig. 6B-ii). The results indicated that the developed paper-based devices have a favorable potential application in the study of the mineralization of osteoblasts.

Moreover, graphene oxide (GO)-modified cellulose (G-C) paper was prepared as scaffolds for the culture of ADSCs and their osteogenic differentiation [88]. The G-C paper was fabricated by using commercially available Kimwipes® cellulose tissue paper as a substrate that was coated by immersion deposition with GO followed by reduction to reduced GO (RGO) without the use of toxic organic solvents. The paper-based device could offer a long-term support of ADSC proliferation (up to 35 days) and enhanced osteogenic differentiation. Furthermore, an "origami-inspired" cell-carrying scaffolds made by folding and rolling the G-C paper substrates laminated with alginate were constructed for 3D cell culture and differentiation (Fig. 6C), allowing future in vitro and in vivo applications.

Conclusions, challenges, and perspectives

The paper biomaterial possesses many favorable properties including low cost, biocompatibility, flexibility, fibrous nature, and high porosity, among others. Due to these unique advantages, paper-based materials have become a promising source in the fabrication of biomedical devices for the construction of in vitro human tissue/disease models with different processing methods. Importantly, these various devices can be obtained by using relatively simple techniques such as wax printing, inkjet and flexographic printing, FFSL, origami, or 3D printing. To date, paper-based devices cultured with numerous types of human cells have been accommodated toward in vitro drug screening and therapeutics development. These paper-based products can also facilitate investigations into cell–matrix interactions and other mechanistic studies better than their traditional counterparts based on 2D planar cultures.

However, limitations associated with these devices are not none. For example, the strong autofluorescence of the paper

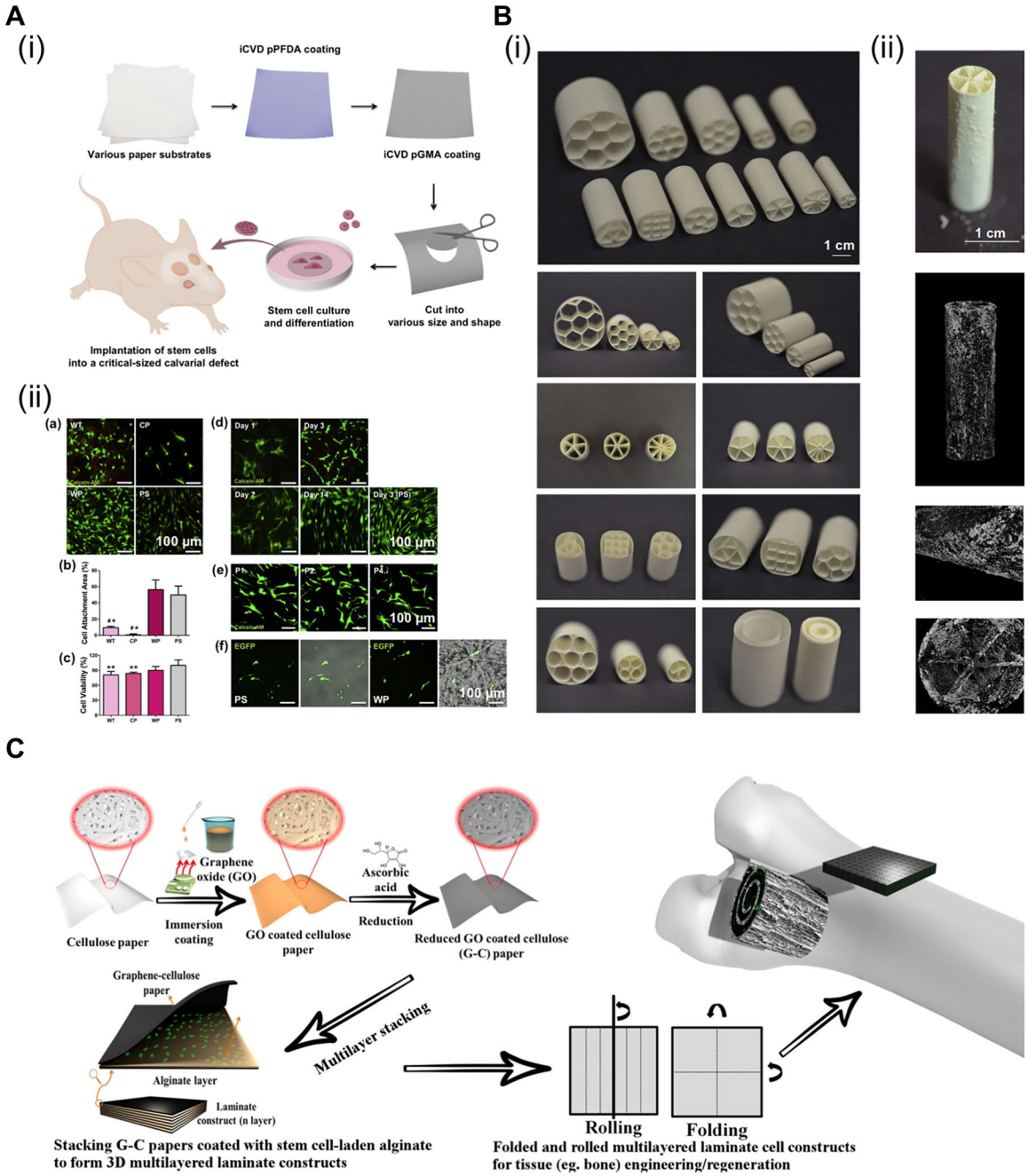

Fig. 6 Paper-based devices for in vitro bone tissue modeling. **A** The paper scaffolds with modified surfaces were fabricated and evaluated for their application in stem cell culture and bone tissue formation (i); the attachment and viability of hADSCs to the paper scaffolds (WT, CP, and WP) and cell culture plate (PS) at 1 day after cell seeding (ii a–c), cell expansion and subculture of hADSCs on the WP scaffolds (ii d, e), and EGFP expressions in hADSCs on the WP scaffolds and PS plates (2 days after transfection) (ii f). Images reproduced with permission [36]. **B** Photographs of the origami-inspired paper scaffolds (i) and characterization of the biomineralization of the osteoblasts in a scaffold by micro-CT on day 21 (ii). Images reproduced with permission [87]. **C** Schematic shows the fabrication of the G-C paper and the origami-inspired 3D cell-carrying scaffolds as well as their application in bone tissue engineering. Images reproduced with permission [88]. *EADSCs* equine adipose-derived stem cells, *EGFP* enhanced green fluorescence protein, *CP* chromatography paper, *GO* graphene oxide, *G-C* GO-modified cellulose, *hADSCs* human adipose-derived stem cells, *iCVD* initiated chemical vapor deposition, *micro-CT* micro-computed tomography, *PS* polystyrene plate, *RGO* reduced GO, *WP* weighing paper, *WT* wiping tissue

biomaterials [89, 90] oftentimes interferes with fluorescence imaging of the cells and tissues residing in these devices, potentially needing careful selection of the fluorophores to minimize such background fluorescence. In addition, even

bright-field optical imaging could be tricky for many paper-based devices due to their opaque nature endowed by the densely entangled cellulose fibrils. This feature of the paper-based devices further leads to their typically small pore sizes making the cell–cell interactions less convenient. Therefore, ways to improve cellular behaviors are also critical in promoting their applications in tissue cultures.

It is believed that with future development, the paper-based substrates combined with the advanced biodesign and manufacturing technologies will find widespread applications in biomedicine in particular in vitro modeling of human tissues and diseases.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Consent for publication All authors have reviewed and approved the manuscript.

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