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2015 J. Micromech. Microeng. 25 055015

(http://iopscience.iop.org/0960-1317/25/5/055015)

IOP Publishing Journal of Micromechanics and Microengineering
J. Micromech. Microeng. **25** (2015) 055015 (6pp) doi:10.1088/0960-1317/25/5/055015

A Janus-paper PDMS platform for air–liquid interface cell culture applications

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Received 11 October 2014, revised 23 January 2015
Accepted for publication 10 February 2015
Published 17 April 2015

Abstract

A commercially available Janus paper with one hydrophobic (polyethylene-coated) face and a hygroscopic/hydrophilic one is irreversibly bonded to a polydimethylsiloxane (PDMS) substrate incorporating microfluidic channels via corona discharge surface treatment. The bond strength between the polymer-coated side and PDMS is characterized as a function of corona treatment time and annealing temperature/time. A maximum strength of 392 kPa is obtained with a 2 min corona treatment followed by 60 min of annealing at 120 °C. The water contact angle of the corona-treated polymer side decreases with increased discharge duration from 98° to 22°. The hygroscopic/hydrophilic side is seeded with human lung fibroblast cells encapsulated in a methacrylated gelatin (GelMA) hydrogel to show the potential of this technology for nutrient and chemical delivery in an air–liquid interface cell culture.

Keywords: paper, rapid prototyping, PDMS bonding, cell culture, air–liquid interface

(Some figures may appear in colour only in the online journal)

1. Introduction

There is a plethora of structural materials at the disposal of the microfluidic and lab-on-a-chip community, with polydimethylsiloxane (PDMS) and glass being the most popular and widely used ones [1]. More recently, paper has garnered significant attention as a low-cost disposable platform for chemical and biological assays [2, 3]. Paper-based devices are often fabricated either by using a hydrophilic paper that is patterned and impregnated with wax [4, 5] or other hydrophobic materials to create water-repelling regions [6, 7], or alternatively by laser-treating a hydrophobic paper to create hydrophilic regions [8, 9]. In both cases, the hydrophilic/hygroscopic pattern is then used to perform colorimetric or electrochemical analyses on aqueous samples [10, 11]. In addition to chemical and biological assays [12], researchers have also utilized paper to create complex 3D scaffolds for tissue engineering or platforms for cellular pathology (e.g. breast cancer cell) studies [12].

In this work, we introduce a commercially available paper (Janus paper) with one hydrophobic (polyethylene-coated) face and a hygroscopic/hydrophilic one as a substrate for

microfluidic and lab-on-a-chip applications. Janus papers can be used to develop microsystems with enhanced functionality and improved biomaterial integration capabilities. This is particularly true if it can be easily coupled with traditional microfluidics materials and system [13]. A unique application for such hybrid (e.g. paper/PDMS) systems is a cell culture platform for air–liquid interface (ALI) applications. ALI cultures offer cost-effective *in vitro* drug screening platforms for epithelial tissues such as pulmonary airways [14], providing a more clinically relevant structure compared to other *in vitro* models such as trans-well immersion system [15, 16]. Although various ALI models have been reported using PDMS [17], hydrogel [18], polyester [19], and porous fiber sheets, they typically require complex and time-consuming fabrication processes [20, 21]. The Janus-paper/PDMS platform takes advantage of the porous mesh structure of the hydrophilic side of the paper for easy seeding of hydrogel-encapsulated cells while simultaneously utilizing the PDMS microchannels to deliver nutrients and drugs to the cells, enabling proper nutrition and sufficient air environment for ALI cell culture.

2. Janus-paper and its characterization

A paper exhibiting Janus property is the freezer paper used to store food. It is composed of a cellulose fiber mesh coated with hydrophobic polyethylene on one side, leaving the opposite side hydrophilic/hygroscopic. As with many polymers, the polyethylene coating on the freezer paper exhibits a low surface energy preventing its bonding to other surfaces. Corona treatment can be used to alter the polyethylene surface energy and promote its adhesion/bond to the PDMS. A similar surface activation with a plasma cleaner may provide a more uniform surface treatment; however, a key advantage of using a hand-held corona discharge unit is its simplicity, which allows treatment without the need for expensive equipment or a vacuum environment. This latter point is especially important since the ability to process at atmospheric pressures makes the technique easily adaptable to large-scale manufacturing processes such as roll-to-roll fabrication.

In order to characterize the effect of corona treatment on surface energy, we used a goniometry test setup to measure the water contact angle before and after surface modification. For these experiments, multiple samples of freezer paper were cut into 2 cm × 2 cm pieces and their polymer-coated sides were exposed to corona discharge for various durations (0 – 300 s). Immediately afterwards, the contact angle of each sample was measured by placing a 4 μl water droplet on the surface. As is the case with PDMS, the corona treatment causes a significant reduction in the contact angle on the surface of polyethylene coated side. The goniometry test results are shown in figure 1, which plots the contact angle change in response to corona treatment as a function of exposure time, with the contact angle changing from 98° to 22° after 150 s of treatment (inset pictures in figure 1). This decrease can be attributed to the generation of oxygen polar groups on the polyethylene surface by plasma exposure [22, 23]. It is interesting to note that the contact angle does not change after

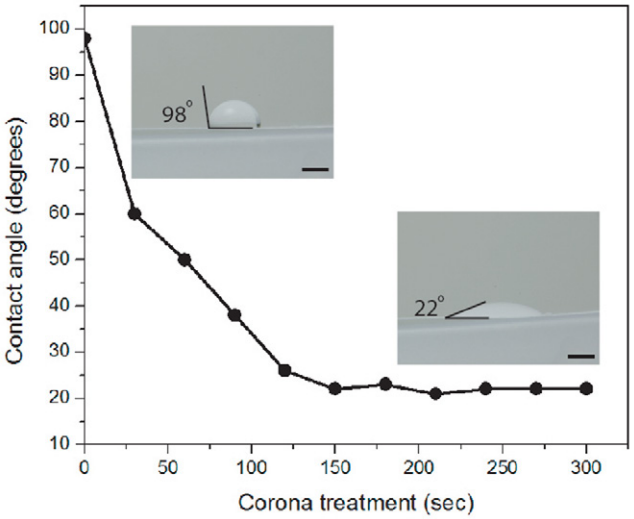

Figure 1. Contact angle of a 4 μl water droplet on the hydrophobic surface of a freezer paper as a function of corona treatment time. All scale bars are 1 mm.

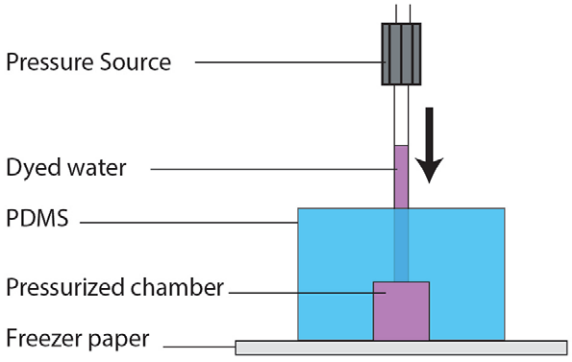

Figure 2. Test setup for measuring bond strength between the freezer paper and PDMS. A gas source provides the pressure.

2 min of surface treatment; probably due to the saturation of all the available surface states.

We bonded the surface-treated freezer paper to PDMS and evaluated its quality as a function of annealing time and temperature. For this purpose, we fabricated multiple test devices and tested them with the setup illustrated in figure 2. The test setup consisted of a cylindrical chamber in a block of PDMS bonded to the freezer paper substrate. The PDMS block (15 mm × 15 mm × 5 mm) was formed from a master mold laser-cut out of an acrylic cylinder (5 mm diameter × 2 mm height) adhered onto a petri dish with UV-curable epoxy (Loctite® 3105). An 800 μm-diameter port was cored-out of the center of the PDMS block to create an inlet for the application of pressurized gas during the bond test. A sheet of freezer paper was cut to match the dimensions of the PDMS mold. The PDMS and paper were then bonded after 2 min of corona treatment.

The optimal combination of annealing time and temperature required for a strong bond was determined by measuring the bond strength of the PDMS-paper structure discussed above. The annealing temperatures and durations were varied between 40 – 140 °C and 0 – 180 min, respectively. To assess the bond quality, an 800 μm-diameter syringe needle was inserted into

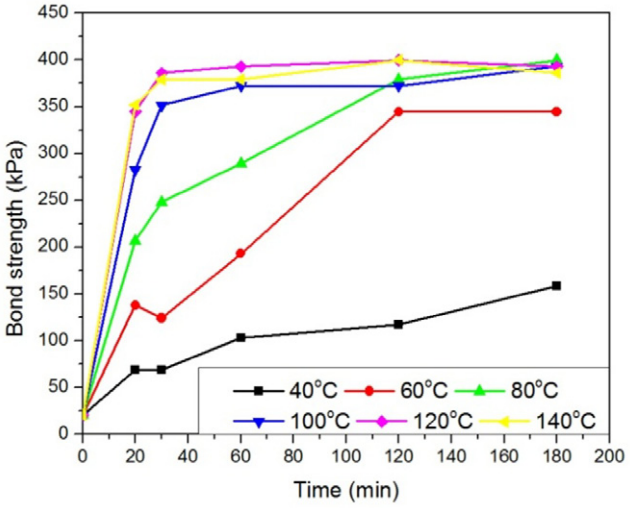

Figure 3. Bond strength as a function of annealing time and temperature. Note that at temperatures above 100 °C annealing times beyond 20 min do not increase the bond strength. Maximum strength occurs within 60 min at $T > 80$ °C (for $T > 100$ °C 20 min annealing time is adequate).

the inlet port of the PDMS block and its perimeter was sealed with a polyurethane-based adhesive (Gorilla Glue™, Gorilla Glue Inc.). The needle was connected to a syringe filled with dyed water; the open end of which was connected to a pressure gauge and a flow-regulated nitrogen source in order to control the pressure in the PDMS/paper chamber. The pressure was gradually increased at a rate of 250 Pa s^{-1} until the first signs of fluid leakage was visually observed. Bond failure was identified either by the delamination of the two layers or by the failure of the paper (tearing at the interface).

The effects of annealing temperature and duration on bond strength are plotted in figure 3. The data show that lower temperatures and shorter annealing times result in weaker bonds; in such cases, device failure occurs at the paper/PDMS interface. By increasing the annealing temperature and time, the bond strength also improves up to an observed maximum of 392 kPa (at this point device failure occurs as a tear in the surface of the paper rather than at the bond interface). This is comparable to the bond strengths achieved via traditional oxygen plasma bonding and adhesive tape-PDMS techniques [24, 25]. The data also reveal an optimal annealing temperature and time of 120 °C and 60 min for creating a strong irreversible bond between the paper and the PDMS.

3. Design and fabrication of Janus-paper/PDMS microfluidic platforms

Figure 4 illustrates the fabrication process used for creating PDMS/Janus-paper devices. First, a CO_2 laser engraving system (Universal Laser Systems Inc., Scottsdale, AZ) is used to directly write microchannel patterns on a substrate of crosslinked PDMS (Dow Corning Sylgard® 184, 10 : 1 ratio, cured at 60 °C for 5 h) by surface ablation (power 15 W, speed 4 mm ms^{-1}). The resulting channels have a Gaussian cross-section (200 μm wide, 100 μm deep); the shape can be further

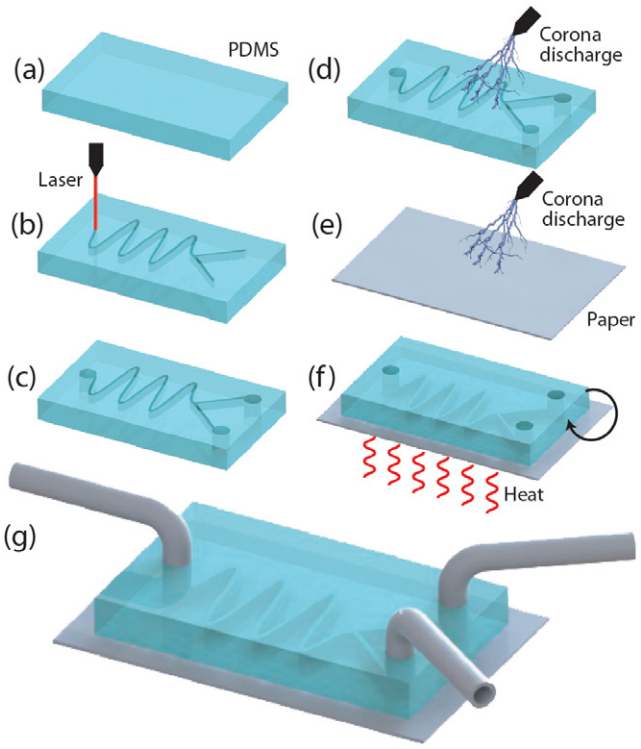

Figure 4. Conceptual illustration of the fabrication process for Janus-paper/PDMS devices: (*a*) cast, crosslink, and release the PDMS substrate; (*b*) laser-define the microfluidic network; (*c*) punch inlet and outlet ports; (*d*) treat the surface of PDMS and (*e*) the freezer paper with corona discharge or plasma; (*f*) flip the PDMS and bring it into contact with the treated paper, press and anneal to bond; (*g*) attach tubing to microfluidic ports.

modified (e.g. rounder or deeper) by using an unfocused laser beam or multiple passes of the beam. After defining the channels on the PDMS, two 800 μm-diameter holes are punched out to create inlet and outlet ports. Pieces of the Janus paper (Freezer Paper, Reynolds Consumer Products, Inc.) are also cut to the same size as the PDMS sample using the laser system; for the ALI platform, a small hole (250 μm-diameter) is cut on the freezer paper to connect the microfluidics in the PDMS layer to the hydrophilic side of the paper, figure 5. Next, the PDMS and the freezer paper are surface-treated using a hand-held corona discharge unit (BD-10A High Frequency Generator, Electro-Technic Products, Inc.). The two are subsequently brought into contact, bonded, and annealed on a hotplate. Finally, 800 μm-diameter pieces of Tygon® tubing are attached to the inlet and outlet ports.

We fabricated various standard microfluidic designs as a proof-of-concept demonstration. Figures 6(*a*)–(*d*) shows two structures with channel depths and widths of 100 and of 200 μm using a single pass of the laser. The performance of each device was tested by injecting dyed water into the inlet ports at a flow rate of 20 μl min^{-1} and observing the flow behavior. Figure 6(*a*) shows an empty *Y*-junction with a 15 mm-long microchannel. Figure 6(*b*) shows two liquids being pumped into the channel inlets; the insets highlight the laminar flow that was maintained throughout the channel length. Figure 6(*c*) presents a hybrid PDMS/paper microfluidic

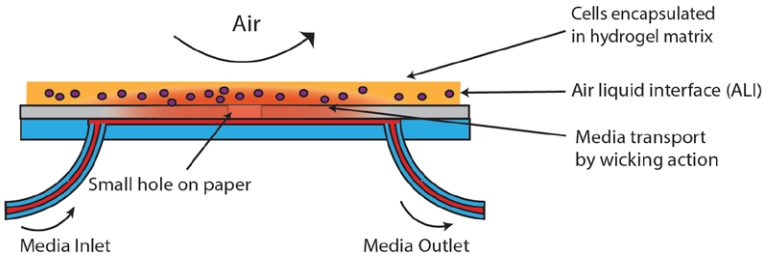

Figure 5. Cross-sectional schematic view of the PDMS/paper platform for ALI cell culture. Chemicals and nutrients are continuously delivered to the encapsulated cells through the holes connecting the microfluidic channels to the top surface.

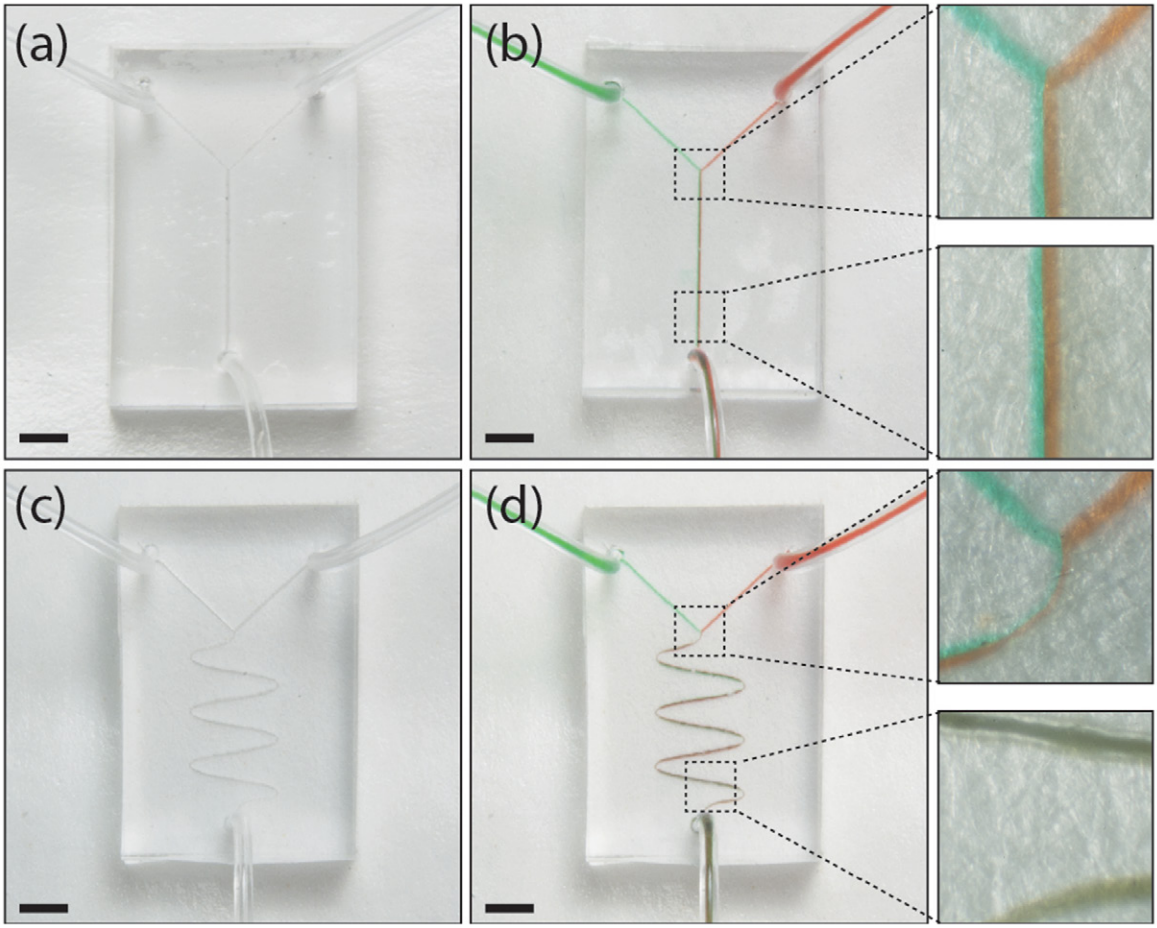

Figure 6. Various microfluidic devices fabricated using PDMS and freezer paper. All channels have a Gaussian cross-section (200 μm width and 100 μm depth), (*a*) an empty *Y*-junction connected to a straight channel imaged from the PDMS side and (*b*) with liquid flowing through (the insets show a 10 × magnified view of the laminar flow); (*c*) an empty serpentine channel imaged from the PDMS side and (*d*) with two dyed streams flowing and mixing together (the insets show a 10 × magnification highlighting the mixing). All scale bars are 2 mm.

mixer consisting of a *Y*-junction with a serpentine channel. Figure 6(*d*) depicts red and green liquids entering the microchannel with a laminar flow, each at a flow rate of 10 μl min^{-1} and mixing together in the serpentine channel; the insets show the resulting mixed solution at the end of the channel.

4. Cell culture and ALI experiments

To determine the capability of the platform to be used as an ALI platform, human lung fibroblasts (MRC-5) were encapsulated in methacrylated gelatin (GelMA) and cultured on the hydrophilic side of the freezer paper, figure 5. The fibroblast (MRC-5) cell lines (LGC Standards cell, UK) were cultured in a humidified incubator (37 °C, 5% CO_2) with Minimum Essential Medium Eagle media (Sigma), supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1% pen/strep, and 1% non-essential amino acid solution. The GelMA was prepared by dissolving 10% GelMA in a 0.25% photo-initiator solution (2-hydroxy-1-(4-(hydroxyethoxy)phenyl)-2-methyl-1-propanone purchased from Sigma) at 60 °C. The GelMA was then cooled to 37 °C before mixing with the cells. Next, the

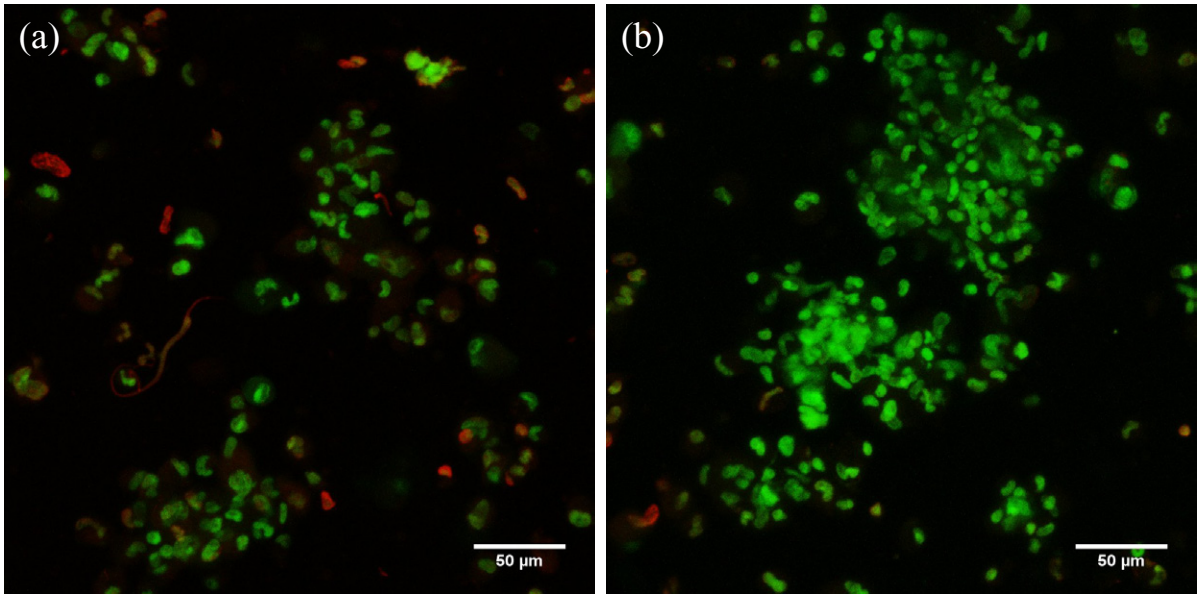

Figure 7. Human lung fibroblasts encapsulated in GelMA and cultured on the PDMS/paper ALI platform for 24 h under static (*a*) and flow (*b*) conditions in which cell culture media is passed through the microchannels. SYTOX 10 stains all cells (green), dead cells are labeled with ethidium homodimer-2 (red). Live/dead staining shows reduced cell viability under static conditions (*a*) compared to flow conditions (*b*) where >95% of cells are viable after 24 h.

cells were mixed in 60 μl of GelMA solution; they were then seeded on the hydrophilic side of the paper at a density of 5×10^6 cells per sample and subsequently exposed to UV light for 14 s to cross-link the GelMA. The ALI platform and the tubing connections were sterilized by UV radiation and 70% ethanol and allowed to dry under sterilized conditions before the cell seeding process.

After seeding, the ALI platform was transferred to an incubator, the inlet tubing was connected to syringes containing the culture media, and the outlet tubing was connected to a collection vessel. Media was flown into the microchannels at a rate of 10 μl min^{-1} using a syringe pump (Harvard Apparatus PHD 2000 Syringe Pump). The cell cultures were maintained for over 24 h and fluorescently labeled to assess their viability. The cell viability assay (LIVE/DEAD Reduced Biohazard Viability/Cytotoxicity Kit, purchased from Molecular Probes) utilized the differential permeability of live and dead cells to a pair of fluorescent stains. The green fluorescent nucleic acid dye, SYTO 10, is highly membrane permeant and labels all cells. DEAD Red (ethidium homodimer-2), a red fluorescent nucleic acid stain, is cell-impermeant and only labels cells with compromised membranes, thereby specifically staining dying cells.

The cells were stained *in situ*, while encapsulated in GelMA upon the ALI platform. Briefly, at the end of the experiment, cells were washed in phosphate-buffered-saline (PBS). The dye solution was prepared by mixing the nucleic acid stains SYTO 10 (1 : 500 dilution) and DEAD Red (1 : 1000 dilution) in a common volume of PBS. A sufficient volume (500 μl) of the dye solution was added to submerge the cells followed by incubation in a dark environment for 15 min at room temperature. The dye solution was then removed and cells were washed with PBS. Finally, cells were fixed by immersion in a 4% formaldehyde solution for up to 1 h before imaging. Samples were imaged using a 20 × objective on a Zeiss LSM710 META confocal microscope.

The cell viability was evaluated after 24 h under both static (no flow in the inlet-feeding PDMS microchannels) and flow conditions. Figure 7 shows fluorescent live and dead staining images of the ALI cell culture experiments. The human lung fibroblasts cells had >95% viability under dynamic flow conditions after 24 h based on the proportion of cells labeled with the red fluorescent dye. However, under static media conditions, the cells exhibited a significant reduction in viability observed from an increase in the number of cells stained red. This result can be attributed to the differences in toxic waste processing. In the dynamic flow conditions, the cells were continuously receiving nutrition, and their waste byproducts were eliminated via the channel outlet; in the static condition, however, the waste byproducts accumulated in the vicinity of cells, thus resulting in toxicity and cell death.

These results show that our platform provides a reliable method for creating inexpensive ALI structures with several advantages over current ALI cell-culturing standards. Current *in vitro* techniques for ALI require periodic replacement of media; in addition, traditional trans-well immersion cultures do not provide a realistic environment which can faithfully model cell micro-environment in tissue under physiological flow conditions (e.g. blood vessel-rich tissue). These properties make freezer paper ideal for ALI applications.

5. Conclusions

We successfully implemented several microfluidic devices using a commercially available Janus paper (freezer paper) with one hydrophobic face and a hygroscopic one bonded to a PDMS backing with embedded microfluidic channels.

A strong paper-PDMS bond was achieved by corona treating the hydrophobic side of the paper prior to the bond followed by an optimized thermal annealing time and temperature. The hygroscopic side of the paper was used to seed human lung fibroblast cells encapsulated in gelatin, creating an air liquid interface cell culture platform.

Acknowledgments

The authors thank the staff of the Birck Nanotechnology Center for their assistance. This work was funded by the National Science Foundation under the EFRI Program, grant #1240443.

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