Video Article

Using Adhesive Patterning to Construct 3D Paper Microfluidic Devices

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Abstract

We demonstrate the use of patterned aerosol adhesives to construct both planar and nonplanar 3D paper microfluidic devices. By spraying an aerosol adhesive through a metal stencil, the overall amount of adhesive used in assembling paper microfluidic devices can be significantly reduced. We show on a simple 4-layer planar paper microfluidic device that the optimal adhesive application technique and device construction style depends heavily on desired performance characteristics. By moderately increasing the overall area of a device, it is possible to dramatically decrease the wicking time and increase device success rates while also reducing the amount of adhesive required to keep the device together. Such adhesive application also causes the adhesive to form semi-permanent bonds instead of permanent bonds between paper layers, enabling single-use devices to be non-destructively disassembled after use. Nonplanar 3D origami devices also benefit from the semi-permanent bonds during folding, as it reduces the likelihood that unrelated faces may accidently stick together. Like planar devices, nonplanar structures see reduced wicking times with patterned adhesive application vs uniformly applied adhesive.

Video Link

The video component of this article can be found at https://www.jove.com/video/53805/

Introduction

In recent years, paper microfluidics has garnered considerable popularity for its potential to provide low-cost point of care (POC) diagnostic devices.[1-3] POC devices offer functionality similar to those of lab-based tests in a format that allows results to be obtained relatively quickly. POC devices made from paper are low-cost, lightweight, and easy-to-use alternatives to expensive microfluidic chips and miniaturized laboratories, making them ideal for use in resource-limited settings. The most common paper microfluidic devices are one-dimensional lateral flow devices, but planar three-dimensional (3D) paper microfluidic devices hold promise to provide multiplexed diagnostic devices[4] that take up a much smaller footprint than would be required by a 2D device[5] and correspondingly use a smaller sample volume.

Initially, planar 3D paper microfluidic devices were assembled individually, layer-by-layer with patterned paper layers alternating with laser-cut double-sided tape. Carefully aligned holes cut in the tape layer were filled with cellulose powder to ensure inter-layer fluid transport.[4] A number of alternate methods were subsequently developed,[6-9] each improving different aspects of the devices. In particular, by eschewing adhesives, devices could be folded via origami techniques with layers held together by an external clamp.[8] This eliminates any potential adhesive interference in a diagnostic test and allows the device to be unfolded post-use, potentially allowing even smaller sample volumes by displaying results internally. Alternatively, by using an aerosol adhesive applied between each paper layer, sheets of devices could be assembled simultaneously, without time-consuming patterning and alignment of tape.[9]

However, by applying an aerosol adhesive through a stencil, it is possible to gain the benefit of both of these techniques. By spraying the adhesive through a stencil, only a fraction of the adhesive is applied to the device, minimizing any potential interference with interlayer fluid transfer. Additionally, with careful stencil selection, a pattern of adhesive can be applied that results in semi-permanent adhesive bonding, allowing devices to be unfolded after use, while still providing sufficient interlayer contact to allow fluid to wick between layers.

Finally, applying aerosol adhesives through a stencil eases the construction of nonplanar 3D paper microfluidic devices, by minimizing the amount of adhesive applied to adjacent faces that may require frequent folding and unfolding during construction.[10] Additionally, the use of patterned adhesive enables device to be unfolded after use for more convenient storage. Nonplanar 3D paper microfluidic devices are expected to be used for tasks that would otherwise be impossible in a planar 3D device. **Figure 1** depicts the general process flow used to construct both planar and nonplanar 3D devices.

Protocol

1. Planar 4-layer Device (Stacked Layers) Construction

1. Print arrays of each layer of the device[9] onto each piece of filter paper using a solid ink printer.[11,12] Place each filter paper on a hotplate at 170 °C for 2 min. This will melt the wax-based ink and allow it to fully penetrate the thickness of the paper, forming hydrophobic barriers.
NOTE: The exact designs used are available as supplemental files.
2. Remove filter paper from hotplate and allow it to cool to RT.
3. Deposit 4 µl of 5 mM dye (red: Allura Red; yellow: tartrazine; blue: erioglaucine disodium salt; green: 10:1 mix of tartrazine:erioglaucine disodium salt) in each branch (one color per branch) of layer 3 (third layer from the top of the completed device) using a micropipette.
4. Begin with the bottom-most layer. Clamp the filter paper between the stencil and a stiff backing, such as a piece of plate glass, using binder clips, or another similar temporary method. Ensure that the stencil is flat against the paper. This will minimize any spray shadows cast by the stencil onto the paper.
5. Apply adhesive (see list of materials and equipment) with an approximately 1.33 sec (a four-count at 180 bpm) spray from about 24 cm.[9,10] During this time, move the can of adhesive across the stencil at a medium pace. Too slow of travel across the stencil will cause adhesive to accumulate on the stencil itself, clogging it. Too rapid of travel will fail to deposit sufficient adhesive on the paper. Four passes during this time (up-down-up-down) are sufficient in preventing spray shadows.
6. Remove stencil and place the next layer of the device (numbered layers are available as supplemental files) atop the freshly sprayed layer, aligning the edges of the paper. Firmly press the two layers together.
7. Replace stencil and repeat the spraying process for each layer of the device. Remove the stack of devices and place packing tape across the bottom layer. This prevents any fluid leakage from the device. Cut individual devices from the sheet using scissors, following the edge of the printed region.

2. Planar 4-layer Device (Origami Folded Layers) Construction

1. Print sheets containing all layers of the device onto filter paper using a solid ink printer. Place filter paper on a hotplate at 170 °C for 2 min. Remove filter paper from hotplate and allow it to cool to RT.
NOTE: The exact designs used are available as supplemental files.
2. Deposit 4 µl of 5 mM dye (red: Allura Red; yellow: tartrazine; blue: erioglaucine disodium salt; green: 10:1 mix of tartrazine:erioglaucine disodium salt) in each branch (one color per branch) of layer 3 (third layer from the top of the completed device) via micropipette.
3. Clamp the sheet of devices between the stencil and a stiff backing, such as a piece of plate glass, using binderclips, or another similar temporary method. Ensure that the stencil is flat against the paper.
4. Apply adhesive (see list of materials and equipment) with an approximately 1.33 sec (a four-count at 180 bpm) spray from about 24 cm. Four passes during this time (up-down-up-down) are sufficient in preventing spray shadows.
5. Remove stencil and turn the sheet over. Replace stencil and spray back side of the paper. Remove the sheet of devices and begin folding in an accordion pleat, as depicted in **Figure 1**. Cut each device out from sheet using scissors, following the edge of the printed region. Place packing tape across the bottom layer.

3. Nonplanar (Origami) Device Construction

1. Print device (**Figure 2A**) onto filter paper using a solid ink printer and place the filter paper on a hot plate at 170 °C for 2 min. Remove device from hotplate and allow it to cool to RT.
NOTE: The exact designs used are available as supplemental files.
2. Print crease pattern (**Figure 2C**) onto printer paper using a solid ink printer and cut to the size of the filter paper. Place crease pattern on a hotplate at 170 °C for 2 min, to melt the wax, causing the pattern to be visible from both sides of the paper. Remove crease pattern from hotplate and allow it to cool to RT.
3. Align the edges of the crease pattern to the edges of the paper containing the channel patterns and attach the two pieces of paper using binder clips, or another similar temporary method.
4. Trace the crease pattern with a blunt stylus, applying enough force that marks appear on the device sheet, but not so hard that the crease pattern paper rips. If that occurs, the device risks being damaged. Precreasing causes the paper to fold much more easily and allows for greater accuracy and precision in folding.
5. Begin folding the device with mountain and valley folds according to the crease pattern. Once the adhesive has been applied, the entire device must be assembled very quickly, so folding the device as much as possible before adhesive application is very helpful.
6. Once the device is folded, unfold the device to expose the portions of the device that require adhesive. Cut out masks (**Figure 2D**) that limit where on the device adhesive may be applied, using a razor blade.
7. Clamp the device between the stencil and mask and a stiff backing, such as a piece of plate glass. Ensure that the stencil is flat against the device. Apply adhesive (see list materials and equipment) with an approximately 1.33 sec (a four-count at 180 bpm) spray from about 24 cm. Four passes during this time (up-down-up-down) are sufficient in preventing spray shadows. Remove stencil and turn the sheet over. Replace stencil and mask and spray back side of the paper.
8. Immediately remove device from stencil and begin folding the device. Once the device is completely folded, apply pressure to the adhesive containing portion until the adhesive has dried.
NOTE: The drying time of the adhesive is very sensitive to ambient humidity, so in locations with low humidity, folding in a humidity controlled chamber allows more time to fold the device.

4. Wicking Test for 4-layer Devices

1. Randomly select 20 devices, previously assembled according to the above protocols. Place devices in a location shielded from any wind or breezes to minimize evaporation. Deposit 40 µl water at the inlet of each device. Record the time it takes for each device to have all of its outlets completely filled with dye.

5. Origami Wicking Comparison

1. Construct two origami peacocks — one according to the above protocol (Section 3), and the other without the use of a stencil during adhesive application.
2. Insert one end of a small paper lead (approximately 5 mm wide by 5 cm long) into the body of each peacock.
3. Place both peacocks in a chamber kept at a high relative humidity (>90%) to minimize evaporation. Place each leg and lead of each peacock into a container filled with 5 mM dye (red: Allura Red, yellow: tartrazine, blue: erioglaucine disodium salt). Record wicking process with a digital camera.

Representative Results

The 4-layer device tests were performed in a sealed chamber, shielding them from any wind or breezes that might cause excessive evaporation of the limited deposited fluid volume. The majority of the wicking in the 4-layer devices is in the middle layers of the device, so differences in wicking speeds due to evaporation were expected to be minimal. Additionally, there is minimal lateral wicking, with only 13 mm between the inlet and any individual outlet, suggesting that variations in wicking times are likely due to vertical, interlayer fluid transfer. Average wicking times and success rates for 4-layer devices constructed with different amounts of applied adhesive are shown in **Table 1**.

In stacked devices, uniform adhesive coverage resulted in relatively high success rates that decreased as we increased the quantity of adhesive. Patterned adhesive coverage resulted in very low success rates when adhesive was only applied to one side, but had much higher success rates and faster wicking times when the patterned adhesive was applied to both sides. Typical successes are depicted in **Figure 3A**. There are several potential explanations for this observed behavior, any combination of which may be applicable. The applied adhesive may be physically blocking, either partially or completely, the pores at the surface of the paper, resulting in a smaller effective contact area between paper layers. Also, the adhesive itself may act as another porous substrate, so heavier coatings of adhesive result in a thicker adhesive layer that fluid must wick through, leading to longer wicking times. Patterning the adhesive, on the other hand, creates adhesive 'dots' that only partially occlude the contact areas, allowing more fluid to wick from paper layer to paper layer directly, which decreases wicking times. However, this very reduction in adhesive coverage also decreases the strength of the adhesive bond between paper layers, resulting in decreased success rates when swelling fibers and unfolding creases cause layers to separate enough that they are no longer in contact. By doubling the size of the border around the channels (increasing the overall device area by ~30%), success rates for both single- and dual-sided adhesive applications increased. A comparison between the two sizes is shown in **Figure 4**. Typical stacked device failure was characterized by outlets that failed to completely fill with dye, or took longer than 5 min to fill. This is depicted in **Figure 3B**.

In origami folded devices, uniform adhesive coverage resulted in low success rates with complete failure resulting when applying the equivalent amount of adhesive present in the stacked, uniform, single-sided adhesive devices. Patterned adhesive coverage resulted in much lower success rates; however, this decrease was offset by using slightly larger devices that had 3 mm borders. Typical origami device failure was characterized by outlets that failed to fill with any amount of dye. These outlets were exclusively located along the two sides of the device that contained the creases. This is depicted in **Figure 3C**.

The masses of adhesive applied under different spray methods are shown in **Table 2**. The above-described spray duration of 1.33 sec (a four count at 180 bpm) deposits 0.26 mg/cm^2 (dry mass) of adhesive when sprayed uniformly across the sheet of devices, while only depositing 0.02 mg/cm^2 (dry mass) when sprayed through a stencil that was 23% open.

In nonplanar 3D structures, uniform adhesive coverage resulted in more difficult folding, as adjacent faces prematurely stuck together. The layers inside the structure could not be unfolded once the adhesive dried, and attempts to do so resulted in shredded paper. Patterned adhesive coverage made folding much easier, as any accidental adhesion was easily undone. Once the adhesive dried, the layers could be pulled apart without any ripping or tearing of the paper. Both methods of adhesive application resulted in devices that successfully routed liquid the length of their channels and without mixing; however, the device with uniformly applied adhesive was noticeably slower. A time-lapse of this wicking is shown in **Figure 5**. Wicking was performed in a humidity controlled chamber kept at >90% relative humidity to minimize evaporation, as evaporation increases with decreasing relative humidity. Due to the long channels present in this design, up to 165 mm long, evaporation can significantly increase the wicking time, even with an infinite fluid reservoir.

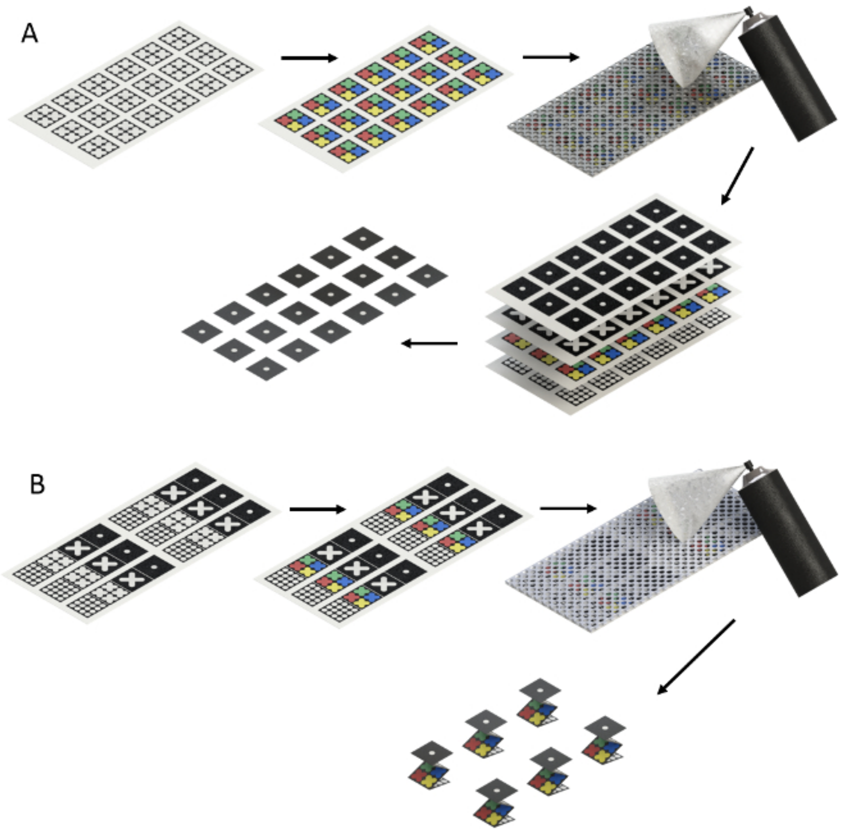

Figure 1. Device Fabrication Process Flow. (**A**) Stacked device fabrication. (**B**) Origami device fabrication. Please click here to view a larger version of this figure.

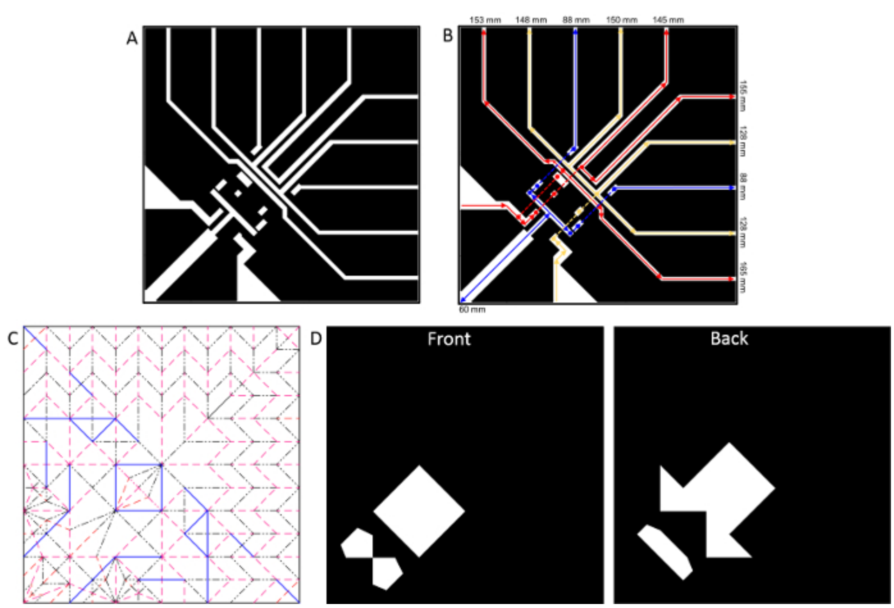

Figure 2. Peacock Patterns. (**A**) Channel pattern, where black indicates hydrophobic regions. (**B**) Arrows indicate the path taken by each dye. Circles indicate the point of contact between layers and the dotted lines indicate the vertical wicking paths. The length of each channel from its respective inlet to edge of the tail is indicated in millimeters. Channel widths averaged between 2 and 3 mm in the tail region. (**C**) Crease Pattern (modified from[13]). Red lines correspond to mountain folds in the final structure; black lines correspond to valley folds; blue lines correspond to creases that are not folded in the final structure, but aid in preliminary folding steps. (**D**) Masks placed between the origami device and the metal stencil during adhesive application, where the white portions are removed. Please click here to view a larger version of this figure.

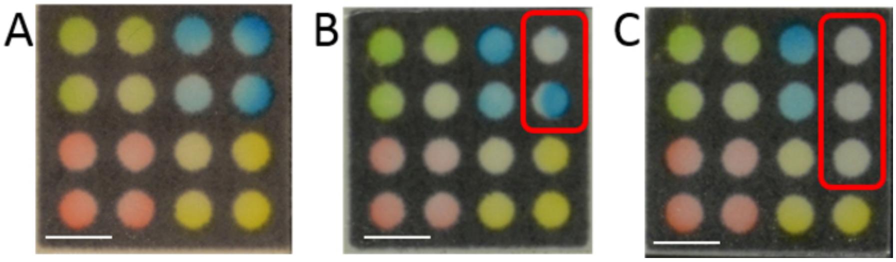

Figure 3. Typical Successes and Failures. (**A**) Typical Success — all outlets completely filled with dye. (**B**) Typical stacked failure — outlets that failed had no apparent pattern in their distribution. (**C**) Typical origami failure — all outlets that failed to fill were located along the left-most or right-most column, closest to the creases. All scale bars are 5 mm. Please click here to view a larger version of this figure.

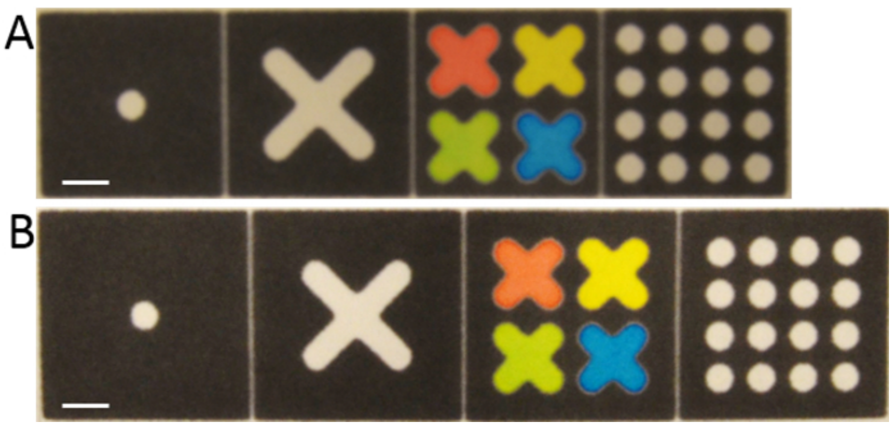

Figure 4. Device Size Comparison. (**A**) Smaller device (1.6 mm border). (**B**) Larger device (3 mm border). All scale bars are 5 mm. Please click here to view a larger version of this figure.

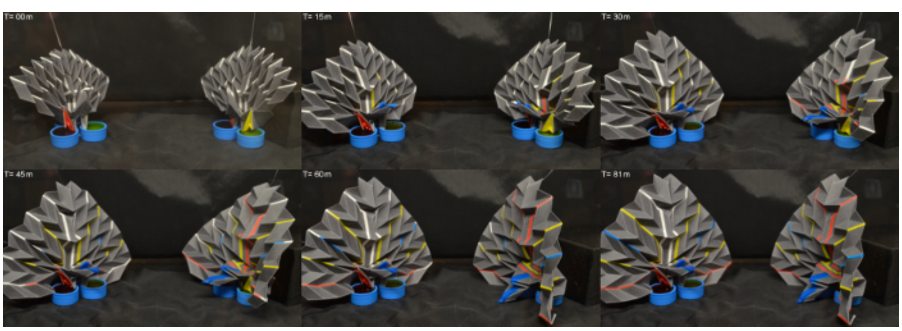

Figure 5. Time Lapse of Origami Peacock. Left: uniform adhesive coverage. Right: patterned adhesive coverage. Please click here to view a larger version of this figure.

Device Style	Adhesive Type (Duration/Border/ Sides)	Average ± SD (sec)	Success Rate
Origami	Uniform (1.33 sec / 1.6 mm / Double)	44 ± 14	45%
	Uniform (0.67 sec / 1.6 mm / Double)	0 ± 0	0%
	Patterned (1.33 sec / 1.6 mm / Double)	41 ± 13	15%
	Patterned (1.33 sec / 3 mm / Double)	64 ± 50	40%
Stacked	Uniform (1.33 sec / 1.6 mm / Single)	152 ± 66	80%
	Uniform (1.33 sec / 1.6 mm / Double)	119 ± 68	60%
	Patterned (1.33 sec / 1.6 mm / Single)	164 ± 75	25%
	Patterned (1.33 sec / 1.6 mm / Double)	81 ± 25	80%
	Patterned (1.33 sec / 3 mm / Single)	116 ± 63	85%
	Patterned (1.33 sec / 3 mm / Double)	80 ± 55	100%

Table 1. Four Layer Device Performance. Average wicking time and success rates for different adhesive application conditions. N=20.

Adhesive Coverage	Spray Duration (sec)	Average Mass ± SD (mg/cm^2)
Uniform	1.33	0.26 ± 0.05
Uniform	0.67	0.14 ± 0.03
Patterned	1.33	0.02 ± 0.01
None	0	-0.01 ± 0

Table 2. Applied Adhesive Amounts. Average adhesive thickness (dry mass) applied over a 9x9 cm square under different spray conditions. N=10.

Discussion

The above protocols use perforated metal sheets as stencils for applying aerosol adhesives to construct planar and nonplanar 3D paper microfluidic devices. In planar devices, this has the advantage of allowing devices to be completely unfolded after the adhesive has dried without destroying the device. In other adhesive based construction techniques, this is almost impossible, although, some designs allow for partial destructive disassembly by unpeeling two halves held together with a removable adhesive.[14] Adhesiveless construction does allow devices to be unfolded after use, but requires custom clamps or housings for each device.[8]

In devices with primarily lateral wicking, adhesives can significantly slow wicking. By patterning the adhesive, the amount of adhesive applied to the wicking regions can be significantly reduced, limiting any potential wicking interference. Devices with predominantly vertical wicking also exhibit similarly slow wicking caused by adhesive, although to a much lesser extent. Design of a stencil that completely blocks out all wicking regions, limiting adhesive application to hydrophobic regions only, may eliminate any potential wicking interferences, but may also add considerable alignment time to the construction process.

In nonplanar devices, the patterned adhesive dramatically eases folding, as the quantity of adhesive that is applied to the paper is decreased, making folding significantly easier than with a uniformly applied adhesive layer. Paper completely covered in adhesive is far more difficult to fold when any incidental contact between different areas of the paper causes adhesion that must be undone before continuing.

For planar 3D multilayer devices that have a large wicking area relative to the hydrophobic area, origami folding paired with an aerosol adhesive is likely not the optimal construction technique due to the adhesive's inability to hold wetted paper layers together while overcoming the tendency of the creases to unfold. Devices with designs that include sufficient hydrophobic borders will increase the success rate of origami-folded devices. Using a stronger bond-strength adhesive may also help to solve this issue, preventing water from weakening the paper-adhesive bond.

Stacked layer devices overall performed better, as they lack creases, which tend to unfold the device. Further, the use of a stencil during adhesive application reduces the total amount of adhesive applied, dramatically reducing the time required for fluid to wick between layers.

In designing nonplanar 3D paper microfluidic devices, there are a number of issues to consider. It is important to compare the crease pattern of the folded device to the layout of the channels, as placing channels along a crease will force the crease open upon water imbibition, due to swelling cellulose fibers. Depending on the design of the specific device, though, this may or may not be desired behavior. Device storage at ambient conditions is not favorable to device viability,[10] thus long-term storage under dry air is recommended to prevent the adhesive bond between layers from weakening.

As previously noted by Lewis *et al.,*[9] the use of aerosol adhesives provide an efficient means to rapidly produce large quantities of 3D paper microfluidic devices. By patterning such adhesives, new devices can be more rapidly developed that take advantage of being able to be unfolded after use.

Further, patterning enables the construction and development of nonplanar 3D paper microfluidic devices. Such devices are expected to be able to provide functionality not previously found in planar paper microfluidics, such as integrated actuation and sensing. For example, actuation can be achieved by creating a bilayer from a water reactive polymer film[15] and a patterned paper substrate. In a device constructed from such a bilayer, actuation would be generated when water wicks along the device's channels and interacts with the film. Once the film dries, the device would return to its initial configuration, leaving it ready to be used again.

Disclosures

The authors have nothing to disclose.

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