Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Use of Vacuum Bagging for Fabricating Thermoplastic Microfluidic Devices

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Materials and Reagents

Zeonor® 1420R film and 1020R resin were obtained from Zeon Chemicals (Louisville, KY). COC 8007 film was obtained from TOPAS Advanced Polymers (Florence, KY). CA-61 acrylic resin was obtained from Plaskolite Inc. (Columbus, OH). Vacuum sealant tape, Stretchalon 800 bagging film, and polyester breather were obtained from Fibre Glast Development Corp. (Brookville, OH). Glucose, and horseradish peroxidase (HRP) were obtained from Fisher Scientific (Pittsburgh, PA). Glucose oxidase (GOx) was obtained from MP Biomedical (Solon, OH). 3-Aminophthalhydrazide (luminol) was obtained from Acros Organics (Morris Plains, NJ). Fluorescein solutions were prepared using water from a Barnstead Thermolyne Nanopure water purification system (Asheville, NC).

Device Fabrication

COC/COP Thick Substrate Device Fabrication

Thick substrate devices used for burst pressure testing were constructed by molding and thermal lamination, presented previously,[1] or by molding and vacuum bonding (described in this work). Briefly, 1020R resin was compressed against a metal mold using a heated hydraulic press to create channel layers with an approximate thickness of 1.5 mm. Individual chips measuring 1” by 3” were then machined from the substrates using a CNC milling machine. Thick substrate devices were made using either thick substrate covers or thin cover films . The thick substrate covers were made by compressing 1020R resin between glass plates to create smooth covers with a thickness of 1.5 mm. Film covers were made from Topas 8007, and were cut from 100 μm sheet material using a craft-cutter (Graphtec Craft Robo-S, Graphtec Corporation).

Once cut, the channel layers and covers were cleaned with ethanol for 15 minutes in an ultrasonic cleaner, rinsed with deionized water, placed in soapy water for 15 minutes in an ultrasonic cleaner, rinsed three times with deionized water, and finally soaked in deionized water in an ultrasonic cleaner for 15 more minutes. The cleaned channel layers and covers were dried with compressed air, and then the dry layers were aligned between a glass slide and a piece of Mylar film, and placed on top of a metal backing plate (Figure S1**b**). A vacuum bag was created by covering the film layers with vacuum bagging film and sealing it to the backing plate using vacuum sealant tape (Figure S1c). Vacuum was applied through a two-piece vacuum port and the entire assembly was placed in an oven (Barnstead/Thermolyne 48000, Thermo Fisher Scientific) to soak at 100 °C (the T_g of 1020R is 105 °C) (Figure S1**d**). After three hours the assembly was removed from the oven, re-pressurized, and allowed to cool to room temperature.

COC/COP Film Device Fabrication

The fabrication process used to produce film devices is similar to that presented previously.[2-4] Film layers were designed using AutoCAD (Autodesk Inc.) and then exported as DXF files to the craft cutter controller software, ROBO Master-Pro (Graphtec Corporation). Zeonor® 1420 film was affixed to an adhesive carrier sheet and cut with a single high force pass using a cutting plotter (Graphtec Craft Robo-S, Graphtec Corporation).

Once cut, the scrap material was removed and the film layers were cleaned with ethanol for 15 minutes in an ultrasonic cleaner, rinsed with deionized water, placed in soapy water for 15 minutes in an ultrasonic cleaner, then rinsed three times with deionized water, and finally soaked in deionized water in an ultrasonic cleaner for 15 more minutes. Cleaned film layers were dried with compressed air, after which, the dry layers were aligned with each other between a glass slide and a piece of Mylar film and placed on top of a metal backing plate (Figure S1b). A vacuum bag was created by cov-

ering the film layers with vacuum bagging film and sealing it to the backing plate using vacuum sealant tape (Figure S1c). Vacuum was applied through a two-piece vacuum port and the entire assembly was placed in an oven (Barnstead/Thermolyne 48000, Thermo Fisher Scientific) to soak at 133 °C (the T_g of 1420R is 136 °C) (Figure S1d). After two hours the assembly was removed from the oven, re-pressurized, and allowed to cool to room temperature.

Acrylic Thick Substrate Device Fabrication

The fabrication process used to produce thick substrate devices using acrylic resin was similar to the one used for thick substrate COC devices. CA-61 acrylic resin was compressed against a metal mold using a heated hydraulic press to create channel layers with an approximate thickness of 1.5 mm. Individual parts measuring 1” by 3” were then machined from the substrates using a CNC milling machine. Once cut, the channel layers and covers were cleaned with ethanol for 15 minutes in an ultrasonic cleaner, rinsed with deionized water, placed in soapy water for 15 minutes in an ultrasonic cleaner, rinsed three times with deionized water, and finally soaked in deionized water in an ultrasonic cleaner for 15 more minutes. The cleaned channel layers and covers were dried with compressed clean air, and then the dry layers were aligned between a glass slide and a piece of Mylar film, and placed on top of a metal backing plate (Figure S1b). A vacuum bag was created by covering the film layers with vacuum bagging film and sealing it to the backing plate using vacuum sealant tape (Figure S1c). Vacuum was applied through a two-piece vacuum port and the entire assembly was placed in an oven (Barnstead/Thermolyne 48000, Thermo Fisher Scientific) to soak at 97 °C (below the Vicat softening temperature of 102 °C) (Figure S1d). After four hours the assembly was removed from the oven, re-pressurized, and allowed to cool to room temperature.

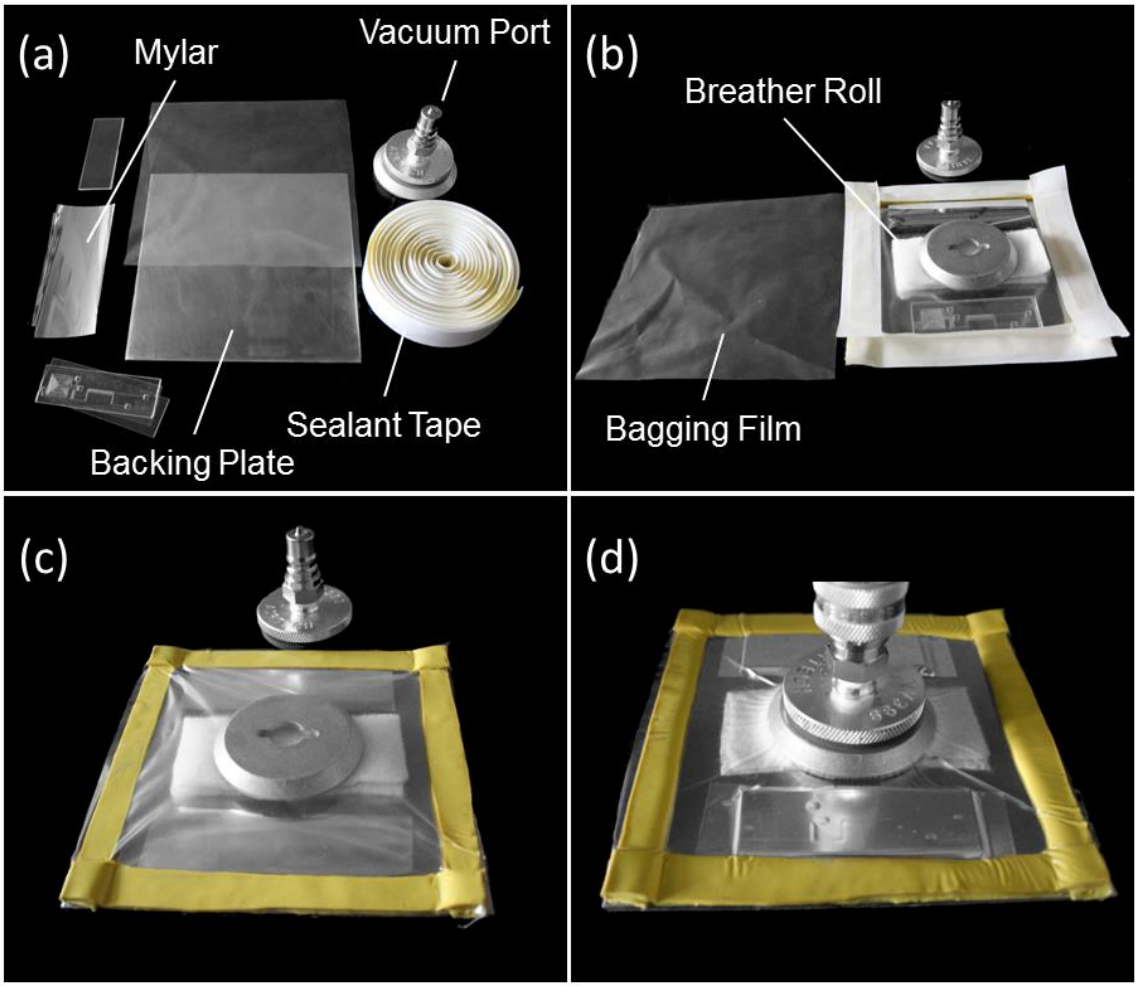

Figure S1 Assembly of a vacuum bag for device bonding. (a) Materials required to construct a vacuum bag include: breather roll, bagging film, COC film, mylar film, a backing plate, microscope slides, a two piece vacuum port, and sealant tape. (b) COC devices are aligned and placed on top of a glass slide on the backing plate and covered with mylar film. (c) The bagging film is adhered to the backing plate using the sealant tape. (d) Vacuum is applied to the completed bag through the vacuum port and the whole assembly is baked under vacuum.

Burst Pressure Testing

Individual chips were produced as described in the thick substrate fabrication section. Each device contained one 54 mm long channel and one 10 mm long channel (Figure S2). Cover films made from Topas 8007 were cut as above and used as described. Laminated chips were preheated for five minutes at 90 °C before passing through the rollers of a heated roll laminator (Catena 35, GBC) set at 118 °C, which results in an actual interfacial temperature of approximately 110 °C. Vacuum bonded devices were baked for two hours at 80 °C, 85 °C, 90 °C, or 95 °C. The bonding temperature range falls above the T_g of 8007 (78 °C) but below the T_g of 1020R (105°C).

Burst pressure testing was accomplished by placing bonded devices in a shallow dish of water and pressurizing a blind 54 mm channel with nitrogen gas. Nitrogen pressure was ramped up at a constant rate of approximately 7 kPa/s until delamination, gas bubble venting, or catastrophic failure was reached. To test for possible channel blockage within bonded devices, deionized water with food dye was passed through the 10 mm channel using vacuum.

Figure S2 shows the failure rate among a total of 6 devices tested at different burst pressures. For example, devices that were bonded at 85 °C have 50% failure rate (i.e., 3 devices) at a burst pressure of 0-50 KPa and 50% failure rate at 51-100 KPa. For devices that were bonded at 95 °C, 1 device failed (17% failure rate) at a burst pressure of 201-250 KPa, 1 device failed at 301-350 KPa, 1 device failed at 451-500 Kpa, and 3 devices (50% non-failure rate) did not fail even at the maximum pressure deliverable by the testing apparatus we used (551 Kpa).

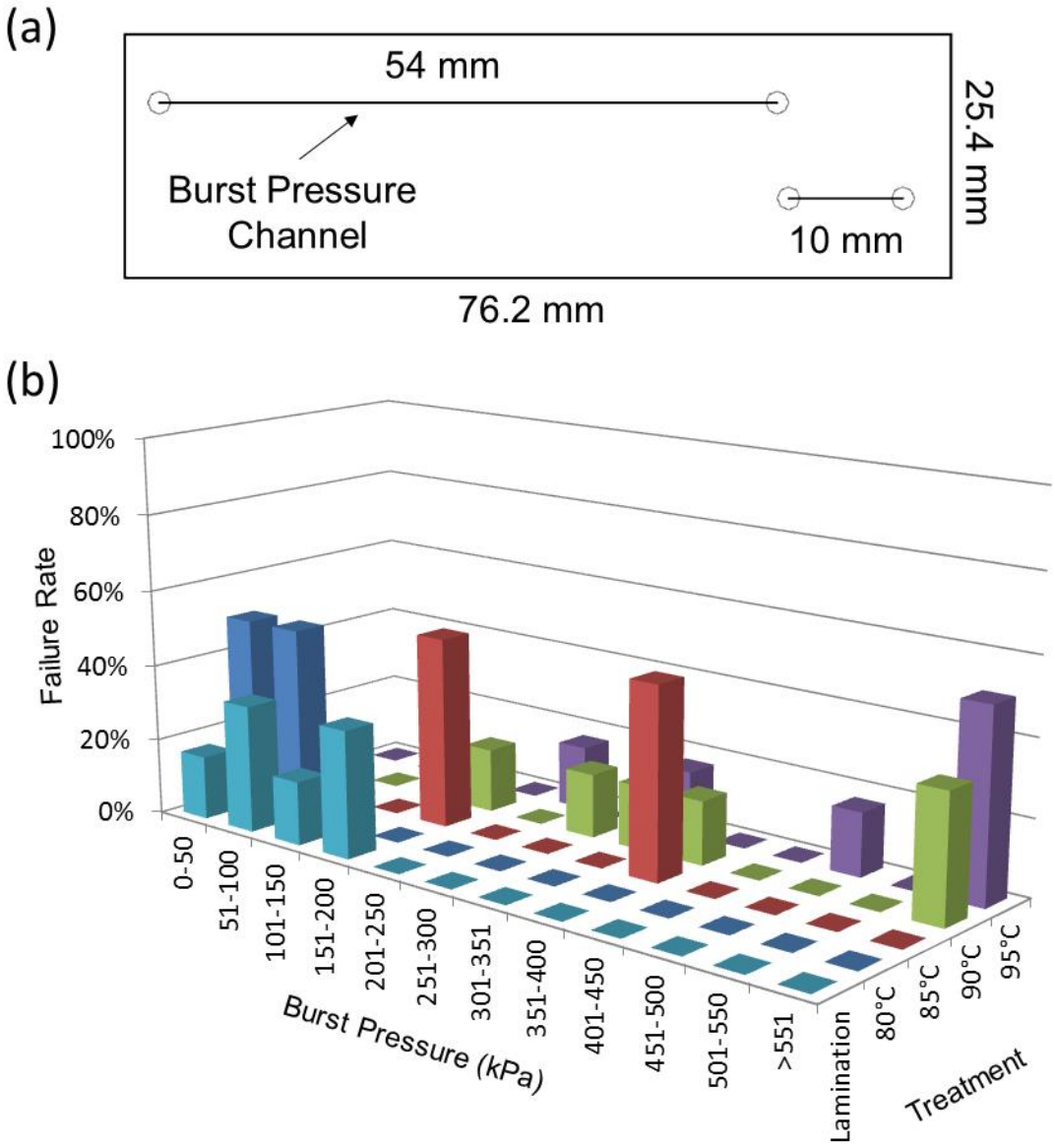

Figure S2 (a) Layout of devices used for burst pressure testing in the long channel (54 mm). The short channel (10 mm) was filled with food coloring after bonding to ensure retention of channel integrity. (b) Burst pressures were obtained using six devices made using Zeonor® 1020R chips bonded to Topas 8007 film using both vacuum bonding and thermal lamination. Some devices did not fail, even at the maximum pressure deliverable by the testing apparatus (551 Kpa). A total of six devices were tested for each temperature.

Tips and Tricks

See the main text for the detail. **Figure S3a** shows trapped moisture expanding to become voids between the layers while **Figure S3b** shows deflected corners and edges of a device.

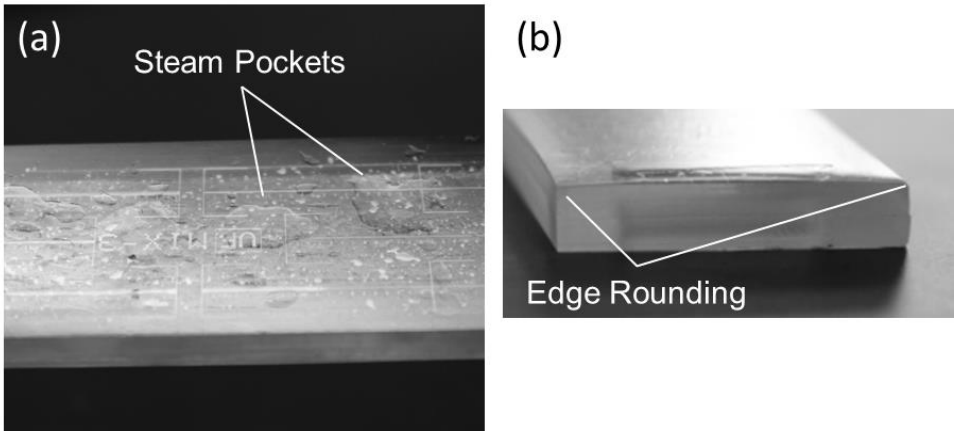

Figure S3. Possible problems encountered when vacuum bonding. (a) Steam pockets during the bonding process by entrapped water. The same thing can occur between device layers, resulting in internal voids. (b) Edge rounding of a device.

Chemiluminescent Assay Protocol

Solutions of glucose in distilled water and 100 μM luminol in sodium hydroxide at pH 12.0 were each introduced into the device through separate inlets at a constant flow rate of 1 mL/hr (or 278 nL/s) for each solution. A steady baseline signal was obtained before a mixture of HRP and GOx in water (100 μg/mL and 500 μg/mL, respectively) was introduced into the device at a constant flow rate of 1 mL/hr. The signal was measured using a photomultiplier tube (Hamamatsu H7360-02, Hamamatsu Photonics K.K) connected to an external counting unit (Hamamatsu C8855-01, Hamamatsu Photonics K.K) set with a 100 ms gate.

Flow Injection Assay

The concept behind the assay is shown in **Figure S4a**. Glucose, GOx, and HRP are mixed within the device and used for in-situ production and catalysis of hydrogen peroxide, which in turn mixes with luminol in the device to produce a chemiluminescent signal within the 3D detection zone.[5]

A representative signal for a 2-μM glucose sample obtained using the multi-layer device is shown in **Figure S4b**. From t = 0 to 100 seconds the signal falls in the range of the noise from the detector as the luminol and glucose solutions were pumped through the device at a continuous rate. At t = 100 seconds the enzyme mixture was added, after which the signal began to increase. At first, the signal increase was unsteady, but once the flow rate stabilized (at around t = 250 seconds), the signal increase began to linearize. The signal continued to increase as the combined solutions filled the entirety of the serpentine mixers, at approximately t = 400 seconds. After 400 seconds the signal reached its maximum and stabilized at about 120,000 counts. This device is at least as sensitive as other microfluidic flow injection analysis devices,[6] and based upon the three order of magnitude difference in signal between the background and the sample, it should be possible to detect glucose concentrations in the low nanomolar range, without significant optimization. This is supported by the signal obtained from a 500 nM sample, shown in Figure S4b, obtained using ten-times dilutions of the enzyme mixtures used for the 2 μM sample.

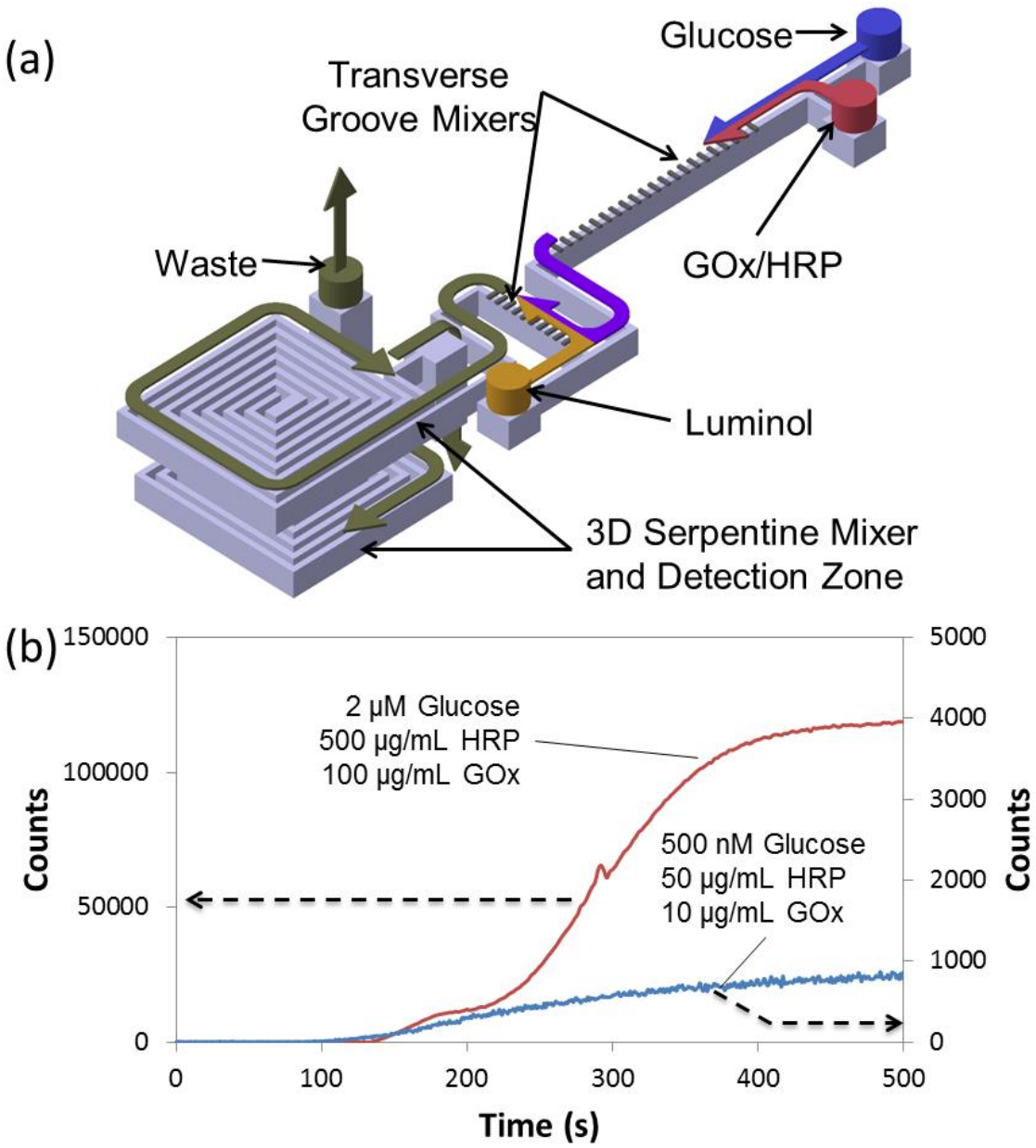

Figure S4. Chemiluminescent glucose assay. (a) Expanded view of the three-dimensional flow path. Hydrogen peroxide is generated in situ by the action of GOx on glucose in solution. Breakdown of hydrogen peroxide is catalysed by HRP, followed by combination with luminol to generate chemi-luminescent signals. (b) Chemiluminescent output for 500 nM and 2 µM glucose samples. The blank signal for these assays fell between 20 and 160 counts, and are not shown.

References

1. Fredrickson, C. K.; Xia, Z.; Das, C.; Ferguson, R.; Tavares, F. T.; Fan, Z. H., Effects of Fabrication Process Parameters on the Properties of Cyclic Olefin Copolymer Microfluidic Devices. *J. Microelectromech. Syst.,* 2006, *15* (5), 1060-1068.
2. Patel, T.; Tencza, L.; Daniel, L.; Criscuolo, J. A.; Rust, M. J. In *A rapid prototyping method for microfluidic devices using a cutting plotter and Shrinky Dinks*, Bioengineering Conference (NEBEC), 2011 IEEE 37th Annual Northeast, 1-3 April 2011; 2011; pp 1-2.
3. Taylor, D.; Dyer, D.; Lew, V.; Khine, M., Shrink film patterning by craft cutter: complete plastic chips with high resolution/high-aspect ratio channel. *Lab Chip* 2010, *10* (18), 2472-2475.
4. Yuen, P. K.; Goral, V. N., Low-cost rapid prototyping of flexible microfluidic devices using a desktop digital craft cutter. *Lab Chip* 2010, *10* (3), 384-387.
5. Liu, W.; Cassano, C. L.; Xu, X.; Fan, Z. H., Laminated Paper-Based Analytical Devices (LPAD) with Origami-Enabled Chemiluminescence Immunoassay for Cotinine Detection in Mouse Serum. *Anal. Chem.* 2013, *85* (21), 10270-10276.
6. Panoutsou, P.; Economou, A., Rapid enzymatic chemiluminescent assay of glucose by means of a hybrid flow-injection/sequential-injection method. *Talanta* 2005, *67* (3), 603-609.