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Fabricating Millimeter-scale Polymeric Structures for Biomedical Applications via a Combination of UV-activated Materials and Daily-use Tools

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Experimental Details

Nitrite ion (NO_2^-) analysis

The nitrite ion assay that we performed was based on the color change from colorless to purple that occurs when nitrates chemically transform to nitrites.[1] To perform the nitrite ion assay on the test zones in a buffer system (i.e., PDMS-based structures or polymeric-based platform), we first spotted 1 μL of a solution of the coupling reagent composed of 50 mM sulfanilamide, 10 mM N-naphthyl-ethylenediamine, and 330 mM citric acid on the test zones. Deionized water with a series of nitrite ion concentrations (0.005, 0.04, 0.17, 0.31, 0.62, 1.25, and 2.5 mM in this study) was then placed on each test zone. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Following a reaction time of 10 minutes, the colorimetric data was recorded using a camera (see supporting information).

Cholesterol assay

Our cholesterol assay, was based on the color change of a reagent (containing both 4-aminoantipyrine and dimethylaminobenzoic acid) from colorless to purple when this reagent oxidized and the oxidized product subsequently reacted with horseradish peroxidase (HRP).[2] To examine whether the cholesterol assay could work on the test zones of a buffer system, we first spotted 1 μL of solution of the reagent composed of 2mM 4-aminoantipyrine and 10mM dimethylaminobenzoic acid on the test zones, and then placed a solution mixture of cholesterol oxidase (4 U/mL) and horseradish peroxidase (3 U/mL) over the reagent. The cholesterol stock solution (600 mg/dL) prepared in 10% Triton X-100 was then diluted with deionized water at concentrations of 100, 150, 200, 300, 400, 500, and 600 mg/dL and then dropped on these test zones. After a reaction time of 10 minutes, the data was recorded with a camera (see supporting information). Once again, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Glucose assay

Our glucose assay was based on the color change of potassium iodide from colorless to brown when iodide enzymatically oxidized (from iodide to iodine) in the presence of glucose in both a buffer system and human serum.[22] To establish glucose diagnosis in our buffer system using PDMS-based structures, we spotted 1 μL of a solution of 0.6 M potassium iodide, and then placed a mixed solution of horseradish peroxidase (15 U/mL) and glucose oxidase (75 U/mL), with a weight ratio of 1 to 5, on the test zones. The deionized water with diluted glucose was then dropped on these test zones at concentrations of 1, 1.5, 2.5, 5, 10, 50, and 500 mM. After a reaction time of 10 minutes, the data was recorded with a camera (see supporting information).

Human serum

We enrolled one volunteer, aged ~ 25 years. Whole blood was drawn and centrifuged at 2000 r.p.m. in BD Vacutainer™ Venous Blood Collection Tubes (Serum tube; Becton, Dickinson and Company, USA) for 10 minutes at 4°C (after we obtained whole blood for 30 minutes), in order to obtain human serum. Human serum was kept at -20°C until performing the following experiments. We diluted a mixed solution of cholesterol oxidase (4 U/mL) and horseradish peroxidase (1.25 U/mL) with human serum at final cholesterol concentrations of 100, 150, 200, 250, 300, 400, and 500 mg/dL to perform the human serum cholesterol diagnosis on our test zones. Once again, we diluted a mixed solution of horseradish peroxidase (15 U/mL) and glucose oxidase (75 U/mL) with human serum at final glucose concentrations of 2.5, 5, 10, 15, 25, 50, and 75 mM, in order to demonstrate glucose diagnosis in human serum on PDMS-based structures. The concentration

of potassium iodide we used for our human serum test was 2.4 M. After a reaction time of 30 minutes, the data was recorded with a camera.

Image recording and data analysis of colorimetric-based metabolic assays in both buffer system and human serum

The colorimetric-based results of our metabolic assays were recorded with a digital camera D3s (Nikon, Japan), and the recorded conditions are listed in Table S1. Image analyses of color intensity on the metabolic assay target zones were performed by importing images into ImageJ (public software from National Institutes of Health; http://rsbweb.nih.gov/ij/) and converting them to grayscale. The mean grayscale intensities were then quantified using the histogram function of ImageJ. The ultimate mean intensity value of each detection zone was obtained by subtracting the measured average detection zone intensity from the mean intensity of the blank control. The results in this study were reported as the mean ± standard deviation.

Table S1 Recorded conditions of colorimetric-based results for metabolic assays using a digital camera D3s.

Recorded Condition	Parameter
ISO	640
shutter speed (second)	1/13
aperture	5.6
distance between camera and assay (cm)	20

Contact angles of UV-activated materials on various substrates

The height of polymeric structures was influenced by the surface tension of UV-activated materials that we used; for example, the height of polymeric structures that we made while using the high-surface-tension UV-activated material (SU-8 2005) was about 25% times higher than the low-surface-tension UV-activated material (OBM-309). The height of polymeric structures could be increased with an increase in the number of adhesive tape layers used. Fig. S1b exhibits the structural heights of the polymeric structures when we used either the high-surface -tension or low-surface-tension UV-activated material (SU-8 2005 versus OBM-309; contact angles are shown in Table S2) with an increase in number of adhesive tape layers from single to double, indicating that, once we used the high-surface-tension UV-activated material to prepare polymeric structures, we could obtain more accurate results (note, the thickness of adhesive tapes that we used was about 100 μm).

To examine the thickness of polymeric structures on different substrates, we have measured contact angles of UV-activated materials (OBM-309 and SU-8 2005) on silicon wafer, glass coverslip, polyethylene terephthalate film, and the adhesive layer of the tape (N = 15; data are mean ± standard deviation). Table S2 shows the contact angles of OBM-309 and SU-8 2005 on different substrates.

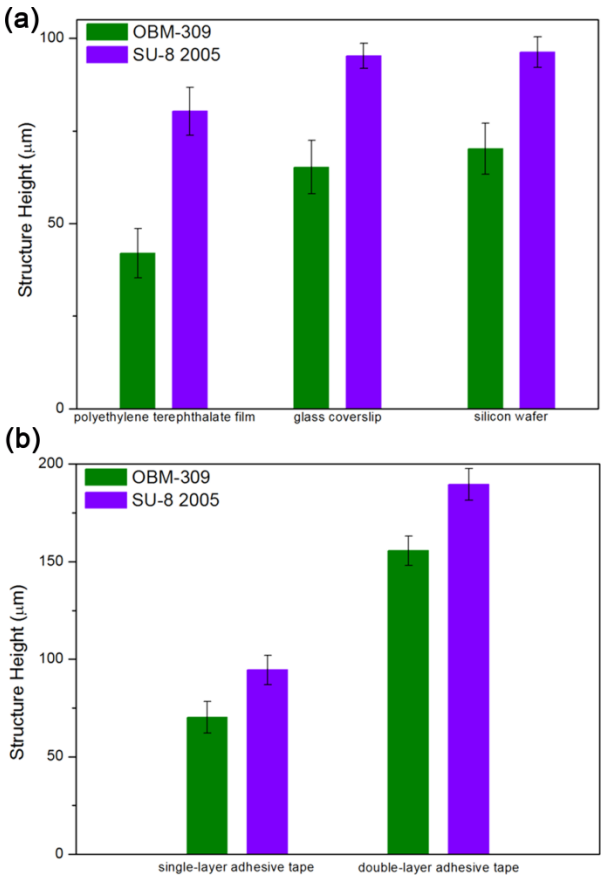

Fig. S1 (a) Structure height of UV-activated materials (OBM-309 and SU-8 2005) on polyethylene terephthalate film, glass coverslip, and silicon wafer. The structure height of UV-activated materials on polyethylene terephthalate film is lower than on glass coverslip and silicon wafer due to the surface energy of substrates (polyethylene terephthalate versus SiO_2). (b) Structure height of UV-activated materials (OBM-309 and SU-8 2005) on glass coverslip made using single-layer adhesive tape and double-layer adhesive tapes.

Table S2 Contact angles of UV-activated materials on various substrates.

Materials	Contact angle (°)	
	OBM-309	SU-8 2005
Silicon wafer	33.5 ± 1.3	56.7 ± 2.1
Glass coverslip	24.2 ± 0.9	48.5 ± 1.9
Polyethylene terephthalate film	22.4 ± 1.4	36.9 ± 3.2
Adhesive tape	36.0 ± 2.6	72.5 ± 2.9

Three-dimensional structures made by multiple-layer adhesive tapes

In this study, a three-dimensional polymeric structure (SU-8 2005) using 10-layer adhesive tapes was demonstrated through our approach. Fig. S2a-c show the images examined via a scanning electron microscope and a camera. The polymeric structure was fabricated on silicon wafer, and the height of the structure is approximately 1 mm. We also can use this approach to fabricate

polymeric structures by using different UV-activated materials, as shown in Fig. S2d.

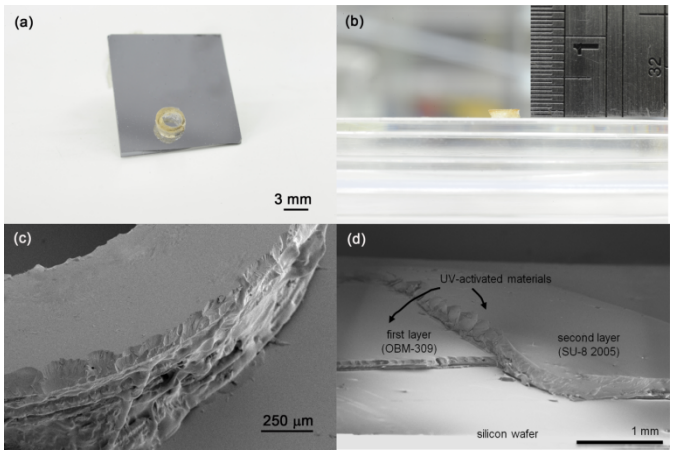

Fig. S2 (a) Three-dimensional polymeric doughnut structure made by SU-8 2005 on silicon wafer using 10-layer adhesive tapes. (b) The height of the polymeric structure is approximately 1 mm (100 μm height for single-layer adhesive tape). (c) A scanning electron microscope image of three-dimensional polymeric structure. (d) A scanning electron microscope image of three-dimensional polymeric structures made via double-tape procedure. The first layer is OBM-309, and the second layer is SU-8 2005.

Estimation of cost per diagnostic test

Cost per device (one well) estimate for the PDMS-based diagnostic test were divided into manufacturing equipment and consumables (Table S3). We estimate the lifetime of manufacturing equipment is 1000 hours (for UV-LED chip). The 7 × 7 arrayed wells (2 mm in diameter) can be fabricated by UV-LED flashlight in a single run (30 minutes), which means a UV-LED flashlight can manufacture 98,000 wells during its lifetime. Therefore, the cost per well of other manufacturing equipment is also estimated by this standard number (i.e., 98,000 wells). In addition, we also evaluated the manufacturing cost while using conventional photolithography process (i.e., the cost of preparing a mask and the UV exposure system). Four hundred polymeric structures (2 mm in diameter) could be made in 30 minutes using a mask aligner (20 minutes for baking, 10 minutes for exposure and development). Eight hundred thousand (400 × 2000) wells can be fabricated in 1000 hours. Consumable pricing is based on cost of materials currently being used at the research level and adjusted for lower prices based on volume discount.

Table S3 Comparison of cost per PDMS-based diagnostic well between our approach and a conventional approach.

	Manufacturing equipment (lifetime = 1000 hours)	**Cost/per well**	
		Our approach	**Conventional approach**
Manufacturing equipment	UV-LED flashlight ($60)	$0.0006	-
	Mask aligner ($150,000)	-	$0.1875
	Oven ($600)	$0.0060	$0.0008
	Vacuum pump ($250)	$0.0026	$0.0003
	Puncher, a pair of scissors ($10)	$0.0001	-
	Spin coater ($100,000)	-	$0.1250
	Total	**$0.0093**	**$0.3136**
Consumables	Battery	$0.0001	-
	Adhesive tape	$0.0008	-
	PDMS	$0.0100	$0.0100
	Reagents (enzymes, chemicals, PR etc.)	$0.0200	$0.0300
	Total	**$0.0309**	**$0.0400**
Clean room	Class 1000 ($100,000)	-	$0.1250
	Total cost	**$0.0403**	**$0.4786**

Demonstration of PDMS-based Microfluidics

To demonstrate PDMS-based microfluidics, PDMS was then poured into a petri dish to produce a piece of PDMS substrate with a thickness of ~1-2 mm after baking at 140°C for 20 minutes in an oven. The mixed PDMS prepolymer was smeared (spin coating) onto the PDMS substrate, and the final PDMS-based microfluidic sample was completed after the PDMS structure was irreversibly bonded onto the PDMS substrate at 90°C for 20 minutes in an oven.

To investigate the axial bonding force of two PDMS structures bonded together with PDMS prepolymer at various spin speeds (1500, 2000, 2500, and 3000 rpm), the bonded PDMS structures were cut as 7 × 7 mm^2 and then fixed onto pre-cleaned screw holders. After that, the test sample was put into the testing machine Tytron-250 (MTS Systems Corporation, MN, USA) and gradual force was applied to determine PDMS bond breaking point. During this experiment, individual PDMS structures sometimes peeled off from one another at the corner, but force was still applied until the PDMS structures broke apart.

Experimental discoveries of the bonding force of two PDMS structures bonded by PDMS prepolymer at various spin speeds is shown in Fig. S3a. The results show a linear relationship between bonding force and spin speed, with bonding force directly increasing with spin speed. This implies that the bonding force of PDMS was stronger when using the thinner PDMS (with higher spin speed) prepolymer. The bonding force using PDMS prepolymer as a glue is strong enough for microfluidics applications. A simple microfluidics device can be made and demonstrated via this physically based approach as shown in Fig.s S3b and c. For identification, red ink was injected from the left inlet and allowed to flow through the microchannel to the right outlet (Fig. S3b). We also can inject two different colorized inks from two inlets via a micropump and allow it to flow through one microchannel to form two separated laminar flows based on low flow rate and low Reynold number (Fig. S3c). This microfluidics system can be used for a cell counting chip or a mixer via different pattern design. These demonstrations, we believe, can simplify the fabrication process of microfluidics devices and provide easy to use tools for early disease detection in less economically developed regions and abroad.

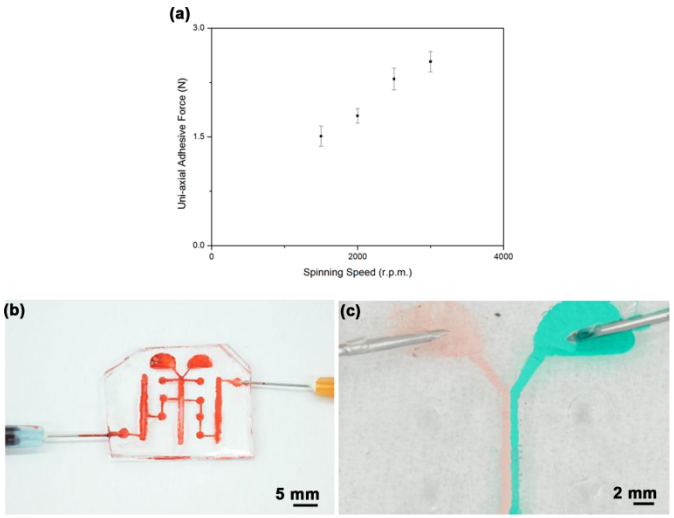

Fig. S3 The PDMS-based microfluidic devices were bonded by two PDMS substrates with PDMS prepolymer and baking at 140°C in an oven. (a) The bonding force of two PDMS structures adhered via PDMS prepolymer at various spin speeds. Data are mean ± standard deviation (N = 5). The bonding force increased with spin speed, indicating that a more uniform PDMS prepolymer may enhance the bonding force. (b) Our PDMS microfluidic device was injected with red ink, and (c) two colorized liquids were injected to form laminar flows in one channel with a pumping rate at 500 μL/minute using a micropump.

Notes and references

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2 N. R. Pollock, J. P. Rolland, S. Kumar, P. D. Beattie, S. Jain, F. Noubary, V. L. Wong, R. A. Pohlmann, U. S. Ryan and G. M. Whitesides, *Sci. Transl. Med.*, 2012, **4**, 152ra129.

