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

Supplementary Information

Selective laser ablation for in-situ fabrication of enclosed channel porous-media microfluidic analytical devices

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Assay protocols for determination of albumin, glucose, and cholesterol in 3D flow-through devices

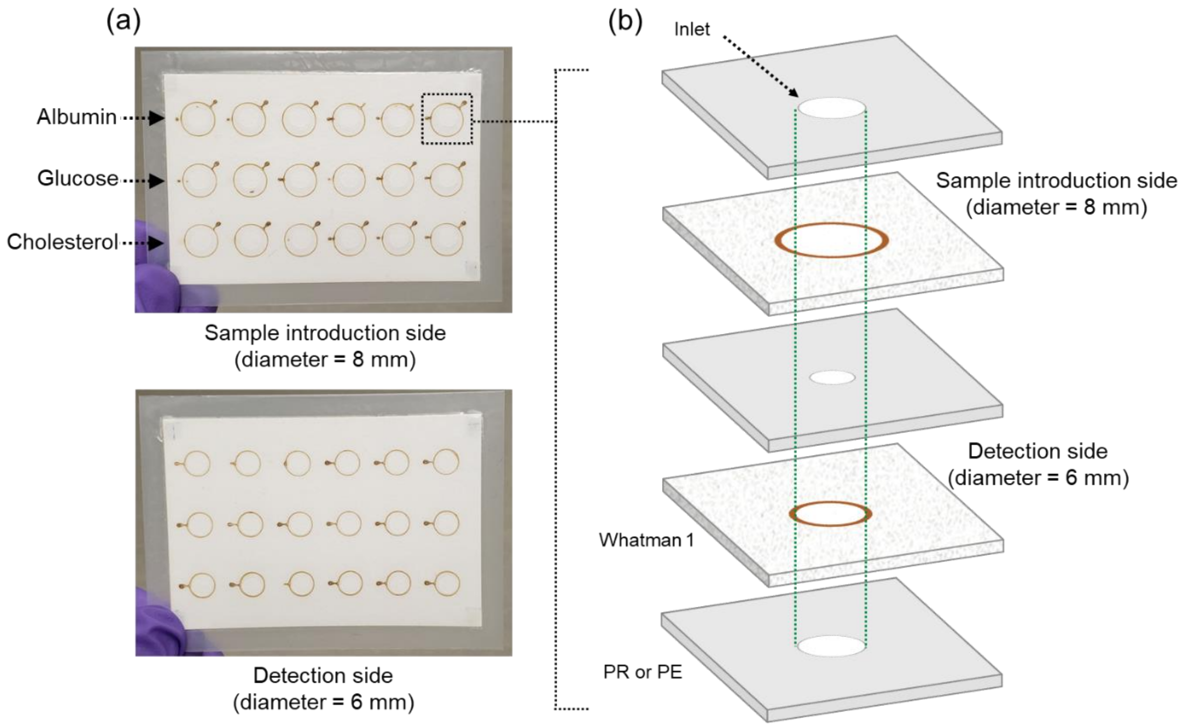


Fig. S1 3D flow-through devices used for analysis of albumin, glucose, and cholesterol. (a) Photographs of the devices in array format. (b) Schematic illustration of a device in an exploded view. Whatman 1 was ablated by a 455 nm laser, and PR or PE was cut using a digital craft cutter.

Albumin assay used in this work based on color changing of an indicator, TBPB, from yellow to green/blue upon formation of protein-indicator complex. In this assay, 2.0 µL of 250 mM of citrate buffer pH 1.8 (92% water, 8% ethanol) was deposited on the sample introduction side and 1.5 µL of 1:1 mixture of the citrate buffer (500 mM) and 5 mM of TBPB (95% ethanol, 5% water) was deposited on the detection side. The device was dried at 30 °C for 10 minutes. BSA solutions (1 - 80 g/L) prepared in PBS pH 7.4 were used as albumin standards. Human serum sample was applied to the device without further dilution. 6 µL sample volume was used when the device was fabricated with PR and 7 µL sample volume was used when the device was fabricated with PE.

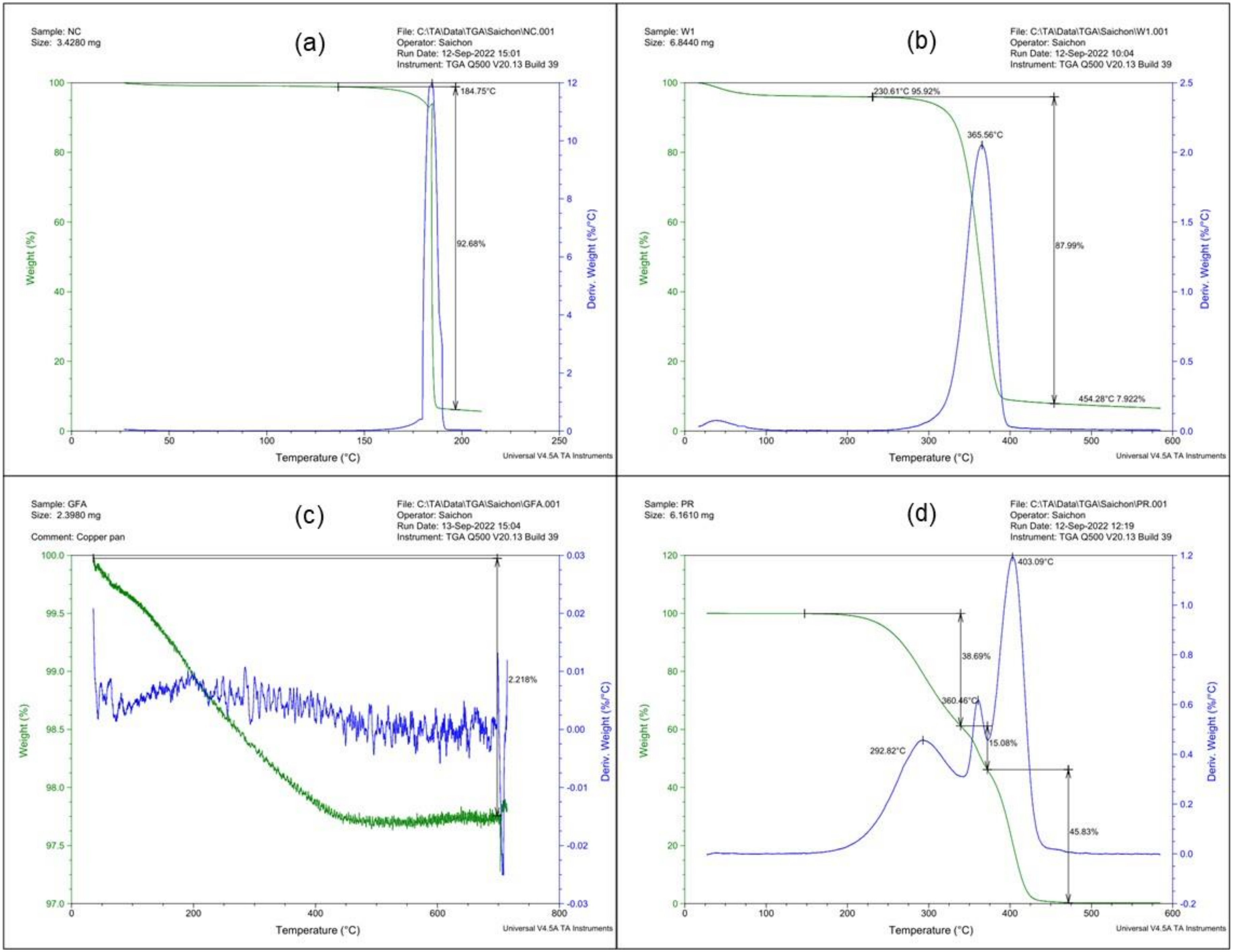
Enzymatic assays were used for glucose determination. 1.5 µL of solution containing 720 U/mL GO and 180 U/mL HRP in PB pH 6 was dried on the detection side. For the devices fabricated with PR, 2.0 µL of solution containing 0.05 M AAP and 0.1 M HBA in 25% (v/v) ethanol was dried on the sample introduction side. For the devices fabricated with PE, 2.5 µL of solution containing 0.2 M AAP and 0.4 M HBA in 25% (v/v) ethanol was dried on the sample introduction side. Glucose standard solutions (0.5 – 12 mM) prepared in PBS pH 7.4 were used as glucose standards. Human serum sample was applied to the device without further dilution. 6 µL sample volume was used when the device was fabricated with PR and 8 µL sample volume was used when the device was fabricated with PE.

Enzyme cascade assays were used for total cholesterol determination. 1.5 µL of solution mixture of 20 U/mL CE, 40 U/mL CO, and 20 U/mL HRP in PB pH 7 containing 0.1% (v/v) Triton X-100 was dried on the detection side. 2.0 µL of 0.075 M ABTS was dried on the sample introduction side for the devices fabricated with PR and 2.5 µL of 0.15 M ABTS in 50% (v/v) ethanol was dried on the sample introduction side for the devices fabricated with PE. The stock solution of cholesterol (5 mM) was prepared in water containing 3% (v/v) isopropanol and 3% (v/v) Triton X-100. The solution was kept in a water bath at 60°C until cholesterol was dissolved, and a clear solution was obtained. The cholesterol standard solutions (0.2 – 2 mM) were prepared by dilution of the stock solution with PB pH 7 containing 1% (v/v) Triton X-100. Human serum sample was diluted five times with PB pH 7 containing 1% (v/v) isopropanol and 1% (v/v) Triton X-100. The diluted serum was heated in a water bath at 40°C for a few minutes and then vortex for 30 seconds to ensure cholesterol was soluble in the solution. 7 µL of standards or sample solutions were applied to the device for cholesterol determination.

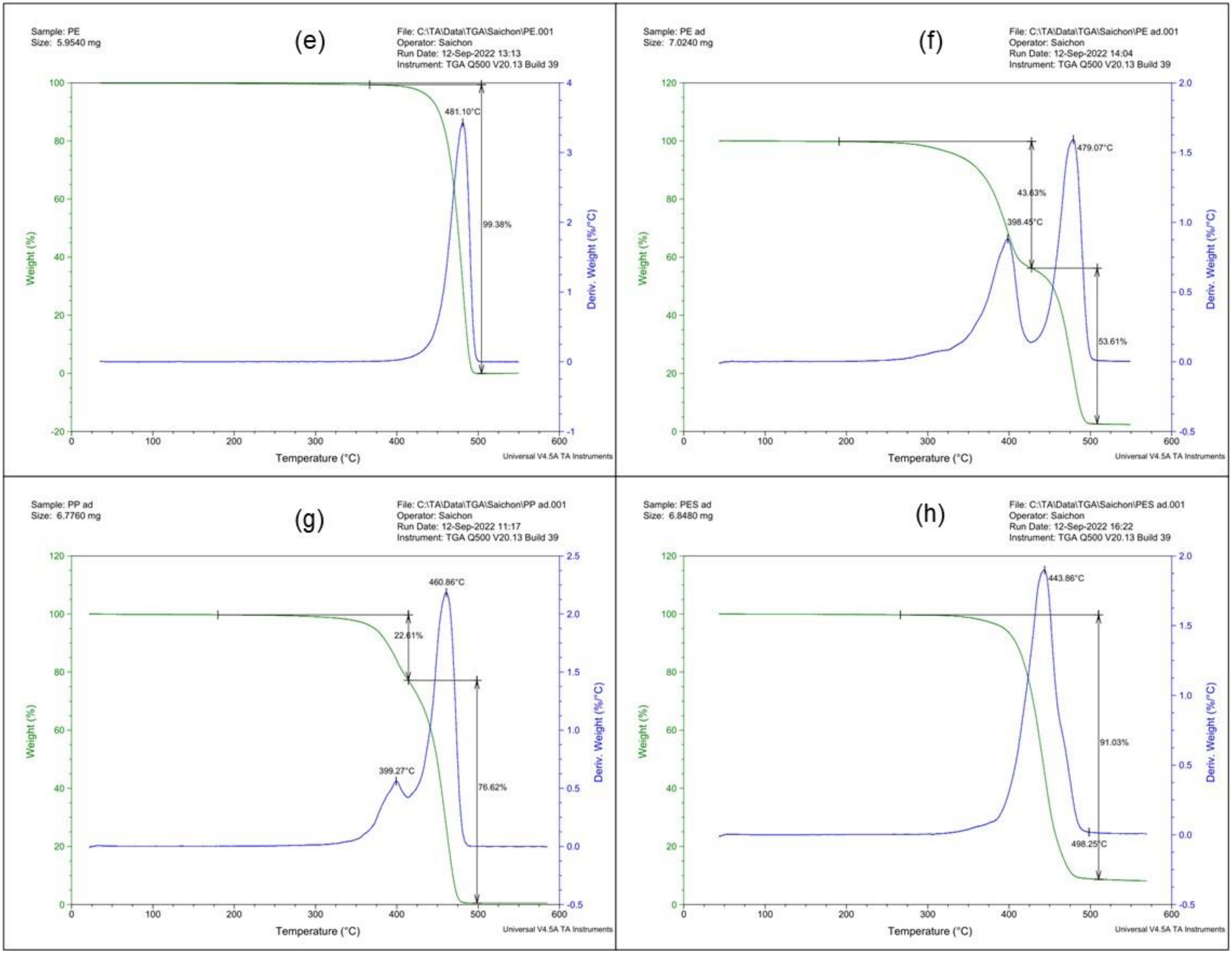
Thermogravimetric analysis of porous substrates and polymeric films

Table. S1 Summary of decomposition temperatures of porous substrates and polymeric films obtained from thermogravimetric analysis (TGA).

Materials	Thickness (mm)	Decomposition temperature, D_T (°C) [Mass loss (%wt)]		
		D_{T1}	D_{T2}	D_{T3}
Nitrocellulose (NC)	0.12	184.75 [92.68%]	-	-
Cellulose* (W1)	0.18	365.56 [87.99%]	-	-
Glass microfiber (GF/A)	0.26	>725 [2.218%]	-	-
Parafilm (PR)	0.12	292.82 [38.69%]	360.46 [53.77%]	403.09 [99.6%]
Polyethylene (PE)	0.13	481.10 [99.38%]	-	-
Polyethylene + adhesive (PE Ad)	0.13	398.45 [43.63%]	479.07 [97.24%]	-
Polypropylene + adhesive (PP Ad)	0.06	399.27 [22.61%]	460.86 [99.23%]	-
Polyester + adhesive (PES Ad)	0.07	443.86 [91.03]	-	-
Silicone + adhesive (Si Ad)	0.78	349.22 – 391.66 [20.68%]	549.19 – 599.22 [78.57%]	-



Sample: NC
Size: 3.4280 mg
(a)
File: C:\TA\Data\TGA\Saichon\NC.001
Operator: Saichon
Run Date: 12-Sep-2022 15:01
Instrument: TGA Q500 V20.13 Build 39
184.75°C
92.68%
Weight (%)
Deriv. Weight (%/°C)
Temperature (°C)
Universal V4.5A TA Instruments
Sample: W1
Size: 6.8440 mg
(b)
File: C:\TA\Data\TGA\Saichon\W1.001
Operator: Saichon
Run Date: 12-Sep-2022 10:04
Instrument: TGA Q500 V20.13 Build 39
230.61°C 95.92%
365.56°C
87.99%
454.28°C 7.922%
Weight (%)
Deriv. Weight (%/°C)
Temperature (°C)
Universal V4.5A TA Instruments
Sample: GFA
Size: 2.3980 mg
Comment: Copper pan
(c)
File: C:\TA\Data\TGA\Saichon\GFA.001
Operator: Saichon
Run Date: 13-Sep-2022 15:04
Instrument: TGA Q500 V20.13 Build 39
2.218%
Weight (%)
Deriv. Weight (%/°C)
Temperature (°C)
Universal V4.5A TA Instruments
Sample: PR
Size: 6.1610 mg
(d)
File: C:\TA\Data\TGA\Saichon\PR.001
Operator: Saichon
Run Date: 12-Sep-2022 12:19
Instrument: TGA Q500 V20.13 Build 39
403.09°C
38.69%
360.46°C
15.08%
292.82°C
45.83%
Weight (%)
Deriv. Weight (%/°C)
Temperature (°C)
Universal V4.5A TA Instruments


Sample: PE
Size: 5.9540 mg
(e)
File: C:\TA\Data\TGA\Saichon\PE.001
Operator: Saichon
Run Date: 12-Sep-2022 13:13
Instrument: TGA Q500 V20.13 Build 39
481.10°C
99.38%
Weight (%)
Deriv. Weight (%/°C)
Temperature (°C)
Universal V4.5A TA Instruments
Sample: PE ad
Size: 7.0240 mg
(f)
File: C:\TA\Data\TGA\Saichon\PE ad.001
Operator: Saichon
Run Date: 12-Sep-2022 14:04
Instrument: TGA Q500 V20.13 Build 39
479.07°C
43.63%
398.45°C
53.61%
Weight (%)
Deriv. Weight (%/°C)
Temperature (°C)
Universal V4.5A TA Instruments
Sample: PP ad
Size: 6.7760 mg
(g)
File: C:\TA\Data\TGA\Saichon\PP ad.001
Operator: Saichon
Run Date: 12-Sep-2022 11:17
Instrument: TGA Q500 V20.13 Build 39
460.86°C
22.61%
399.27°C
76.62%
Weight (%)
Deriv. Weight (%/°C)
Temperature (°C)
Universal V4.5A TA Instruments
Sample: PES ad
Size: 6.8480 mg
(h)
File: C:\TA\Data\TGA\Saichon\PES ad.001
Operator: Saichon
Run Date: 12-Sep-2022 16:22
Instrument: TGA Q500 V20.13 Build 39
443.86°C
91.03%
498.25°C
Weight (%)
Deriv. Weight (%/°C)
Temperature (°C)
Universal V4.5A TA Instruments

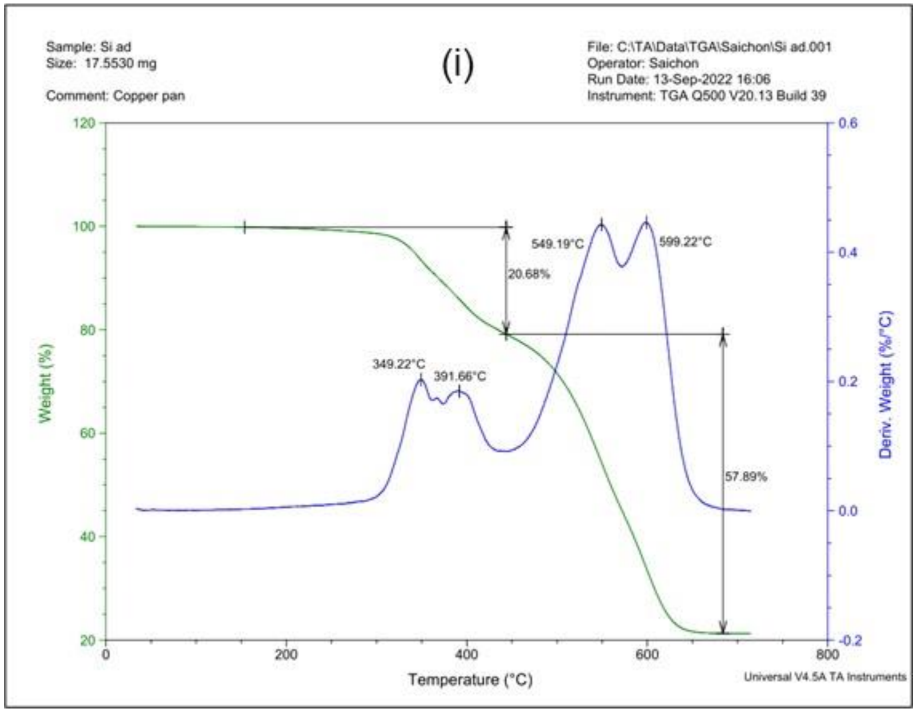


Fig. S2 Thermalgravimetric analysis of (a) Whatman 1, (b) Nitrocellulose, (c) Glass microfiber, (d) Parafilm, (e) Polyethylene, (f) Polyethylene + adhesive, (g) Polypropylene + adhesive, (h) Polyester + adhesive, and (i) Silicone + adhesive. The green curves represent the % weigh loss due to increasing of temperature. The blue curves represent derivative of the % weight loss with respect to temperature.

Combinations of porous substrate and polymeric film tested for fabrication of 2D enclosed devices

Dumbbell-shaped devices were made of the combinations of materials that applicable to selective laser ablation. Stitched composite photographs of the dumbbell-shaped devices are shown in the middle row. Top view and cross-section view microscope images are shown in the top row and bottom row, respectively. Square-shaped devices were made of the combinations of materials that inapplicable to selective laser ablation. Photographs of the square-shaped devices and cross-section view microscope images are shown in the top row and bottom row, respectively.

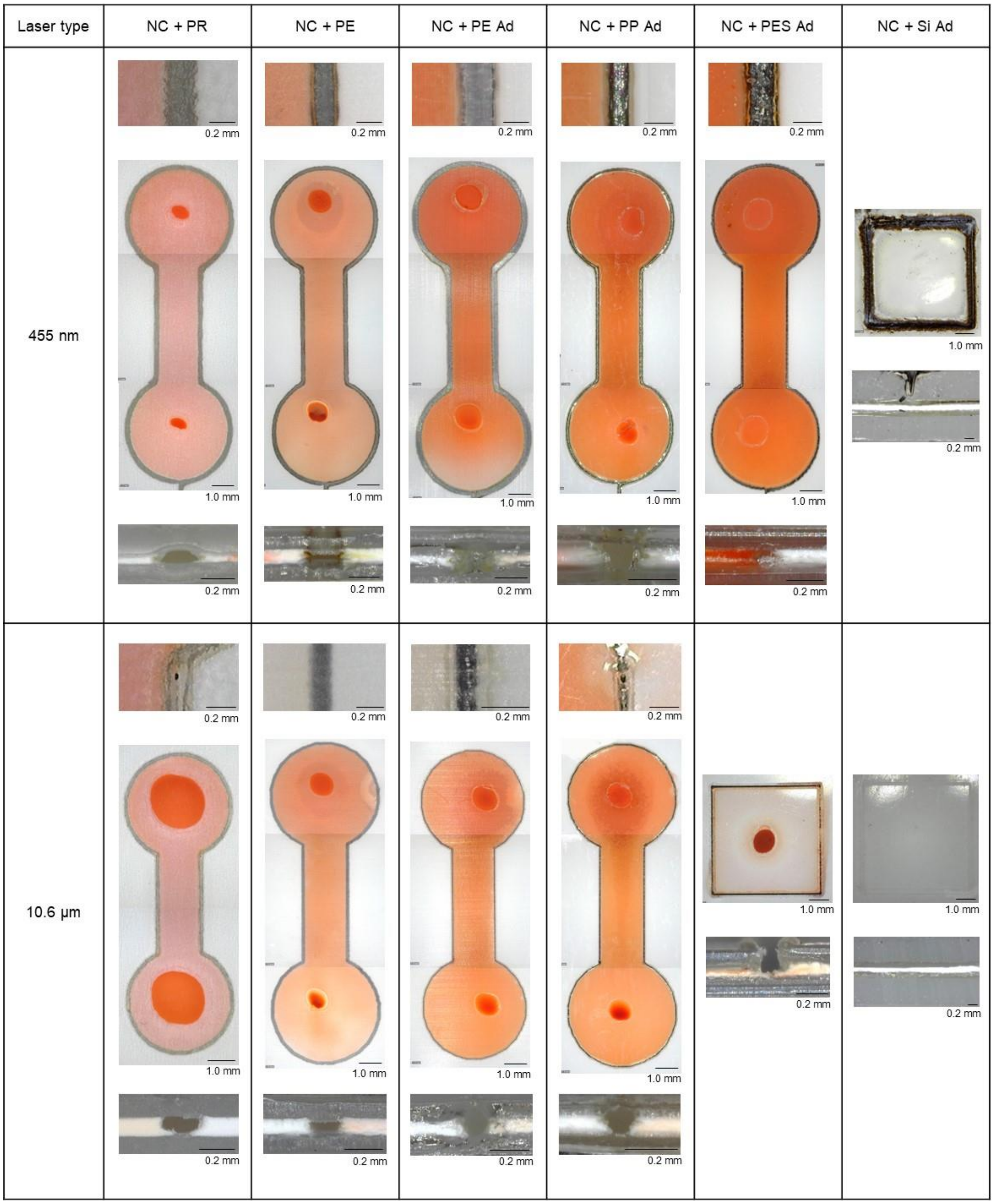


Fig. S3 Photographs of 2D enclosed devices made of nitrocellulose as a porous substrate and Parafilm, polyethylene, polyethylene + adhesive, polypropylene + adhesive, polyester + adhesive, or silicon + adhesive as a polymeric material. Minute cut-through holes on the top layer of the PR and PP Ad films when the devices were fabricated using a 10.6 µm laser were observed.

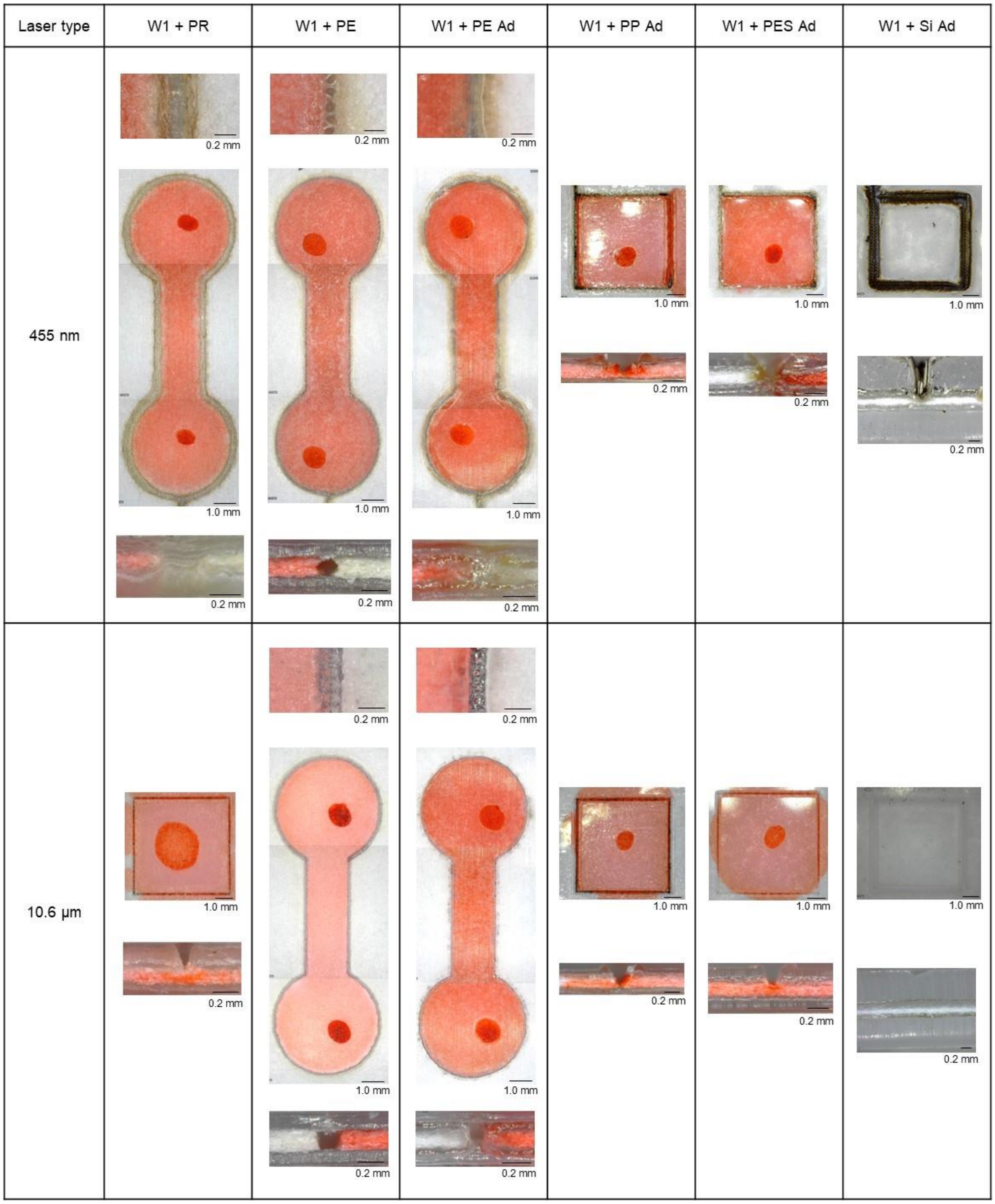


Fig. S4 Photographs of 2D enclosed devices made of Whatman 1 as a porous substrate and Parafilm, polyethylene, polyethylene + adhesive, polypropylene + adhesive, polyester + adhesive, or silicon + adhesive as a polymeric material.

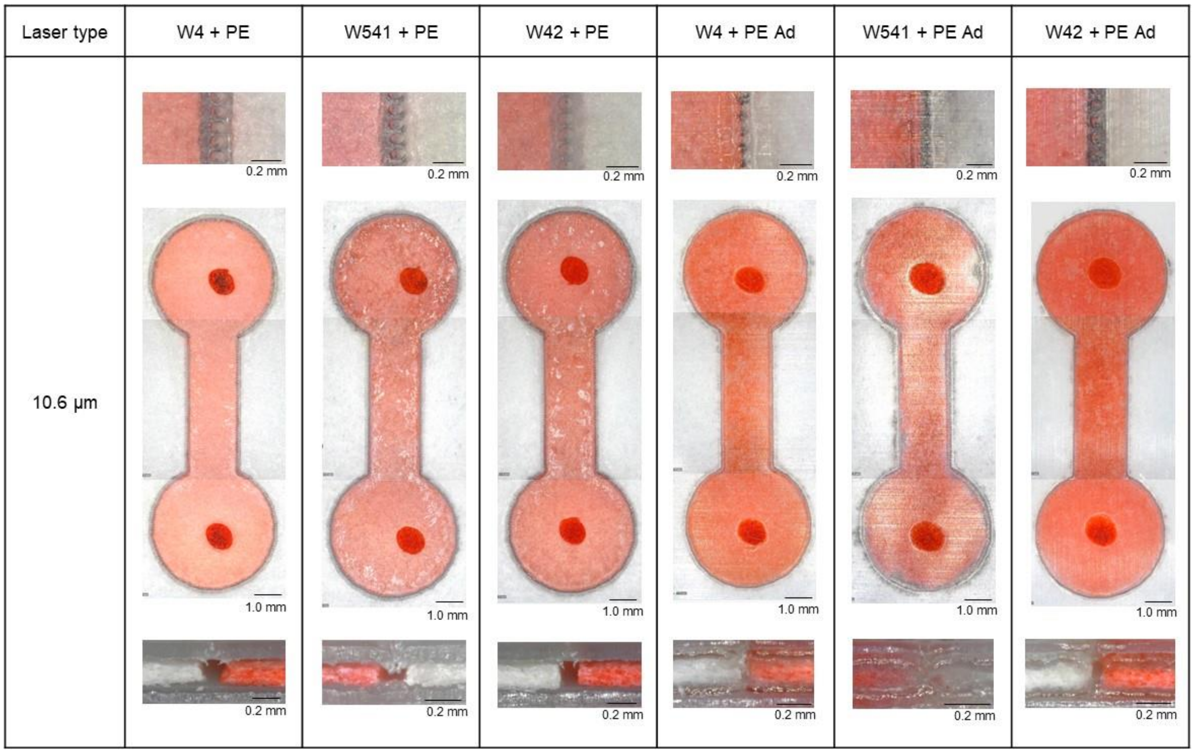


Fig. S5 Photographs of 2D enclosed devices made of Whatman 4, Whatman 541, or Whatman 42 as a porous substrate and polyethylene, or polyethylene + adhesive as a polymeric material.

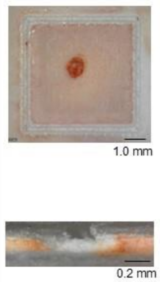
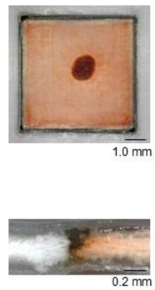
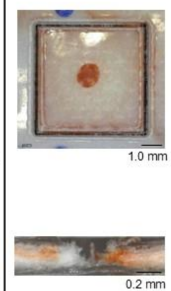
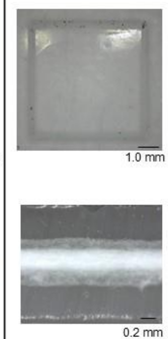
Laser type	GF/A + PR	GF/A + PE	GF/A + PE Ad	GF/A + PP Ad	GF/A + PES Ad	GF/A + Si Ad
10.6 µm						

Fig. S6 Photographs of 2D enclosed devices made of glass microfiber grade GF/A as a porous substrate and Parafilm, polyethylene, polyethylene + adhesive, polypropylene + adhesive, polyester + adhesive, or silicon + adhesive as a polymeric material.

Table. S2 Summary of material combinations, laser cutting parameters, % success rate, obtained channel and barrier widths of the 2D enclosed devices fabricated with 455 nm diode laser in Fig. S3 to Fig. S5.

No	Materials			Cutting parameters			Success rate (%)	Channel width (µm)	Barrier width (µm)
	Top	Middle	Bottom	Power (Watt)	Speed (mm/s)	Focal point (mm)			
1	Parafilm	Nitrocellulose	Parafilm	1.25	4.17	3	100 (n=13)	1835 ± 22	228 ± 20
2	PE	Nitrocellulose	PE	2.50	4.17	3	100 (n=14)	1789 ± 14	190 ± 12
3	PE Ad	Nitrocellulose	PE Ad	2.50	4.17	3	100 (n=14)	1852 ± 19	251 ± 27
4	PP Ad	Nitrocellulose	PP Ad	1.25	4.17	3	100 (n=15)	1920 ± 8	120 ± 15
5	PES Ad	Nitrocellulose	PES Ad	1.5	4.17	3	86 (n=14)	1975 ± 13	76 ± 8
6	Parafilm	Whatman 1	Parafilm	3.50	3.33	3	100 (n=12)	1835 ± 34	185 ± 29
7	PE	Whatman 1	PE	5.00	3.33	3	93 (n=15)	1882 ± 19	86 ± 19
8	PE Ad	Whatman 1	PE Ad	5.00	3.33	3	93 (n=15)	1941 ± 27	80 ± 18

Note: The % success rate was determined by a ratio of the number of fabricated devices without cut-through profile and leakage to the total number of fabricated devices multiplied by 100.

Table. S3 Summary of material combinations, laser cutting parameters, % success rate, obtained channel and barrier widths of the 2D enclosed devices fabricated with 10.6 µm CO_2 laser in Fig. S3 to Fig. S6.

No	Materials			Cutting parameters				Success rate (%)	Channel width (µm)	Barrier width (µm)
	Top	Middle	Bottom	Power (Watt)	Speed (mm/s)	Focal point (mm)	Pulses per inch			
1	Parafilm	Nitrocellulose	Parafilm	0.25	11.34	1.30	650	60 (n=15)	1896 ± 14	82 ± 11
2	PE	Nitrocellulose	PE	0.1	12.27	1.20	300	100 (n=25)	1862 ± 15	140 ± 9
3	PE Ad	Nitrocellulose	PE Ad	0.10	6.49	1.30	290	100 (n=25)	1843 ± 10	57 ± 6
4	PP Ad	Nitrocellulose	PP Ad	0.05	7.45	0.70	400	44 (n=25)	1913 ± 12	71 ± 11
5	PE	Whatman 541	PE	0.60	7.45	1.30	250	80 (n=25)	1806 ± 20	132 ± 14
6	PE Ad	Whatman 541	PE Ad	0.40	7.45	1.30	250	100 (n=20)	1962 ± 15	51 ± 7
7	PE	Whatman 1	PE	0.65	7.45	1.30	250	80 (n=25)	1790 ± 22	149 ± 11
8	PE Ad	Whatman 1	PE Ad	0.45	7.45	1.30	250	92 (n=25)	1909 ± 19	55 ± 6
9	PE	Whatman 42	PE	0.65	7.45	1.30	250	80 (n=25)	1790 ± 18	152 ± 8
10	PE Ad	Whatman 42	PE Ad	0.45	7.45	1.30	250	92 (n=25)	1929 ± 15	43 ± 7
11	PE	Whatman 4	PE	0.65	7.45	1.40	250	92 (n=25)	1792 ± 17	157 ± 10
12	PE Ad	Whatman 4	PE Ad	0.45	7.45	1.40	250	100 (n=25)	1918 ± 20	50 ± 8
13	PE	Glass microfiber	PE	0.85	9.86	1.40	500	92 (n=25)	1765 ± 21	179 ± 22

Note: The devices made of Parafilm and polypropylene + adhesive films resulted in 60 and 40 % success rates. This lower % success rate was a result of minute cut-through holes on the top layer of the films that were observed under a microscope in Fig. S3.

Channel resolution studies on 2D enclosed devices

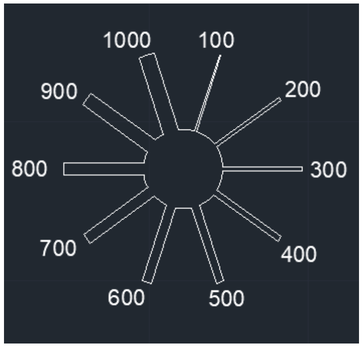


Fig. S7 A Design of a device used for channel resolution studies.

Channels were design with the channel widths ranging from 100 to 1000 µm.

Table. S4 Summary of channel resolution studies including material combinations, laser cutting parameters, and obtained channel widths of the 2D enclosed devices.

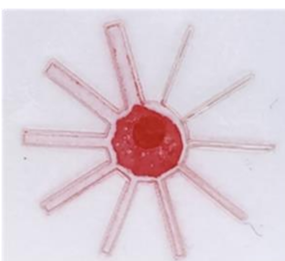
Photo	Combination	Laser parameters				Designed channel width (µm)	Obtained channel width (µm) average ± SD, n=3
		Power (Watts)	Speed (mm/s)	Focal point (mm)	PPI		
	PE GF/A PE 10.6 µm CO_2	0.85	9.86	1.4	500	1000	776 ± 7
						900	737 ± 4
						800	603 ± 14
						700	502 ± 6
						600	446 ± 40
						500	294 ± 36
						400	245 ± 16
						300	N/A
						200	N/A
						100	N/A

Table. S4 Summary of channel resolution studies including material combinations, laser cutting parameters, and obtained channel widths of the 2D enclosed devices (continued).

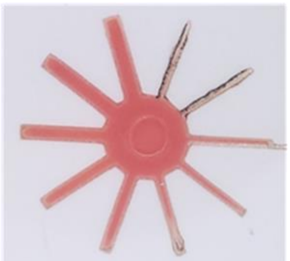
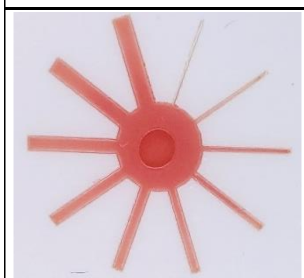
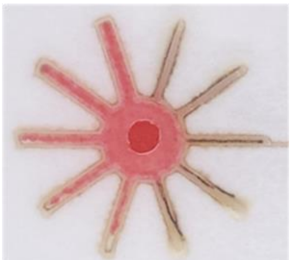
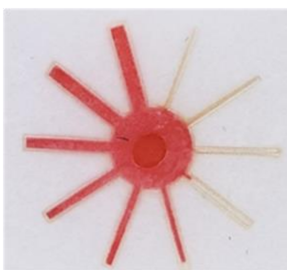
Photo	Combination	Laser parameters				Designed channel width (µm)	Obtained channel width (µm) average ± SD, n=3
		Power (Watts)	Speed (mm/s)	Focal point (mm)	PPI		
	PES Ad NC PES Ad 455 nm diode	1.5	4.17	3	N/A	1000	794 ± 31
						900	801 ± 3
						800	691 ± 12
						700	592 ± 11
						600	506 ± 8
						500	290 ± 22
						400	290 ± 3
						300	189 ± 38
						200	N/A
						100	N/A
	PE Ad NC PE Ad 10.6 µm CO_2	0.1	12.27	1.3	290	1000	918 ± 9
						900	875 ± 10
						800	750 ± 10
						700	620 ± 6
						600	551 ± 10
						500	427 ± 1
						400	367 ± 11
						300	234 ± 2
						200	146 ± 8
						100	N/A

Table. S4 Summary of channel resolution studies including material combinations, laser cutting parameters, and obtained channel widths of the 2D enclosed devices (continued).

Photo	Combination	Laser parameters				Designed channel width (µm)	Obtained channel width (µm) average ± SD, n=3
		Power (Watts)	Speed (mm/s)	Focal point (mm)	PPI		
	PR W1 PR 455 nm diode	3.75	4.17	3	N/A	1000	834 ± 23
						900	811 ± 42
						800	688 ± 35
						700	580 ± 30
						600	540 ± 25
						500	N/A
						400	N/A
						300	N/A
						200	N/A
						100	N/A
	PE W1 PE 10.6 µm CO_2	0.65	7.45	1.3	250	1000	787 ± 18
						900	714 ± 17
						800	590 ± 18
						700	470 ± 14
						600	360 ± 81
						500	310 ± 43
						400	N/A
						300	N/A
						200	N/A
						100	N/A

Combinations of porous substrate and polymeric film tested for fabrication of 3D enclosed devices

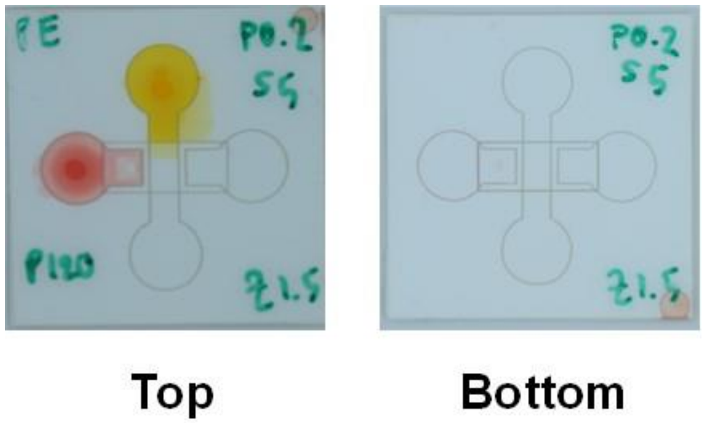


Fig. S8 Photographs of 3D enclosed devices made of the combination of nitrocellulose and polyethylene film after testing with dye solutions. Selective laser ablation was not applicable for this material combination resulting in ablation of top and bottom nitrocellulose layers.

Table. S5 Summary of material combinations and laser cutting parameters for fabrication of the 3D enclosed devices using 10.6 µm CO_2 laser.

No	Materials					Cutting parameters				Function
	1st Layer	2nd Layer	3rd Layer	4th Layer	5th Layer	Power (Watt)	Speed (mm/s)	Focal point (mm)	Pulses per inch	
1	PE	Nitrocellulose	PE	Nitrocellulose	PE	The 4th nitrocellulose layer was ablated while the 2nd nitrocellulose layer was ablating				
2	PE	Nitrocellulose	Parafilm	Nitrocellulose	PE	0.1	12.27	3.0	300	Passive 3D flow
3	PE Ad	Nitrocellulose	Parafilm	Nitrocellulose	PE Ad	0.1	6.49	1.6	290	Compression-activated 3D flow

Results of chemical compatibility of hollow barriers and materials study

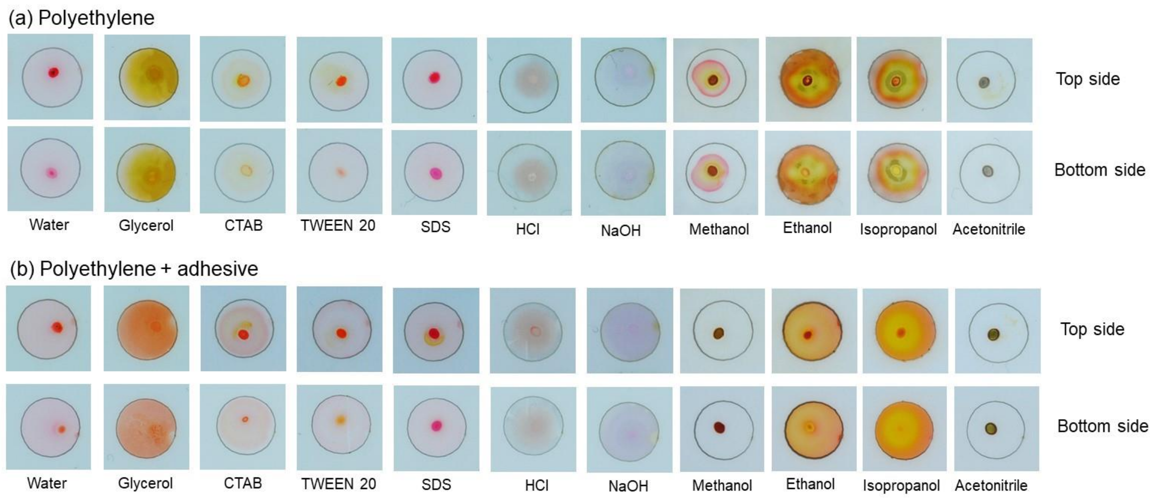


Fig. S9 Photographs show chemical compatibilities of the fabricated 2D enclosed devices with water, glycerol, surfactants (CTAB, TWEEN 20, SDS), acid, base, and common organic solvents. Nitrocellulose was used as a porous material. (a) Polyethylene and (b) Polyethylene + adhesive were used as a polymeric film.

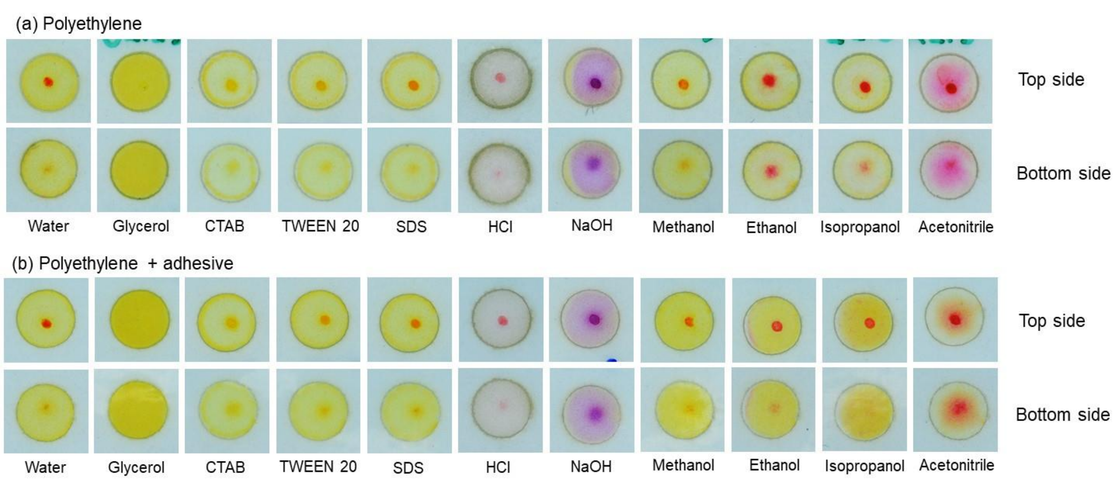


Fig. S10 Photographs show chemical compatibilities of the fabricated 2D enclosed devices with water, glycerol, surfactants (CTAB, TWEEN 20, SDS), acid, base, and common organic solvents. Whatman 4 was used as a porous material. (a) Polyethylene and (b) Polyethylene + adhesive were used as a polymeric film.

Determination of albumin, glucose, and total cholesterol

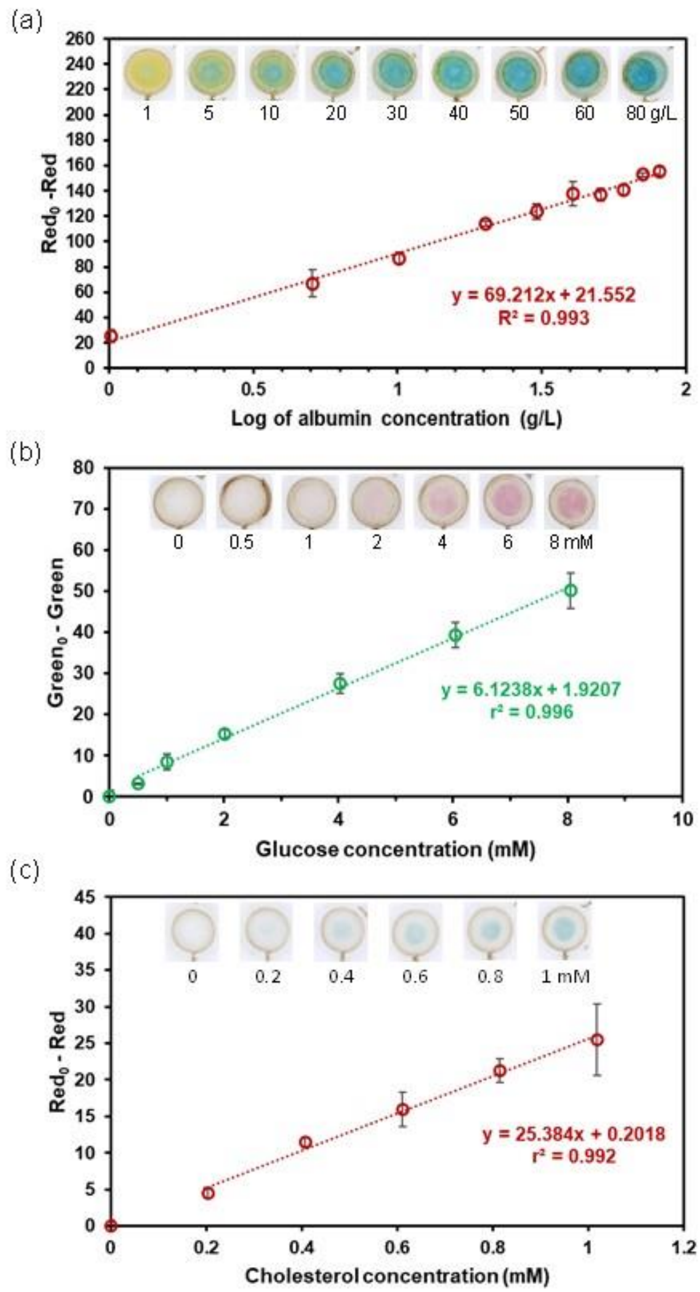


Fig. S11 Calibration curves with the insets of images at the detection zone of the 3D flow-through devices made of Whatman 1 and polyethylene for determination of (a) Albumin, (b) Glucose, and (c) Cholesterol (n=3).

Serum sample preparation for cholesterol assays

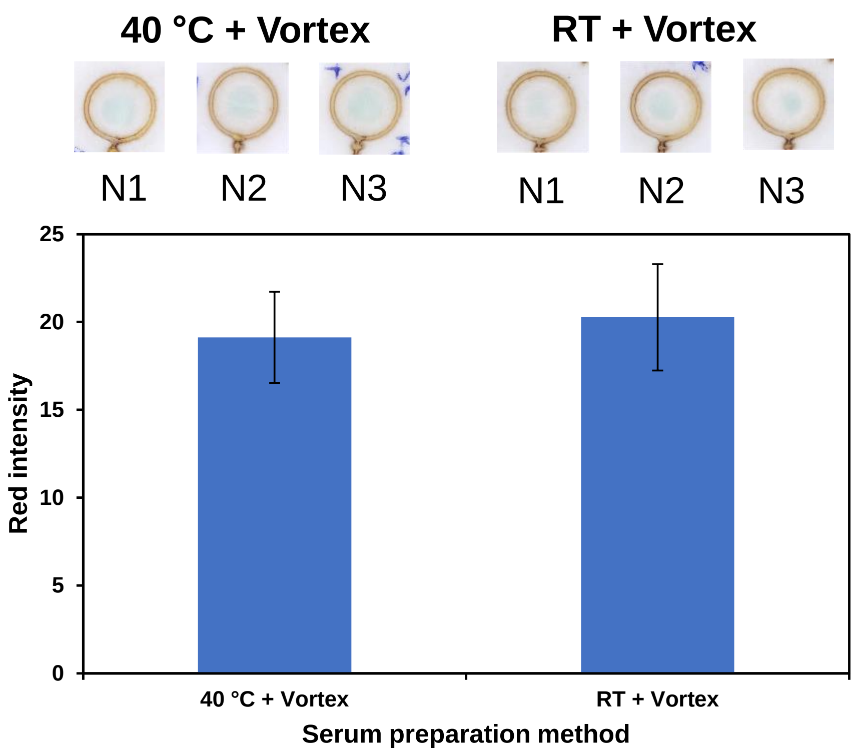


Fig. S12 Red color intensity obtained by different sample preparation methods.