Supplementary Information

High-throughput 3D microfluidic chip for generation of

concentration gradients and solution combinations

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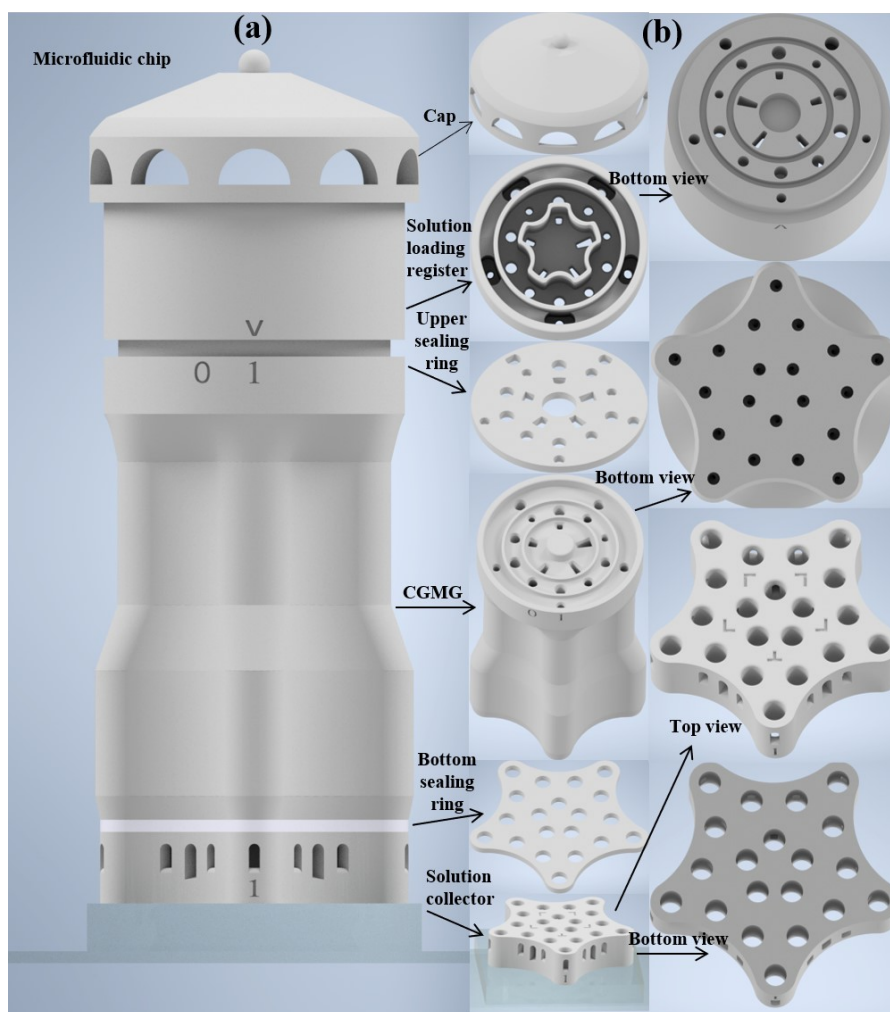
Supplementary Figures:

Fig. S1 Rendering of the external surface of the microfluidic device and its components. (a) 3D rendering of the surface of the microfluidic device. (b) Rendering of the outer surface of each component.
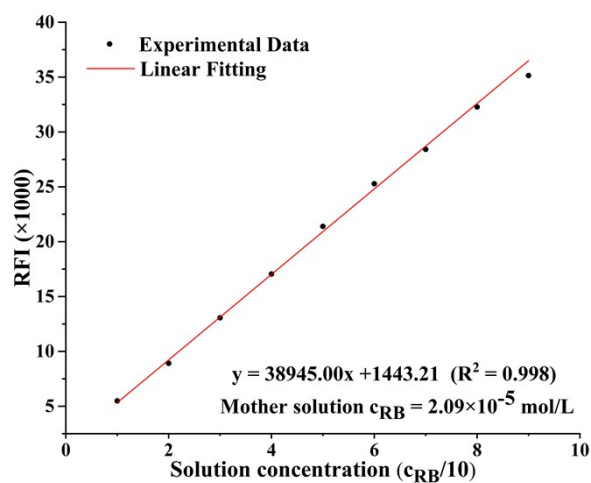

Fig. S2 Configure the linear fitting graph of the solubility value and the fluorescence intensity of the concentration gradient Rhodamine B fluorescent dye. (Where x represents the relative concentration of the Rhodamine B solution (in units of c_{RB}), and y denotes the fluorescence
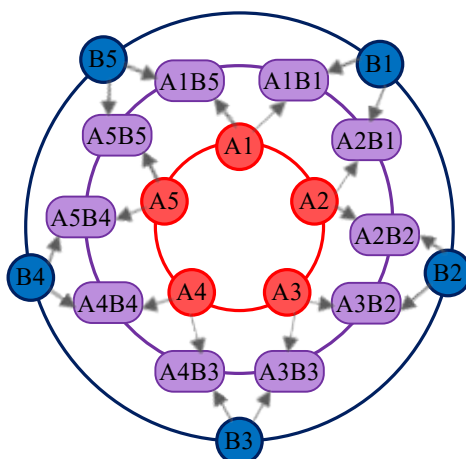
intensity.)

Fig. S3 Inefficient model of mixed structure of gradient solution.

Fig. S4 The chip verification completes the physical map.
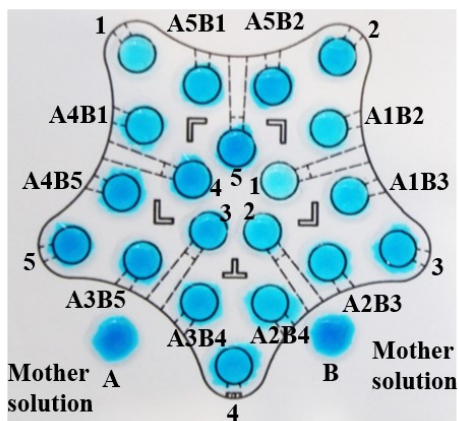
Fig. S5 Image of blue ink solution produced by the chip.
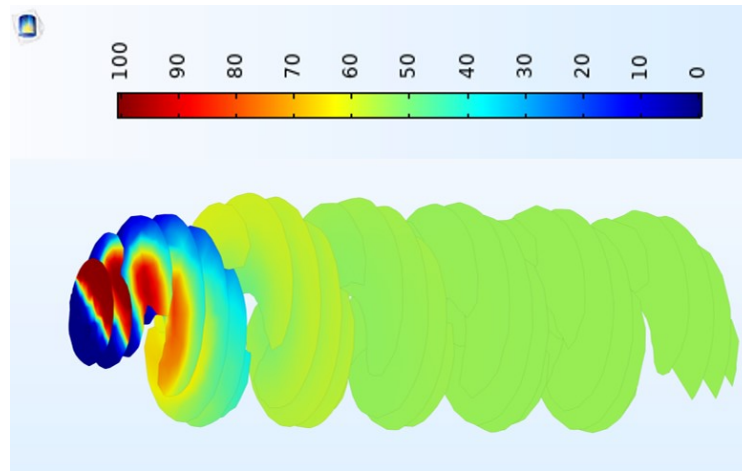
Fig. S6 The simulation of the fluid dynamics was performed for the spiral channels in the chip. A thermal color plot of the concentration distribution when the diffusion coefficient is 4.5 × 10^{-10} m^2/s.

Fig. S7 Single solution loading scheme: (a)(b) Concentration diagram of diluted solutions.

Supplementary Tables:

Table S1 Physical properties table of 3D printed biological application materials.

	Biological application	
Photosensitive resin	BIO	PEGDA
The tensile strength	42MPa	1MPa

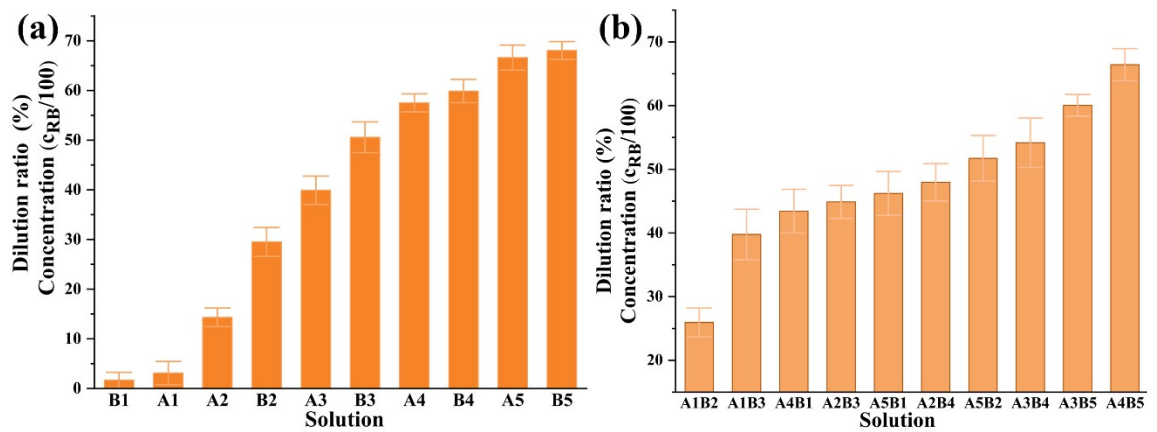| Modulus of elasticity	2.4GPa	35MPa
Elongation at break	2.5%	3.5%
The bending strength	65MPa	-
Bending modulus	2GPa	-
Thermal deformation temperature @0.45MPa	100℃	40℃
Hardness	80 Shore D	27 Shore D
The standard color	Translucent yellow	Translucent yellow, black
other	Biocompatibility	Biocompatible, low modulus materials

Table S2 Gradient solution mixing group table.

Solution combination				
A1B1	A1B2	A1B3	A1B4	A1B5
A2B1	*A2B2*	A2B3	A2B4	A2B5
A3B1	A3B2	*A3B3*	A3B4	A3B5
A4B1	A4B2	A4B3	*A4B4*	A4B5
A5B1	A5B2	A5B3	A5B4	*A5B5*

Table S3 The concentration of Am and Bn and the ratio of solution to volume.

Solution	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5
Concentration ($c_{RB}/100$)	33.4	42.5	60.1	85.7	96.9	31.9	40.1	49.4	70.5	98.3
$V_{r(/100)}$	33.4	42.5	60.1	85.7	96.9	31.9	40.1	49.4	70.5	98.3

Table S4 AmBn concentration and mixing volume ratio.

Solution	A1B4	A1B5	A2B5	A2B1	A3B1	A3B2	A4B2	A4B3	A5B3	A5B4
Concentration ($c_{RB}/100$)	48.28	53.79	56.59	33.57	39.97	45.83	52.05	55.13	60.23	74.06
$V_{r(A_m)}$	0.599	0.686	0.747	0.155	0.285	0.286	0.262	0.158	0.228	0.136
$V_{r(B_n)}$	0.402	0.314	0.253	0.846	0.715	0.714	0.738	0.842	0.772	0.865

Table S5 Solutions A and B were loaded into the inner and outer ring chambers respectively to generate solutions and their concentrations.

The solution produced by the chip										
Solution	**A1**	**A2**	**A3**	**A4**	**A5**	**B1**	**B2**	**B3**	**B4**	**B5**
Concentration (c_A or c_B/100)	33.4 ±2.5	42.5 ±1.8	60.1 ±2.9	85.7 ±1.9	96.9 ±2.3	31.9 ±1.8	40.1 ±2.4	49.4 ±3.1	70.5 ±2.9	98.3 ±1.6
Solution	**A1B4**	**A1B5**	**A2B5**	**A2B1**	**A3B1**	**A3B2**	**A4B2**	**A4B3**	**A5B3**	**A5B4**
A Concentration (c_A/100)	20.0 ±1.5	22.9 ±1.7	31.8 ±1.4	6.6 ±0.3	17.2 ±0.8	17.2 ±0.8	22.5 ±0.5	13.5 ±0.3	22.1 ±0.5	13.1 ±0.3
B Concentration (c_B/100)	28.3 ±1.2	30.9 ±0.5	24.8 ±0.4	27.0 ±1.5	22.8 ±1.3	28.6 ±1.7	29.6 ±1.7	41.6 ±2.6	38.2 ±2.4	60.9 ±2.5

Table S6 Solutions A and B were loaded into the outer and inner ring chambers respectively to generate solutions and their concentrations.

The solution produced by the chip										
Solution	**A1**	**A2**	**A3**	**A4**	**A5**	**B1**	**B2**	**B3**	**B4**	**B5**
Concentration (c_A or c_B/100)	31.9 ±1.8	40.1 ±2.4	49.4 ±3.1	70.5 ±2.9	98.3 ±1.6	33.4 ±2.5	42.5 ±1.8	60.1 ±2.9	85.7 ±1.9	96.9 ±2.3
Solution	**A4B1**	**A5B1**	**A5B2**	**A1B2**	**A1B3**	**A2B3**	**A2B4**	**A3B4**	**A3B5**	**A4B5**
A solution concentration (c_A/100)	28.3 ±1.2	30.9 ±0.5	24.8 ±0.4	27.0 ±1.5	22.8 ±1.3	28.6 ±1.7	29.6 ±1.7	41.6 ±2.6	38.2 ±2.4	60.9 ±2.5
B solution concentration (c_B/100)	20.0 ±1.5	22.8 ±1.7	31.8 ±1.4	6.6 ±0.3	17.1 ±0.8	17.2 ±0.8	22.5 ±0.5	13.5 ±0.3	22.1 ±0.5	13.1 ±0.3

Table S7 Injection of dilution solution into the outer and inner ring chambers, and mother liquor with a concentration of c_{RB} into the central ring chamber. There are the concentrations of the generated solutions.

The concentration of Am, Bn, and AmBn										
Solution	**A1**	**A2**	**A3**	**A4**	**A5**	**B1**	**B2**	**B3**	**B4**	**B5**
Concentration ($c_{RB}/100$)	3.1 ±2.3	14.3 1.9	39.9 ±2.9	57.5 ±1.8	66.6 ±2.5	1.7 ±1.6	29.5 ±2.9	50.6 ±3.1	59.9 ±2.4	68.1 ±1.8
Solution	**A1B2**	**A1B3**	**A2B3**	**A2B4**	**A3B4**	**A3B5**	**A4B5**	**A4B1**	**A5B1**	**A5B2**
Concentration ($c_{RB}/100$)	25.9 ±2.3	39.8 ±4.0	44.9 ±2.6	48.0 ±2.9	54.2 ±3.9	60.0 ±1.7	66.4 ±2.5	43.4 ±3.4	46.2 ±3.5	51.7 ±3.6

Injection of solution A and B into the outer and inner ring chambers respectively, and solution C into the central ring chamber:

Table S8 The concentration of ABC.

The solution of ABC										
Solution	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Concentration ($c_A/100$)	13.1	60.9	22.1	38.2	31.8	24.8	13.5	41.6	22.9	30.9
Concentration ($c_B/100$)	60.9	13.1	38.2	22.1	24.8	31.8	41.6	13.5	30.9	22.9
Concentration ($c_C/100$)	25.9	25.9	39.8	39.8	43.4	43.4	44.9	44.9	46.2	46.2
Solution	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Concentration ($c_A/100$)	22.5	29.6	20.0	28.3	17.2	28.6	17.1	22.8	6.6	27.0
Concentration ($c_B/100$)	29.6	22.5	28.3	20.0	28.6	17.2	22.8	17.1	27.0	6.6
Concentration ($c_C/100$)	48.0	48.0	51.7	51.7	54.2	54.2	60.0	60.0	66.4	66.4

Table S9 The concentration of AC and BC.

The solution of AC or BC

Solution	1	2	3	4	5	6	7	8	9	10
Concentration (c_A or c_B /100)	98.3	96.9	85.7	70.5	60.1	49.4	42.5	40.1	33.4	31.9
Concentration (c_B/100)	1.7	3.1	14.3	29.5	39.9	50.6	57.5	59.9	66.6	68.1