Supporting Information

An all-in-one microfluidic cryopreservation system and protocols with Gradually Increasing CPA Concentration

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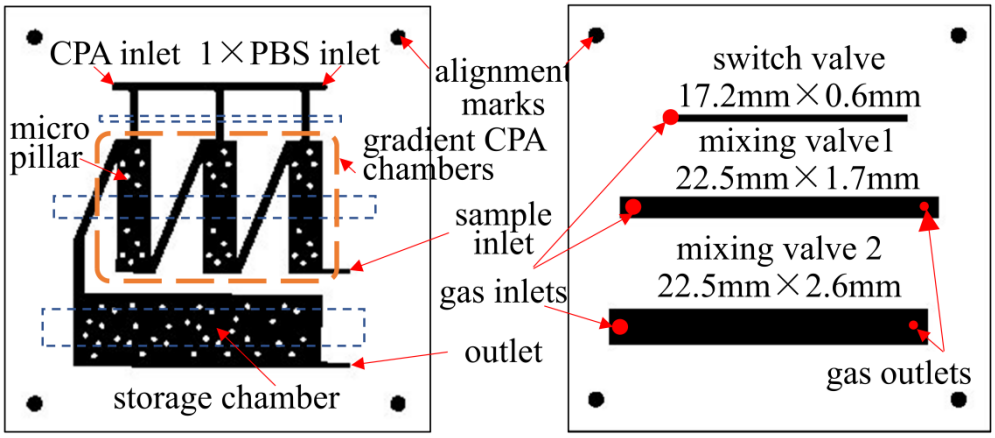

Fig. S1 Function and dimension diagrams of sample processing layer (left) and control layer (right).

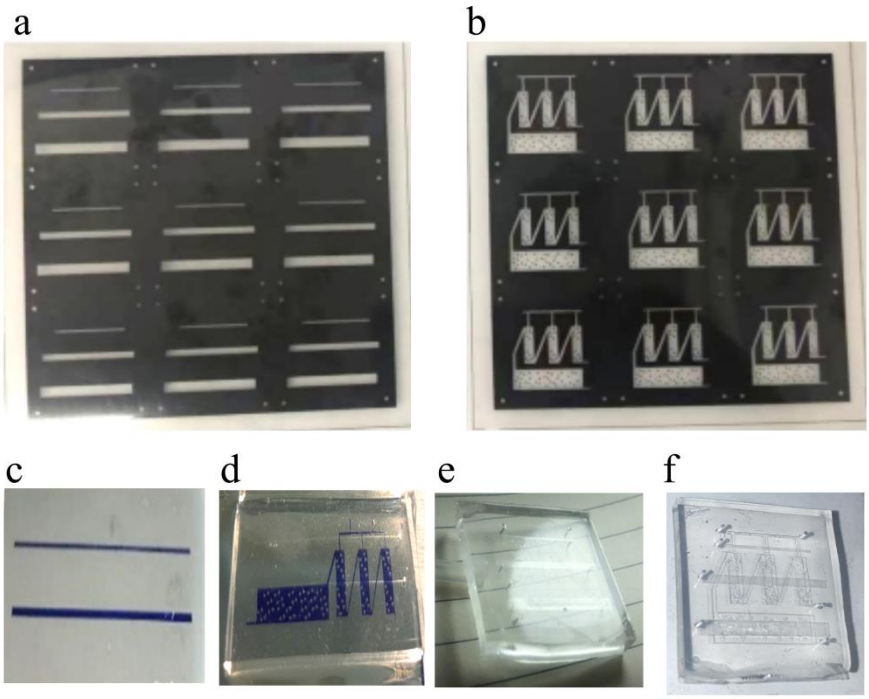

Fig. S2 Details of the integrated microfluidic chip fabrication procedures. Mask films of control layer (**a**) and sample processing layer (**b**) were ink-jet printed. The molds are made by photoresist dry film on stainless steel mirror plate (**c**). The control layer chip is bonded on the silica gel thin film coated on sample processing layer mold to avoid damage of the thin film while peeling off (**d**). The half-assembled chip with control layer and sample processing layer (**e**) is bonded to a glass slide substrate spin-coated with PDMS thin film (**f**).

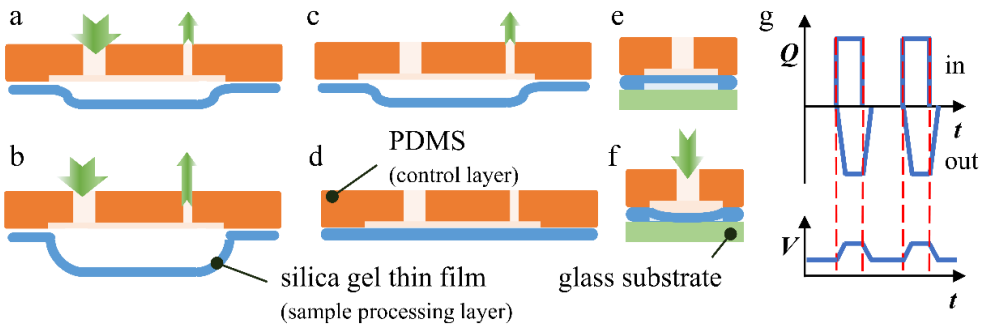

Fig. S3 Working principle of the micro mixing valve.

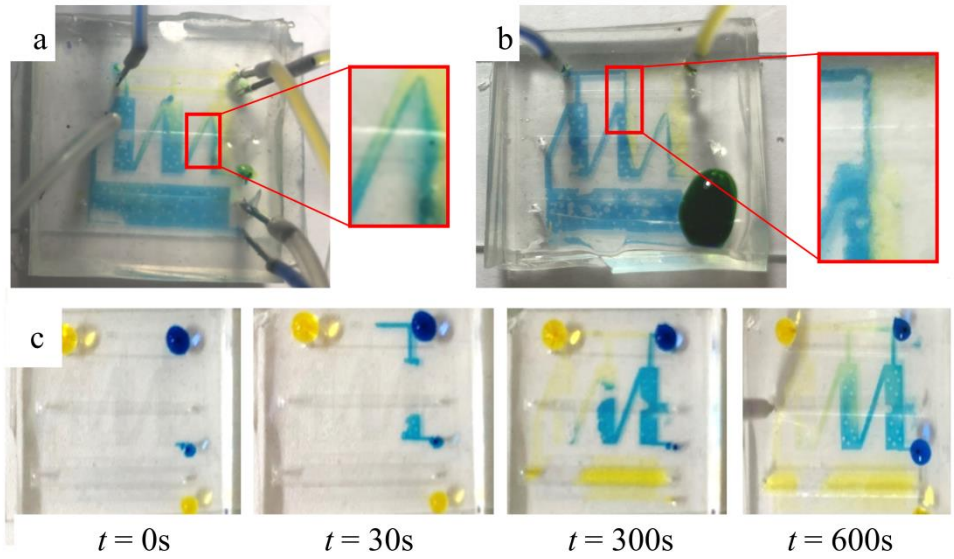

Fig. S4 Reagents loading processes. Unmixing phenomena in connecting channel. (**a**) and in chamber. (**b**) during fast loading with syringe pumps. (**c**) Self-loading process with pre-treated negative pressure.

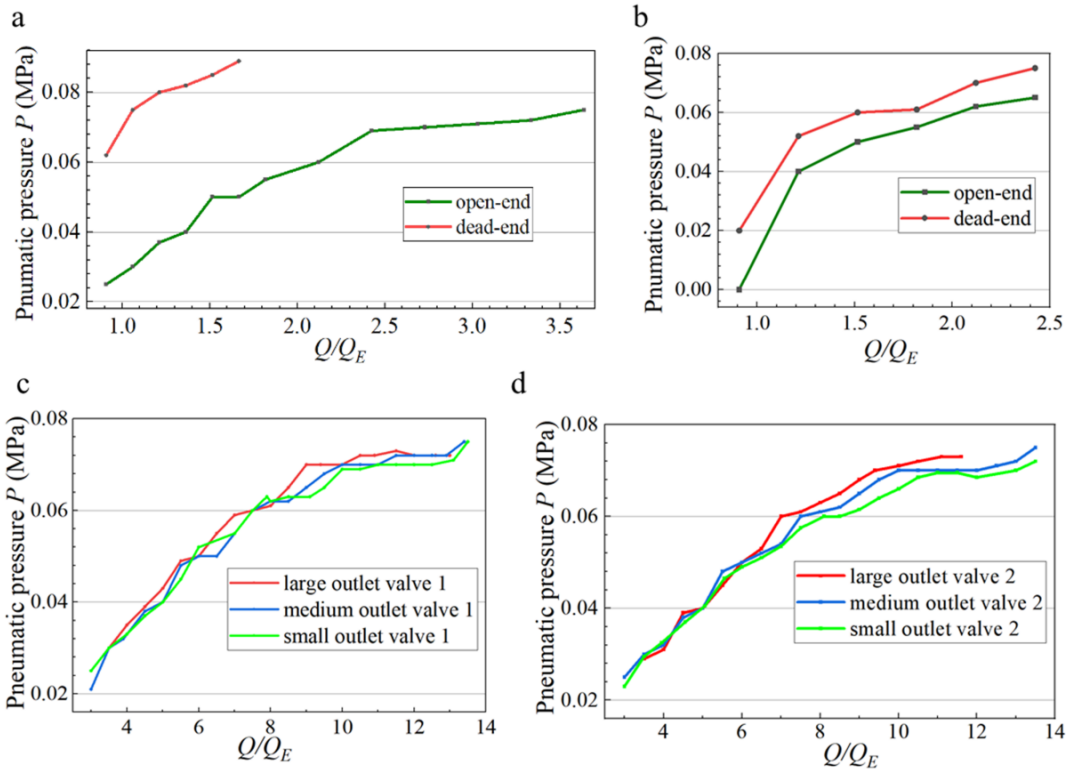

Fig. S5 Comparisons between microvalves with different outlets under increasing flow rates. (**a**) pressure changes of medium valve state with and without outlet orifice. (**b**) pressure changes of large valve state with and without outlet orifice. (**c**) pressure changes of medium valve with different outlet sizes. (**d**) pressure changes of large valve with different outlet sizes.

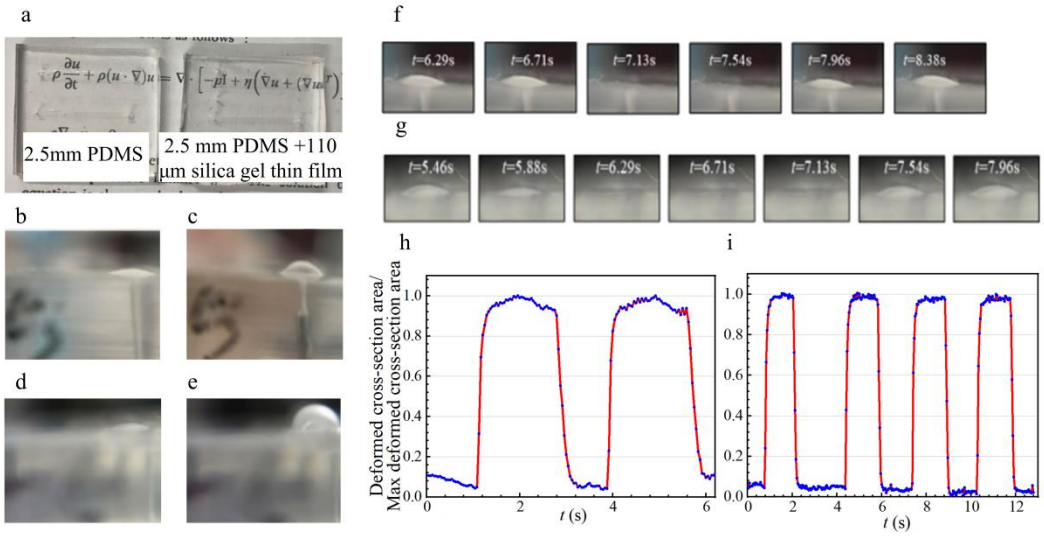

Fig. S6 Comparisons of material properties for the multi-layer chip. (**a**) Transparency comparison between PDMS chip (left) and silica gel thin film (110 μm) + PDMS chip(right). (**b**) Deformed PDMS mixing valve. (**c**) Deformed PDMS dead-end valve before broken down. (**d**) Deformed silica gel mixing valve. (**e**) Deformed silica gel dead-end valve before broken down. (**f**) Side-view of PDMS valve while switching pneumatic pump. (**g**) Side-view of silica gel valve while switching

pneumatic pump. (**h**) Dynamic reaction of PDMS valve while switching. (**i**) Dynamic reaction of silica gel valve while switching. Silica gel thin film sample processing layer (110 μm) + PDMS control layer chip has good elasticity with no obvious sacrifice of transparency and reaction time.

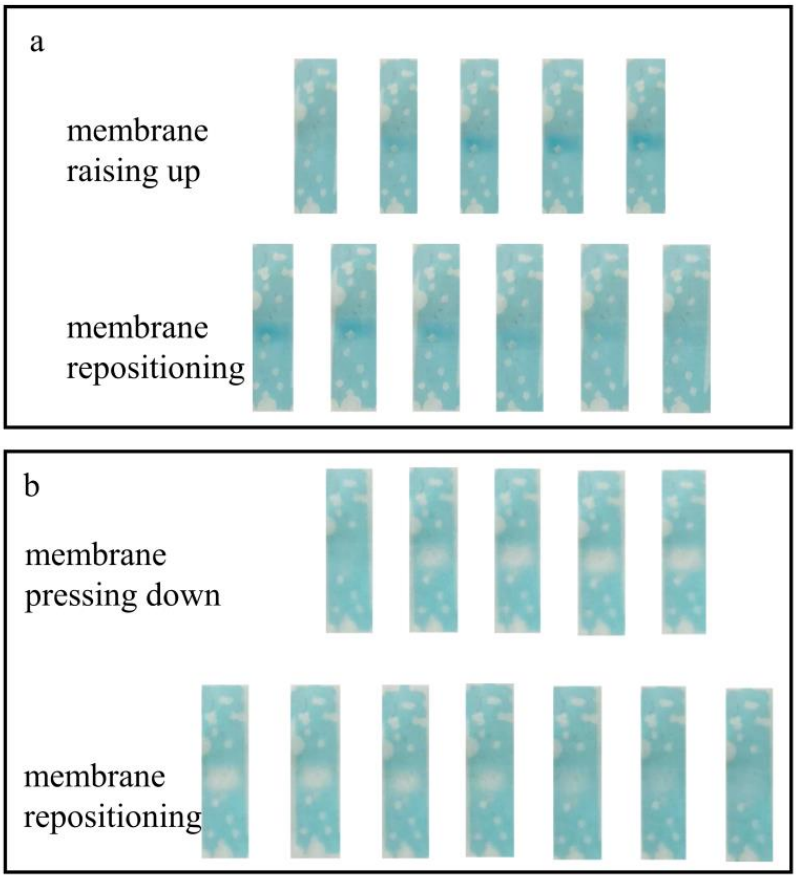

Fig. S7 Deformation processes of assembled and loaded chip. (**a**) Photos while medium elastic membrane is raising up and then repositioning. (**b**) Photos while medium elastic membrane is pressing down and then repositioning. Time gap = 0.083 s.

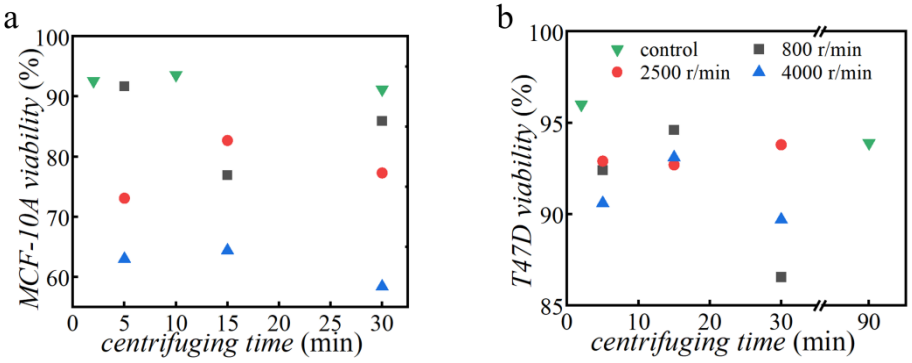

Fig. S8 Measured cell viability to centrifuge speed and centrifuge time at normal-scale. (**a**) MCF-10A cell viability after centrifuging. (**b**) T47D cell viability after centrifuging. Control group cells were not centrifuged; rotating at 800 r/min has 134g on the sample; 2500 r/min has 1307g; 4000 r/min has 3345g.

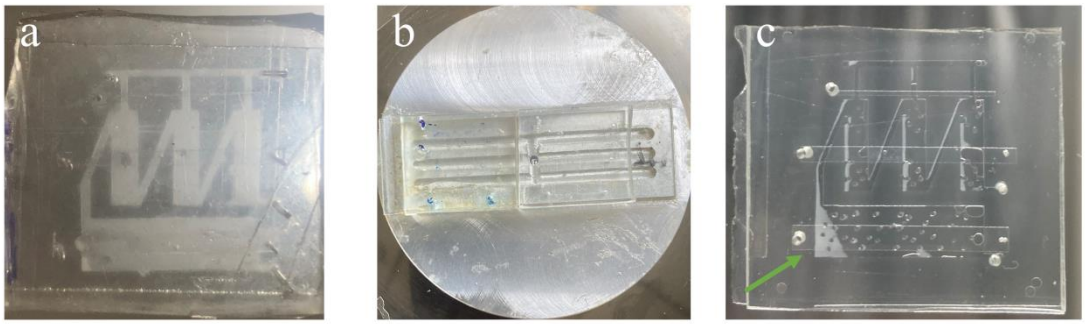

Fig. S9 Separating cell suspension by centrifuging. (**a**) Chip filled with cells suspension before centrifuging. (**b**) A glass slide bonded with 2 chips placed on a spinning plate. (**c**) Bottom-left part of the chip after centrifuging.