Supplementary Information

An on-chip microfluidic pressure regulator that facilitates reproducible loading of cells and hydrogels into microphysiological system platforms

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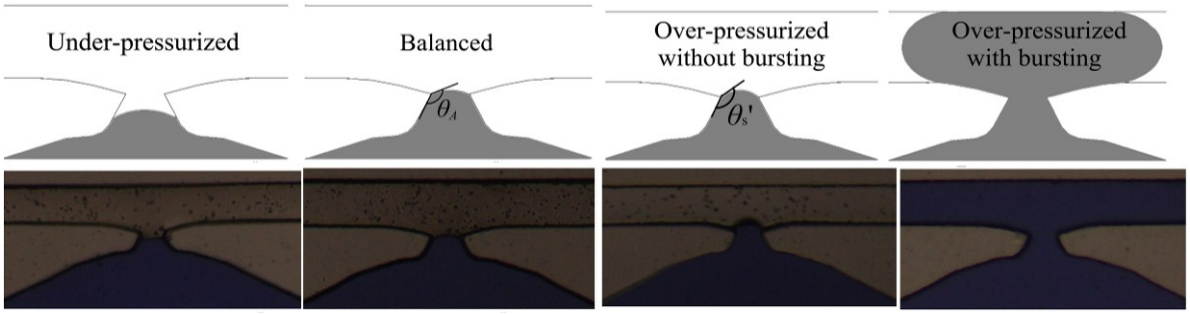

Fig. S1 Different gel loading condition based on the relative pressure values between applied gel injection pressure and Laplace pressure of gel-air interface at perfusion microvalve. If gel injection pressure is equal to the balanced pressure (P_b) with the critical advancing contact angle of θ_A between the interface and internal sidewall, the gel will be positioned at the vertices of perfusion microvalve with gently sloping interface (Balanced). However, when the applied gel injection pressure is not strong enough, the loaded gel will only fill a portion of perfusion microvalve (Under-pressurized). In contrast, when the gel injection pressure is over P_b but less than the burst pressure of perfusion microvalve $P_{valve\text{-}burst}$, the gel interface will bulge to the contact angle of θ_s' ($\theta_A < \theta_s' < \theta_A^*$) with the internal sidewall (Over-pressurized without bursting). Once the contact angle of gel interface with the internal sidewall is above the critical bursting contact angle θ_A^*, the gel will burst into the adjacent microfluidic channel (Over-pressurized with bursting).

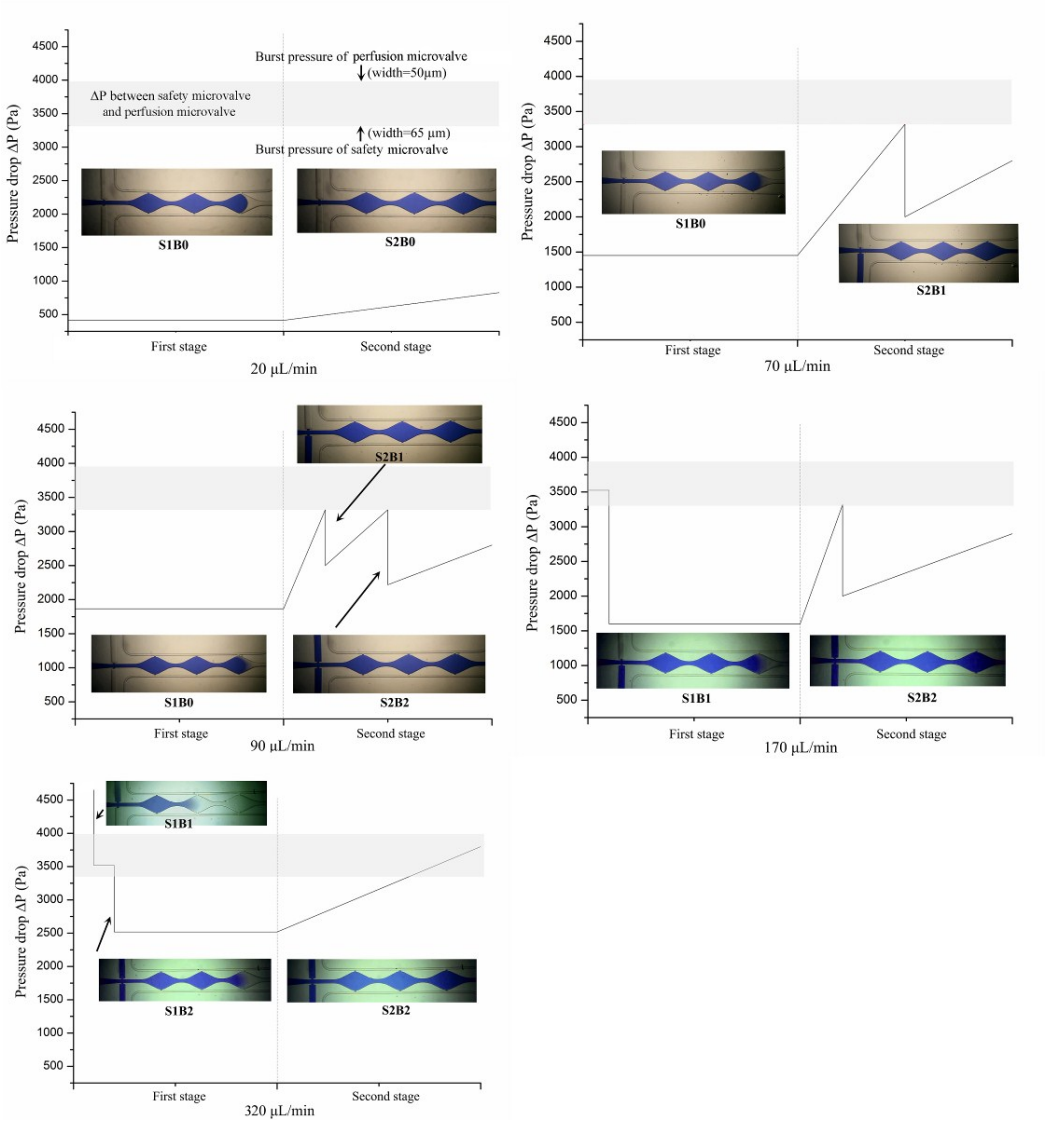

Fig. S2. Hydraulic pressure profile inside gel loading channel at different stages under different flow rates. (1) Due to the low flow rate of 20μL/min, the hydraulic pressure inside gel loading channel was much less than the burst pressure of safety microvalve in the pressure regulator module regardless of stage. Therefore, both safety microvalves would be intact at both the first stage (S1B0) and the second stage (S2B0). (2) During the first stage under the flow rate of 70μL/min, the hydraulic pressure inside gel loading channel was less than the burst pressure of safety microvalve. Therefore, there was no bursting occurred at this stage (S1B0). However, due to the increased fluidic resistance at the second stage, the hydraulic pressure inside gel loading channel would increase accordingly. Once it reached up to the burst pressure of safety microvalve, one would burst first to redirect redundant gel to release the build-up pressure (S2B1). Although certain amount of gels were directed to the diversion channel, a portion of gels would still flow towards the outlet reservoir of gel loading channel. The relative value of gel volume towards diversion channel and gel loading channel depended on the relative fluidic resistance values between diversion channel with safety valves and gel loading channel with certain height medium inside outlet reservoir, respectively. Since certain volume of gel flowed into the diversion channel, the flow rate inside the gel loading channel would reduce. Even though the total fluidic resistance including the gel loading channel and outlet reservoir still increased, the build-up rate of

hydraulic pressure inside gel loading channel would become slow without reaching the burst pressure of safety microvalve again. (3) When flow rate reached up to 90μL/min, two safety microvalves would burst one by one during the second stage. Firstly, one safety microvalve would burst to release the build-up hydraulic pressure (S2B1), just like the process at the flow rate of 70μL/min. Due to the increased flow rate from 70μL/min to 90μL/min, the build-up rate of hydraulic pressure inside gel loading channel became a little bit faster than that of 70μL/min. Therefore, the hydraulic pressure inside gel loading channel would increase to burst pressure of safety microvalve again, which would induce the bursting of the other one (S2B2) to release the build-up pressure. (4) If the flow rate of 170μL/min was applied, its initial hydraulic pressure was over the burst pressure of safety microvalve, which would induce the bursting of one safety microvalve at the first stage (S1B1). Therefore, the flow rate would decrease accordingly to decrease the hydraulic pressure inside gel loading channel. At the second stage, the other one would burst to release the pressure due to the increased fluidic resistance, just like the process of 70μL/min at the second stage. (5) If the applied flow rate was pretty high (e.g. 320μL/min), one pressure safety microvalve would burst first to release the initial hydraulic pressure during the first stage (S1B1). However, the hydraulic pressure inside gel loading channel after releasing was still higher than the burst pressure of safety microvalve, then the other one would burst immediately at the first stage (S1B2). At the second stage, the hydraulic pressure inside gel loading channel would increase continuously because both safety valves were invalid during the second stage. If the hydraulic pressure value inside gel loading channel was between the burst pressure of safety microvalve and burst pressure of perfusion microvalve, the gel interface pinned at the perfusion microvalve would bulge without bursting.

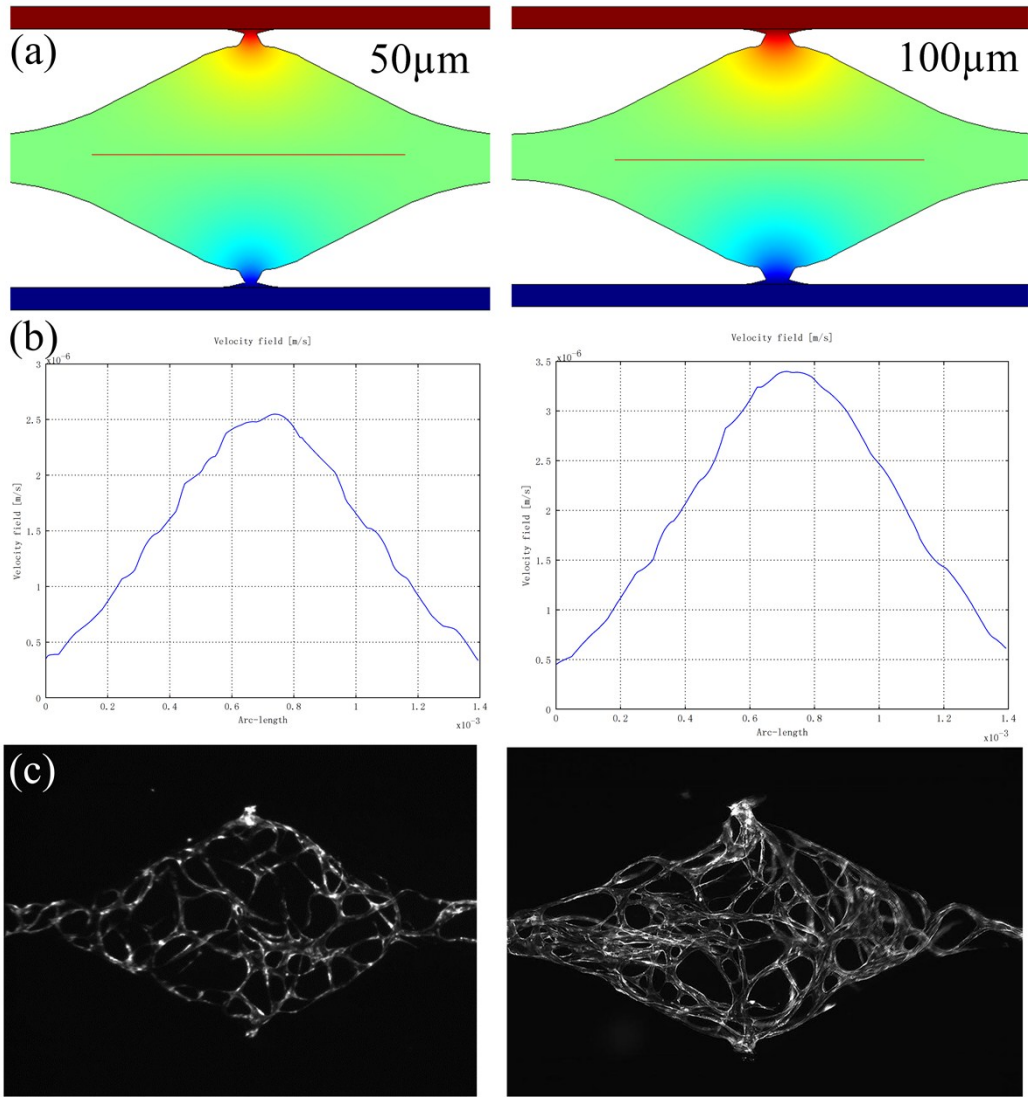

Fig. S3 Comparison results of perfusion microvalve with different widths (Left: 50μm VS Right: 100μm). (a) Simulation results on pressure distribution inside tissue chamber under the same hydrostatic pressure drop (5mm H_2O). (b) The corresponding interstitial flow velocity profile in the middle of tissue chamber horizontally. Since more bulk flow of culture medium transported across the tissue chamber with 100μm wide perfusion microvalve under the same hydrostatic pressure drop, its interstitial flow velocity was higher than that with 50μm width. (c) Vessel network formation inside tissue chamber. The anastomosed vessel at the 100μm perfusion microvalve was wider than that of 50μm perfusion microvalve, which was beneficial to particle/cell perfusion into vessel lumen.

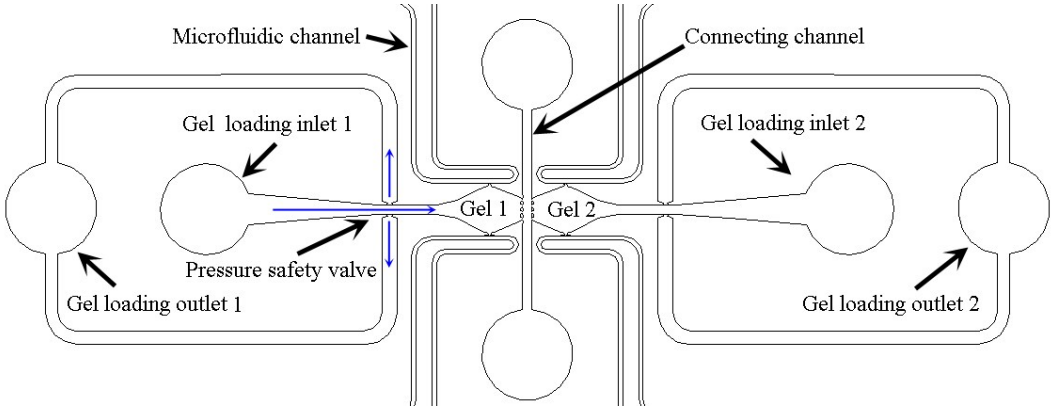

Fig. S4 Schematic of chip design for heterotypic hydrogel injection along gel loading direction by using the diversion outlet in the pressure regulator module as a gel loading outlet. Since the burst pressure of safety microvalve in pressure regulator is smaller than those of perfusion microvalves in tissue chamber, the pressure regulator will burst first to release the redundant gel as gel loading outlet. The central connecting channel can be injected with either gel to interconnect these two heterotypic gels for multi-tissue co-culture study or liquid (medium, drug, etc.) for drug screening application.

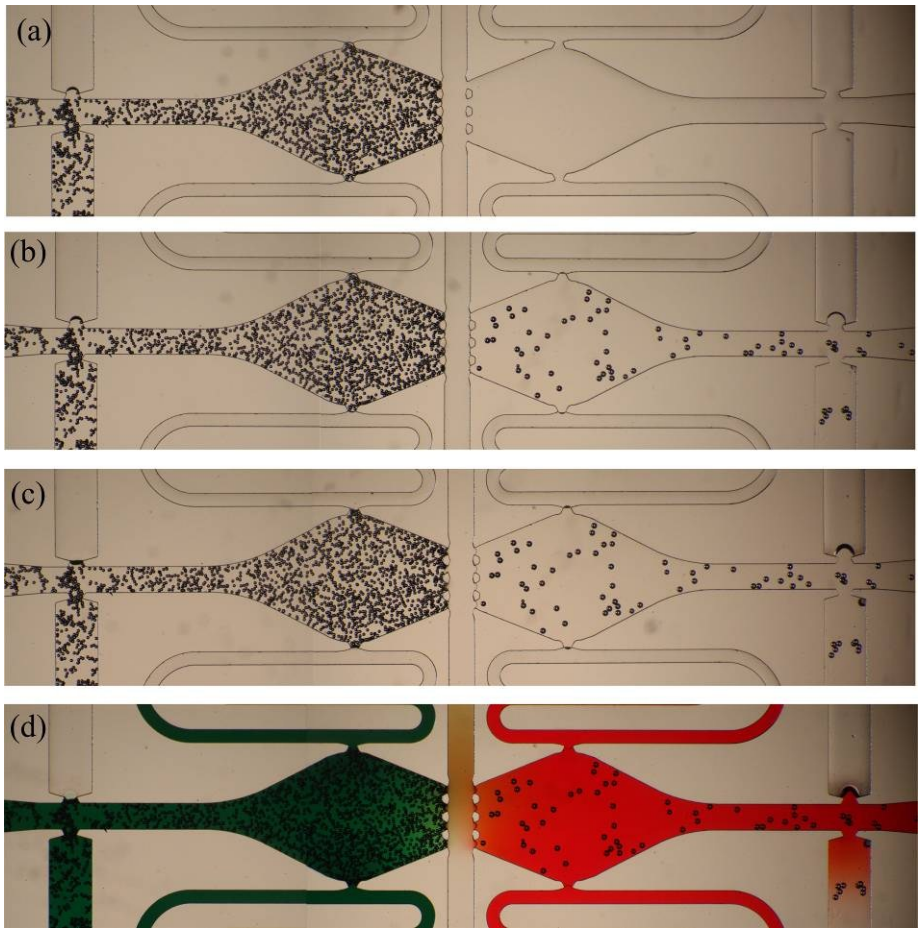

Fig. S5 Experimental result on gel loading into each tissue chamber with different particles and independent microenvironment control. (a) Loading left chamber with 25μm beads in fibrin gel. (b) Loading right chamber with 45μm beads in fibrin gel. (c) Loading central connecting channel with pure fibrin gel to interconnect these two chambers. (d) Loading microfluidic channel with food dye (green on the left, red on the right) independently and perfusion into their respective tissue chamber.

Supplementary movie 1: Two-stage performance of 65μm wide safety microvalve in the pressure regulator module by automatic dye-mixed gel loading with a syringe pump under different flow rates.

Supplementary movie 2: Comparison result of dye-mixed gel loading performance with 55μm and 85μm wide safety microvalves under different flow rates to characterize the sensitivity and working range of pressure regulator module with different safety microvalve width.

Supplementary movie 3: Gel confinement inside 100μm wide perfusion microvalve without bursting into microfluidic channel by using 130μm wide safety microvalve under different flow rates.

Supplementary movie 4: Performance of manual cell-seeded gel loading with a micropipettor under different pipetting speed.