Supplementary information

1. Fabrication process

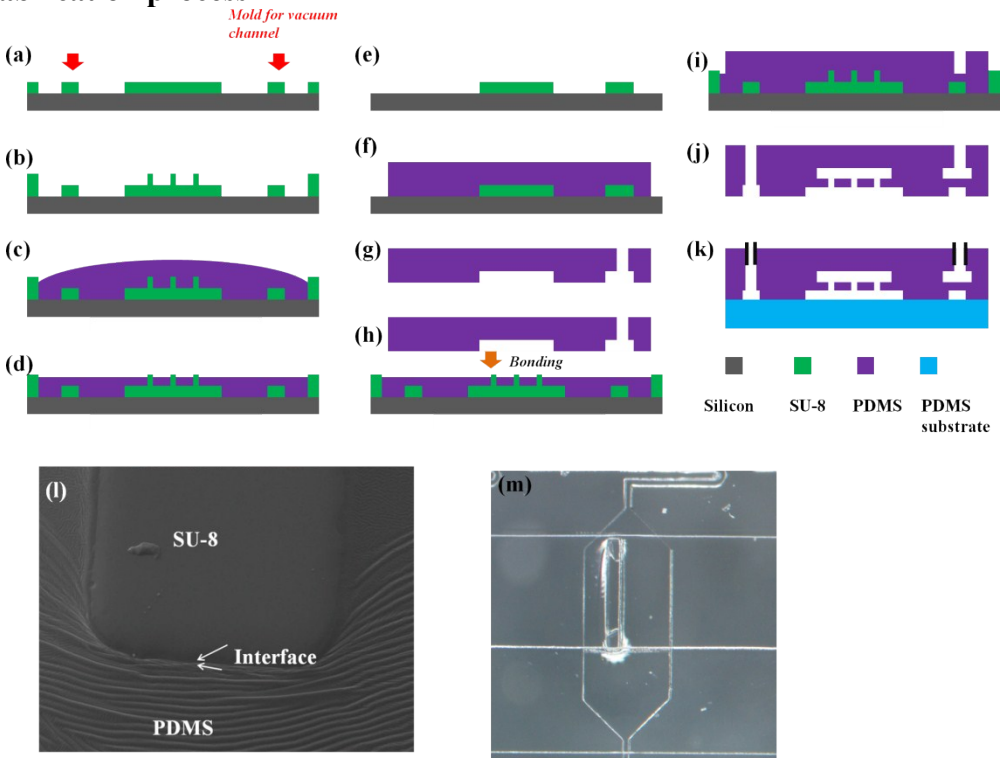

Fig. S1 (a) - (k)Fabrication process; (l) SEM image showing the top the injection port SU-8 mold not covered by PDMS; (m) Optical image showing the top layer with meandering channel aligned with the injection port and bonded onto the bottom layer.

The fabrication process is shown in Fig. S1(a). This device is formed by two PDMS layers. The PDMS layer for the U-shape channel and vertical injection port array is shown from Fig. S1(a) to S1(d). The PDMS layer for the meandering channel network is shown from Fig. S1(e) to S1(g). The bonding process to form the complete device is shown from Fig. S1(h) to S1(k). Firstly, a layer of SU-8 was patterned to form the mold of U-shape channel as shown in Fig. S1(a). In this layer, around the U-shape main channel, another channel network was designed as vacuum channel for applying vacuum to fix and seal the PDMS device onto the substrate.(can combine into last sentence?) Then a second layer was patterned based on the first layer to from the mold of vertical injection ports as shown in Fig. S1(b). After that, PDMS was poured onto the SU-8 mold as shown in Fig. S1(c) and spin coated as shown in Fig. S1(d). After spin coating, the thickness of the PDMS was slightly lower than the thickness of SU-8 layer. The tops of the mold of injection ports were not covered by PDMS due to the hydrophobic surface of SU-8 as shown in Fig. S1(l). After being baked at 95^{o} C for 90 mins, the first PDMS mold was prepared. The SU-8 mold of channel network was patterned in Fig. S1(e). Then PDMS was poured and baked to form the second PDMS layer in Fig. 1(f). The PDMS layer was released from the substrate as shown in Fig. S1(g). This PDMS layer was aligned and bonded onto the first PDMS layer as shown in Fig. 1(h) and S1(i). These two PDMS layers could be released from the substrate together as shown in Fig. S1(j) and fixed onto another PDMS layer by applying vacuum in the vacuum channel network as shown in Fig. S1(k).

2. Gradient profile of both accumulation method and dilution method with the

flow rate 500nl/min of inlet 1.

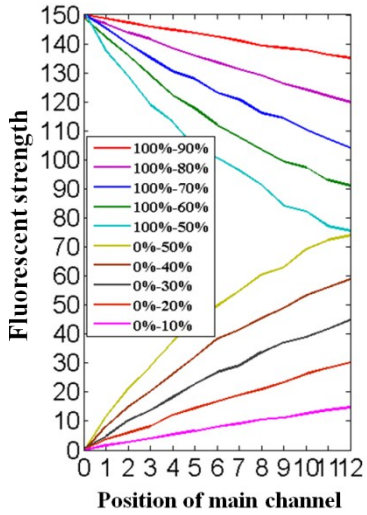

Fig. S2 Experiment results of gradient profile by accumulation method and dilution method.. The flow rate of inlet 1 is 500nl/min. Gradient profiles starting from 100% were by dilution method. Gradient profiles starting from 0% were by accumulation method.

The results by applying 500nl/min flow rate to inlet 1 are shown in Fig. S2. The results are similar with the results by applying 100nl/min flow rate to inlet 1. There is not an obvious relationship between the absolute value of flow rates and gradient profile. Only the ratio of the flow rates can affect the gradient profile.

3. Detailed process for cell culture and immobilization in U- shape main channel.

In the experiment, a PDMS substrate is required for sealing the channels of the device and culture the PC-9 cancer cells. To enable the PC-9 cancer cell culture on the PDMS substrate, the PDMS substrate was treated with fibronectin of a concentration of 50μg/ml for overnight inside the incubator. The device made of PDMS was attached onto the PDMS substrate. The vacuum channels were connected with a tube then the vacuum was applied through the vacuum channel. Due to the flexibility of PDMS substrate, the device could have a conformal contact with the PDMS substrate. By applying the vacuum, the device could be fixed onto the PDMS. All the fluidic channels were well sealed. Then culture media was injected from both inlet 1 and inlet 2 with a high flow rate to get rid of the air bubbles in the fluidic channels. These air bubbles could be extracted from outlet 2.

After removing all the air bubbles, suspension solution of PC-9 cancer cells stained with calcein-AM were introduced from inlet 1. During this process, the pure culture media was also injected from inlet 2 to prevent the PC-9 cancer cells from flowing into the meandering channels through injection ports. After the main channel being filled with PC-9 cancer cells, reduce the flow rate of inlet 1 to 10nl/min for 4 hours. The PC-9 cancer cells were seeded onto the PDMS after that. Then doxycycline solution and culture media were introduced from inlet 1 and inlet 2 to conduct the experiments.

4. Viability test of PC-9 cancer cells of the gradient from 0% to 90% generated by

accumulation method

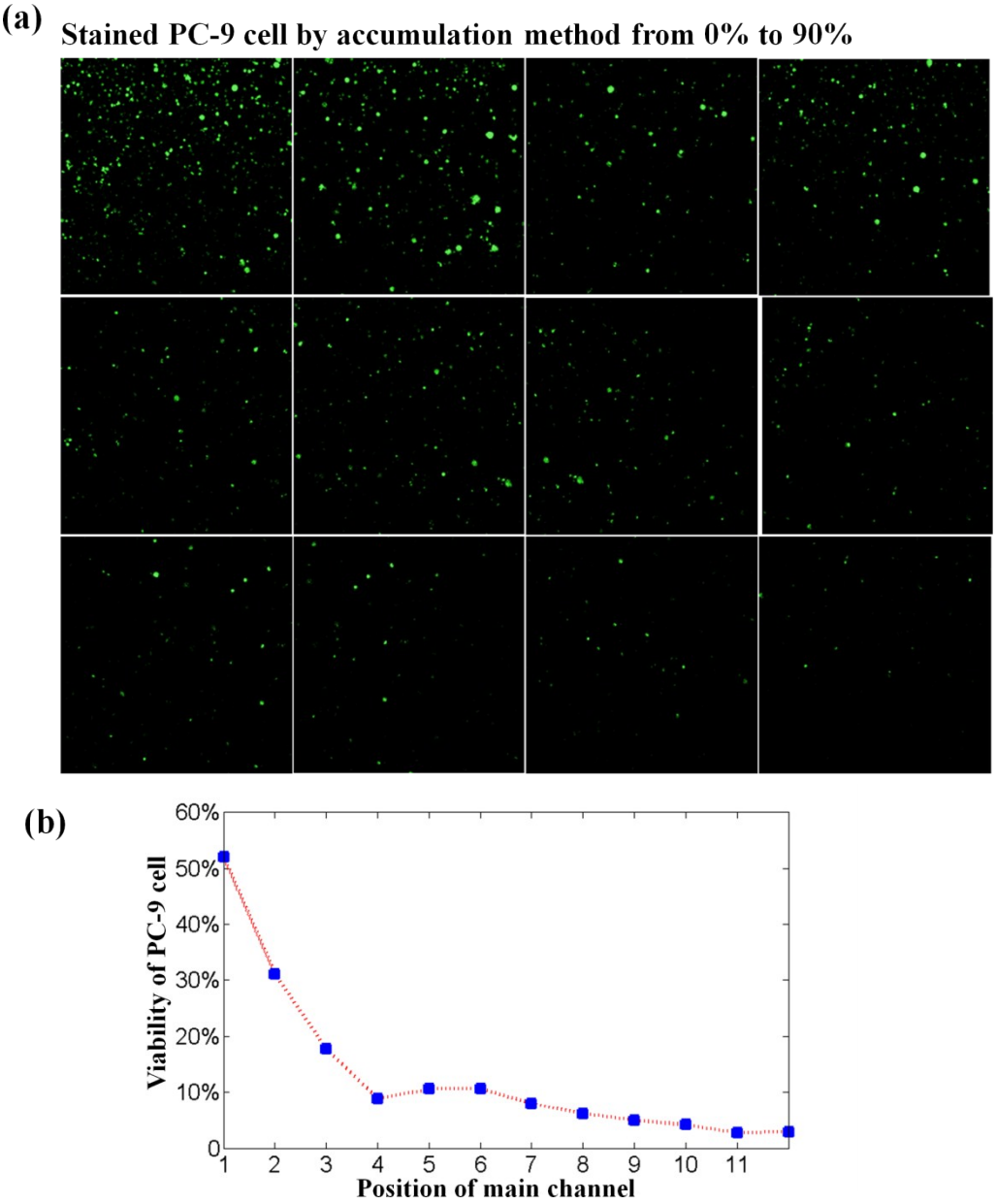

Fig. S3 Experiment of PC-9 cell viability test. (a) Calcein-AM stained PC-9 cancer cells from concentration of 0% to 90% by accumulation method; (b) Viability of PC-9 cancer cells along the main channel.

Fig .S3 shows the viability test results by the gradient from 0% to 90% generated by accumulation method. Due to the nonlinear profile, the concentration of doxycycline was about 40% just after the first injection port and soon saturated to 90% after the fourth injection port. Hence the viability of PC-9 cancer cell soon declined to 10% after the fourth injection port. The viability of PC-9 cancer cells at the concentration lower than 40% cannot be investigated in the gradient generated by this method.