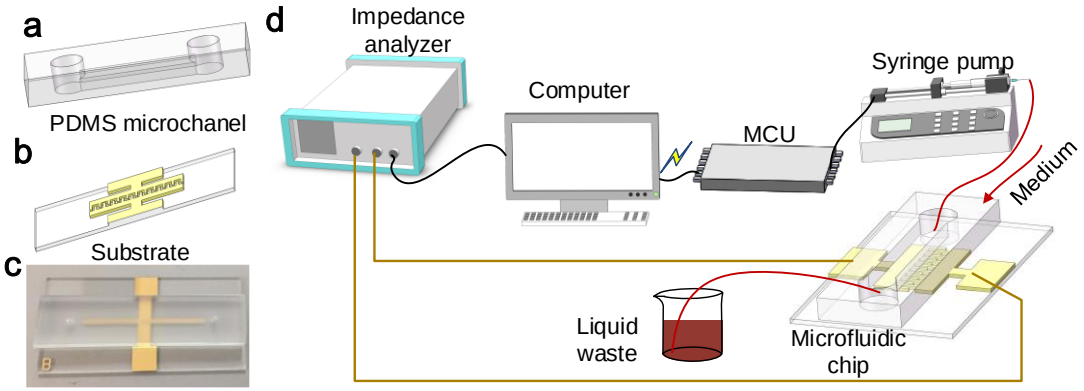

FIGURE. 1. The process of the cell culture assay. (**a**) The PDMS slab of the designed microfluidic chip. (**b**) The substrate of the microfluidic chip. The substrate is sputtered by the interdigital electrode structures. (**c**) The image of designed microfluidic chip. It is composed of PDMS micro-channel and substrate (**d**) The experiment setup of the cell culture incorporated with impedance measurement. Springe pump is connected with MCU to control the flow rate.

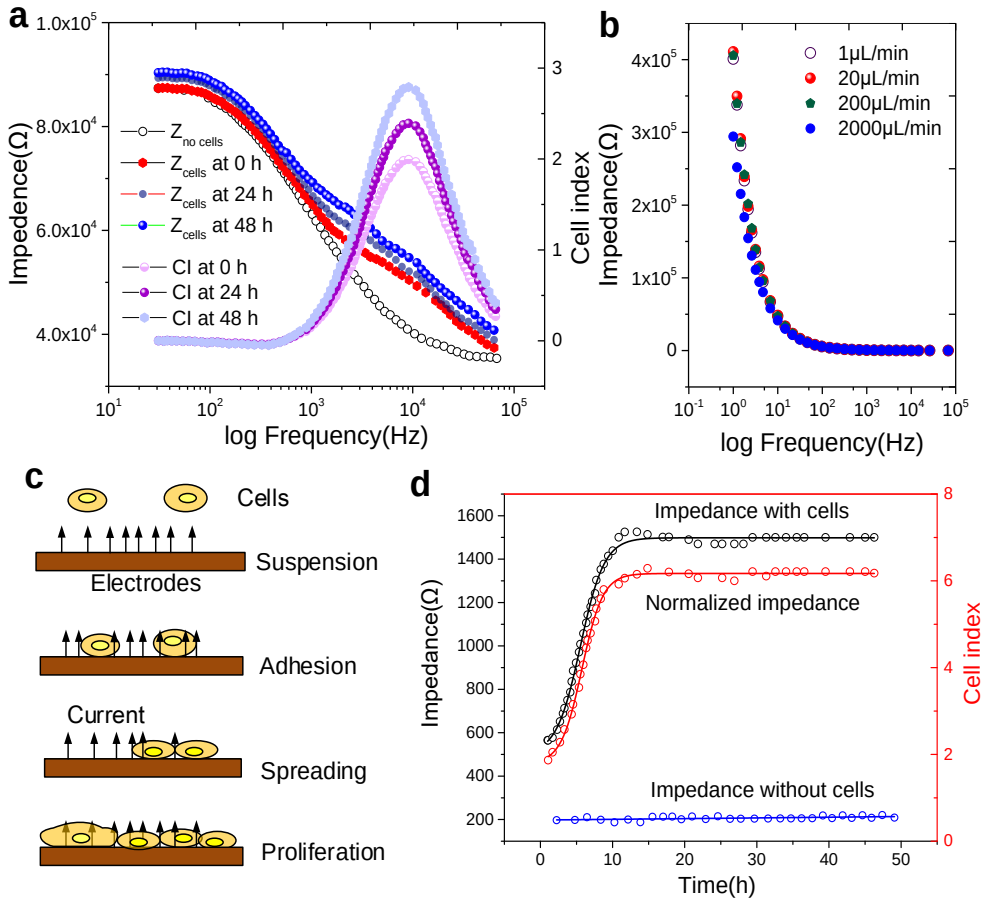

FIGURE. 2. The relationship between impedance and cell proliferation (**a**) The sensitive frequency decided by the impedance spectroscopy. (**b**) The influence of flow rate on impedance without cells. (**c**) The schematic of cell growth on the electrodes. Cells grow from suspension to adhere, and then they are proliferated. (**d**) The impedance measured at 10 kHz by the impedance analyzer. The control group is $Z_{cell\text{-}free}$ meaning impedance without cells. Cell index is described as the normalized impedance to accurate calculation and eliminates the effect during the cell

culture.

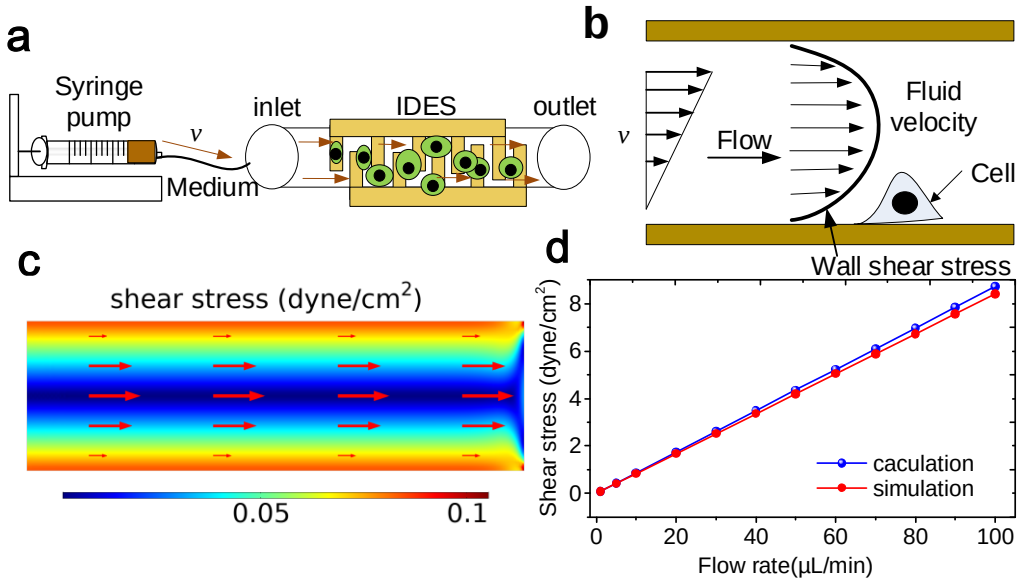

FIGURE. 3. The effect of flow rate on the shear stress. (**a**) The schematic of the shear stress of the adherent cells in medium flow. (**b**) Schematic diagram of wall shear stress. The flow velocity in the bottom of the chip causes the wall shear stress on the cells. (**c**) Shear stress is simulated by giving different flow rate of perfusion using COMSOL Multiphysics 5.2. The arrows denote the flow velocity in the micro-channel. (**d**) The relationship between flow rate and wall shear stress calculated by the Equation. (6) and the COMSOL simulation.

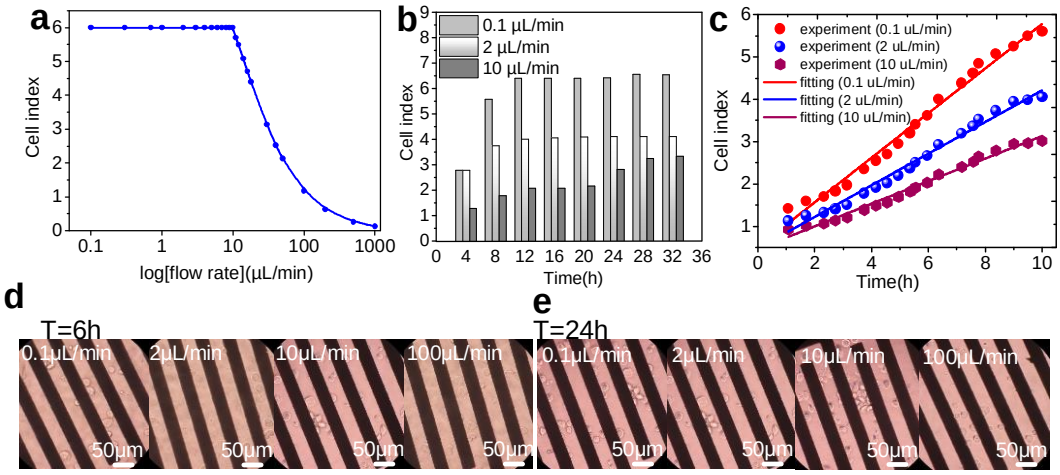

FIGURE. 4. The results of cells cultured in microfluidic chip (**a**) The relationship between flow rate of perfusion and normalized impedance. The flow rate used in the experiments is from 1 μL/min to 100 μL/min (**b**) The cell proliferation affecting by different flow rates of 0.1 μL/min, 2μL/min and 10 μL/min. (**c**) The experiment results (several points) and fitting results (lines) of impedance measurements at the flow rate of 0.1 μL/min, 2 μL/min and 10 μL/min. Data in all panels represent mean of five biological replicates. (d) The images about adherent cells using different flow rates at 6 h. (**e**) The images of cells culturing in the chip at 24 h. The bar is shown in the images.

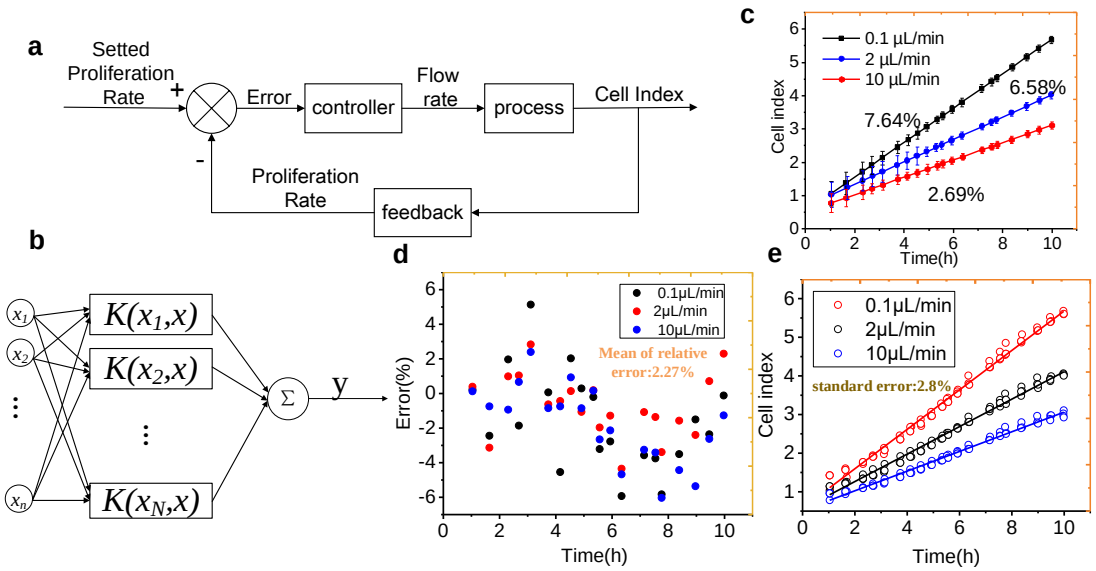

FIGURE. 5. The regulation of cell density by controlling proliferation rate. (**a**) The control block diagram (**b**) The simulation diagram of the control system using the least squares support vector machines method (**c**) The experimental errors in the experiments compared with the desired curves at the 0.1 μL/min, 2 μL/min and 10 μL/min. **(d)** The errors of the simulation results at different flow rates. It can be seen that the designed controller is proper to regulate the cell density. **(e)** The detection results in the repeated assays at different flow rates showing the method can keep consistency of the desired density.