Shear Stress-dependent Cell Detachment from Temperature-responsive Cell Culture Surfaces in Microfluidic Device

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Statement of Purpose: An intelligent surface, which can control cell adhesion and deadhesion by changing temperature, has been proposed by our laboratory. Poly(*N*-isopropylacrylamide) (PIPAAm), is introduced on the surfaces of tissue culture polystyrene (TCPS) dishes (PIPAAm-TCPS) by electron-beam irradiation. At 37 °C, cells were well adhered and spread on PIPAAm-TCPS. By lowering temperature to 20 °C, cells detached from PIPAAm surface spontaneously. In order to quantitatively evaluate cell detachment process from hydrophilic PIPAAm surface, a microfluidic device was constructed on the surface, referring to a parallel plate flow chamber (PPFC). A laminar flow, which could generate shear force within the chamber, was applied to cells in flow chamber, resulting in cell detachment. Shear stress-dependent cell detachment from PIPAAm surface was monitored at various shear stresses. A cell transformation rate constant (C_t) and an intrinsic cell detachment rate constant (k_0) were obtained through studying the effect of shear stress on cell detachment, referring to a peeling model. This approach provided a basis for the theoretical analysis of interaction between cells and PIPAAm surface.[1]

Methods: The microfluidic device had one inlet and five outlets with five parallel test channels (400 μm in width, 50 μm in depth) (Figure 1). By changing the length of test channel, the flow rate of each channel was designed as 3.0 : 2.5 : 2.0 : 1.5 : 1.0 from Outlet A to Outlet E. Shear stress in each channel could be calculated through the equation (1): $\tau=6Q\mu/h^2w$, where τ is shear stress, Q is flow rate, μ is fluid viscosity, h is the height of chamber, and w is the width of chamber. NIH/3T3 mouse fibroblast cells (MFCs) were seeded in each microchannel from Outlet C. After 24 h incubation at 37 °C, the microfluidic device was moved on a cold plate (20 °C). The cold medium (20 °C) was introduced into the microfluidic device, while the flow rate at inlet was 2.0 ml/h. Cell detachment process was monitored by CCD camera for 1h. To study cell detachment kinetics, a set of detachment curves was obtained by plotting the time-courses of percentage of attaching cells for different shear stress. Each of these curves was described by a first-order kinetics equation, referring to a peeling model.

Results: The resultant microfluidic device from Outlet A to Outlet E worked as designed, while the ratio of measured flow rates was 3.1 : 2.3 : 2.1 : 1.4 : 1.1. After incubation for 24 h at 37 °C, MFCs adhered and spread on the substrate of microchannels. By reducing the temperature to 20 °C, MFCs shrunk, and changed their shape from flat to round without flow. These results were found to quite similar to those of general PIPAAm-TCPS dishes, strongly suggesting the fabrication of microfluidic device on PIPAAm-TCPS gave no effect on the temperature-responsive properties of PIPAAm layer. By reducing the temperature to 20 °C and introducing cold medium into the microdevice, MFCs were gradually removed from the surface of microchannels. The time-courses of cells detachments of all channels were summarized in Figure 1. These results strongly suggested that the speed of cells detachment from PIPAAm-TCPS depended on the shear stress in the microchannels. Referring to a peeling model, the decreasing of cell number upon various shear stresses was described by a set of curves, where the kinetics of cell detachment was described by a first-order relationship. In this way, the number of detached cells, n(τ, t), as a function of shear stress τ and time t, was related as the following equation:

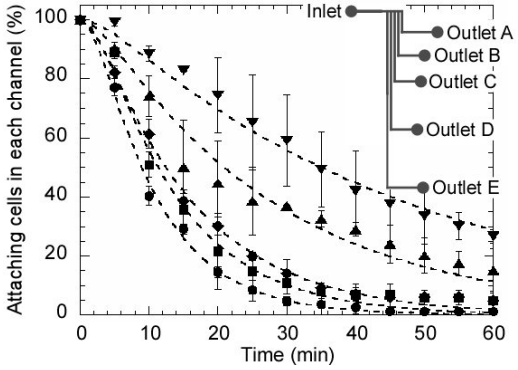

Figure 1. Time-dependent of cell detachment from each channel at 20°C. (●:Channel A, ■:Channel B, ◆:Channel C, ▲:Channel D, ▼:Channel E.) The cell detachment profiles were fitted with equation (4). The design of microfluidic device is shown in the upper right corner.

$$\frac{dn(\tau,t)}{dt}=-kn \qquad 2$$

where k is the detachment rate constant. Due to cell detachment process depended on not only applied shear stress but also shrink of cell itself, cell detachment rate constant k(τ, t) at an applied shear stress was related as the following equation:

$$\frac{dk(\tau,t)}{dt}=-C_t k \qquad 3$$

Thus, the equation (2) could be solved to the equation (4).

$$n(\tau,t)=100\exp\left[\frac{k_s(\tau)}{C_t}(1-\exp(-C_t t)-k_s(\tau)t\right] \qquad 4$$

where $k_s(\tau)$ is the cell detachment rate constant after the reformation of cell cytoskeleton. Two parameters $k_s(\tau)$ and C_t were obtained by fitting the experimental results. Moreover, k_0 was obtained by studying the relationship between $k_s(\tau)$ and τ. C_t and k_0 of MFC were calculated to be 0.34 min^{-1} and 0.58×10^{-2} min^{-1}, respectively.

Conclusions: Microfluidic device has been fabricated on a PIPAAm-TCPS surface successfully. Cells applied with a high shear stress were removed for the substrate more quickly than those applied with a low shear stress. Moreover, C_t, and k_0 were obtained through analyzing the kinetics of cell detachment. The analytic method could be useful for evaluating the interaction between cells and PIPAAm-TCPS.

Reference:

1. Tang ZL. Biomaterial 2012; 33: 7405-7411.