

Creation of a Cell-Based Separation Microdevice Using Human Renal Proximal Tubule Epithelial Cells

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ABSTRACT

Here we report a cell-based separation microdevice using human renal proximal tubule epithelial cells. Near organ-level of separation efficiency and selectivity including reabsorption and clearance by renal tubule cells was demonstrated on chip. In addition, cell performance was directly measured under different operating parameters (flow rate and feeding concentration) in order to optimize the cell separating capability. This cell-based separation microdevice also referred to as bioartificial renal tubule on chip has the potential for drug screening and portable medical care device for kidney failure [1].

KEYWORDS

cell-based separation, renal tubule cell, bioartificial renal tubule, reabsorption, clearance.

INTRODUCTION

Separation modes in microfluidics such as chromatography, electrophoresis, and filtration are mainly based on single parameter including chemical affinity, electronic charge, and molecular size, respectively. When the separation modes above deal with a complicated biological fluid system, one-step separation usually cannot be accomplished due to the complicity and close property of the system. Here, we present a novel cell-based separation mode for the separation of biological fluid samples using the intrinsic physiological functions of cells. Especially, this proposal can as well extend the cellular function into micro unit operations for micro chemical engineering.

EXPERIMENT

Proximal renal tubule reabsorbs nearly 90% of glucose / 70% of electrolytes as nutrients and simultaneously clears wastes such as urea and creatinine (clearance ratio of 70% and 100%). Based on this concept, we constructed a separation microdevice with compartmentalized structure in which human renal proximal tubule epithelial were cultured on the polycarbonate porous membrane (pore size: 3 μm , thickness: 15 μm) between the upper and lower microchannels (Figure 1). Construction of the compartmentalized structure between glass substrates and membrane was achieved by low temperature bonding.

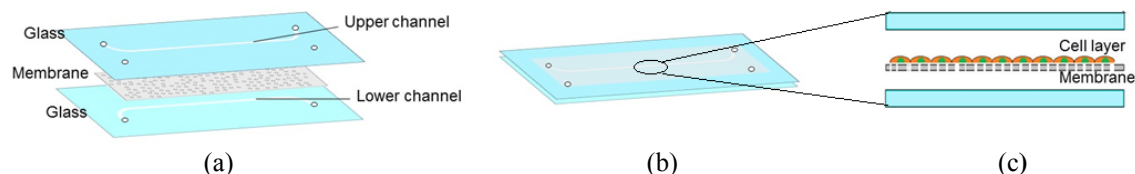


Figure 1. Construction of a sandwich structure for cell-based separation microdevice: (a) basic structure of microchips with upper and lower channel and membrane in the middle (b) realization of structure by glass-membrane bonding (c) cross section of compartmentalized structure

Perfusion culture of renal tubule cells on chip was described as follows: membrane within microchannel was perfused by matrigel dissolved in non-serum renal epithelial basal medium at 0.5 $\mu\text{l}/\text{min}$ for 2 h. Cell suspension was then injected into the microchannel at 5×10^6 cells/ml and cultured for 4-5 days. And then, cells were detected by nuclei staining using DAPI to prove the confluent attachment to membrane. Immunostaining was also made to confirm the tight junction between cells using rabbit anti-ZO-1 as primary and Alexa Fluor 488 conjugated goat-anti-rabbit as secondary.

For the demonstration of separating function including reabsorption and clearance of renal tubule cells, first of all a cell layer leakage test by inulin was made before every run of experiment. Inulin standard solution ($c_0 = 50$ $\mu\text{g}/\text{ml}$) and water were introduced into the upper and lower channels at a flow rate of 2 $\mu\text{l}/\text{min}$, respectively. Leakage ratio was defined as the ratio of inulin concentration (c_i) in the collection and the initial standard solution. After that, reabsorption and clearance of renal tubule cells were demonstrated by the same experimental setup (Figure 2) with that of inulin. Primary urine (solution consist of 50 $\mu\text{g}/\text{ml}$ of glucose, 0.2 M NaCl, 10 $\mu\text{g}/\text{ml}$ of urea and 10 $\mu\text{g}/\text{ml}$ of creatinine) was introduced into the upper microchannel. Reabsorption / clearance ratio was defined as ratio of concentration (C_1 and C_2) in lower reabsorbate / upper waste and the initial feeding concentration (C_0). All the concentrations were determined by colorimetric method with standard assay kits.

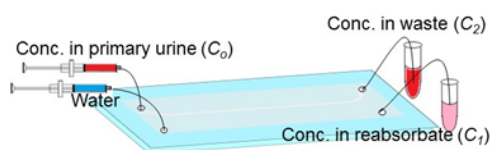


Figure 2. Experimental setup for cell layer leakage test and renal tubule demonstration on chip

Finally for the optimization of operating parameters, a numerical model to describe this cell-based micro separation process was established. Reabsorption and clearance ratio were tested under different feeding concentrations ($C_0 = 0-1000 \mu\text{g/ml}$) and flow rates ($Q_0 = 2-16 \mu\text{l/min}$) by the same experimental setup with above. Control experiment was carried out with a bare membrane.

RESULTS AND DISCUSSION

Renal tubule cells reached confluent monolayer with a coverage ratio over 95% on the membrane inside the microchannel. Specific protein expressing of tight junction ZO-1 was also detected of the cells as shown in Figure 3.

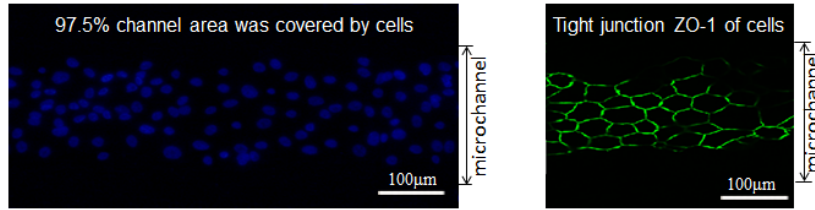


Figure 3. Nuclei staining and immunostaining of renal tubule cells on chip (DAPI: blue, Alexa Fluor 488: green)

For the demonstration of cellular separation on chip, first of all less than 5% leakage was proved. Reabsorption ratio of nearly 60% of glucose / 65% of Na^+ and clearance ratio of 60% of urea / 90% of creatinine were achieved which are more efficient than the macro renal assist device [2] (Figure 4). This is the first successful attempt to integrate renal tubule cells into microspace near *in vivo* level. The reason of high cell performance was explained as much thinner membrane compared with that of macro and the membrane incorporation by rigid glass substrate can prevent membrane deformation so as the ensure an intact cell layer.

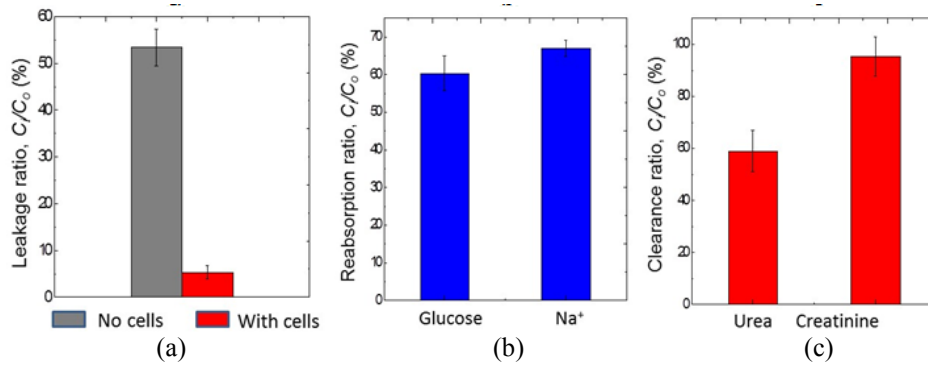


Figure 4. Results of renal tubule demonstration on chip: (a) leakage ratio (b) reabsorption ratio (c) clearance ratio

Reabsorption and clearance by renal tubule cells under different operating conditions were shown in Figure 5 and Figure 6 and the numerical model is based on the following equation, where S is the membrane surface area and ω_m and ω_{cell} is the permeability coefficient of membrane and cell layer, respectively.

$$C_i/C_0 = (\omega_m + \omega_{cell})RTS/Q_0$$

Experimental values and theoretical line agrees well both for bare membrane and cell layer. In the reabsorption experiment of glucose under different feeding concentrations, when C_0 exceeded $800 \mu\text{g/ml}$, a reabsorption saturation value of $0.97 \mu\text{g/min}$ was observed. This is consistent with the “glomeruli-tubular balance” theory [3] that the reabsorption is proportional with the concentration in filtrate from the glomeruli of kidney. But the value of $1.3 \mu\text{g/min}$ is still lower than $1.8 \mu\text{g/min}$ *in vivo* which is common in the *in vitro* cell experiment that declining of cell performance occurs. An enhancement of reabsorption by the cell layer over the bare membrane was observed (Figure 5 (a)). This is explained as that the cell layer offers active transport by the transport proteins and enzymes so as to improve the performance compared to bare membrane.

Interesting phenomena of the reabsorption when varying the flow rate has been observed that when shear stress was increased, an increased reabsorbing quantity was obtained. The permeability coefficient ω_{cell} was switchable to different shear stress that indicating a higher shear stress within the range max can enhance the cell performance as shown in Figure 5 (b). In the first segment of “no activation”, the flow rate is low so that cell performance is lower than the prediction. Corresponding to a higher shear stress, the cell performance was enhanced obviously which is consistent with the cell morphology change also named “cell reorganization” due to the shear stress. [4] In the last segment of flow rate, the cells cultured on membrane cannot endure and began to detach from the membrane which can be improved from the detached cells detection in the outlet solution from the upper microchannel.

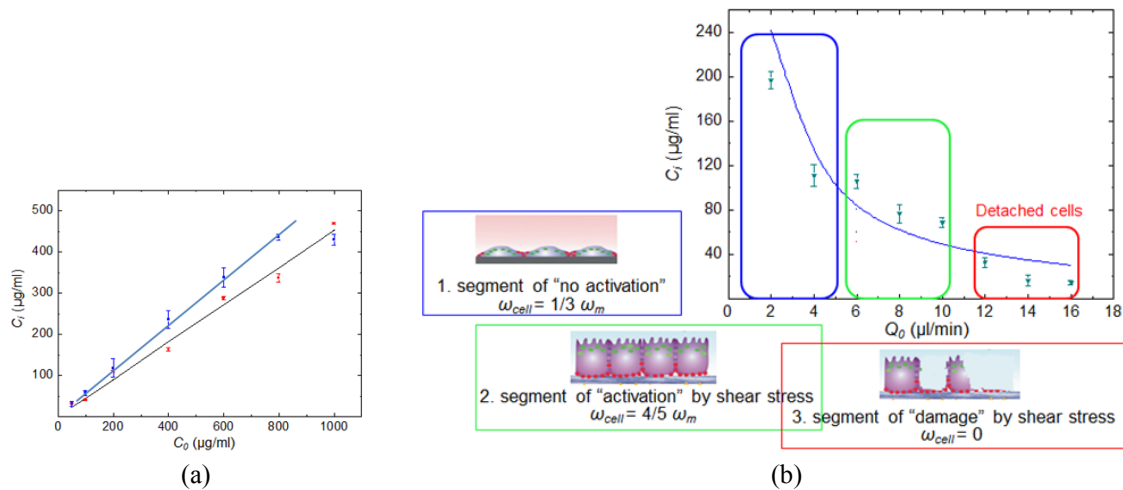


Figure 5. (a) reabsorption under different feeding concentrations (black line: theoretical of bare membrane, red dots: experimental of bare membrane, blue dots: experimental of cell layer, blue line: fitting of cell layer) (b) reabsorption under different flow rates (green dots: experimental of cell layer, blue line: theoretical of cell layer)

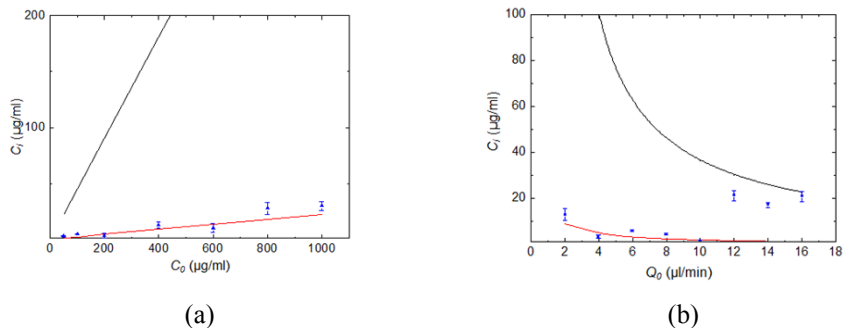


Figure 6. (a) clearance under different feeding concentrations (black line: theoretical of bare membrane, blue dots: experimental of cell layer, red line: theoretical of cell layer) (b) clearance under different flow rates (black line: theoretical of bare membrane, blue dots: experimental of cell layer, red line: theoretical of cell layer)

Creatinine was selected for the clearance experiment. Experimental results and theoretical prediction are shown in Figure 6 (a). Clearance of creatinine under varied flow rates are shown as Figure 6 (b). A similar tendency was observed when the flow is higher than 10 $\mu\text{l}/\text{min}$ that the performance of cell layer begin to decline and this indicated a collapsed layer also illustrated in the reabsorption experiment.

CONCLUSION

A cell-based separation microdevice using renal tubule cells was successfully demonstrated in a compartmentalized structure constructed by glass-membrane low temperature bonding. Near organ-level separation was realized due to the microenvironment supplied for the cell proliferation. Optimization of flow rate and feeding concentration was also studied and a higher shear stress activation effect to the cells was proved by a direct physiological measurement.

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