論 文 の 内 容 の 要 旨

論文題目　Creation of a Cell-Based Separation Microdevice and Its Application to Chemical Processes
（細胞を用いたマイクロ分離デバイスの創成と化学プロセスへの展開）

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In this thesis, a cell-based separation microdevice using renal tubule cells has been successfully created near *in vivo* level. Firstly, reliable compartmentalized structure was constructed by low temperature bonding of glass and membrane. Secondly, cultivation of renal tubule cells was realized on membrane inside the microchip and successful demonstration of the cellular separating function was made. Thirdly, numerical model to describe this mass transfer process was established from the view of engineering and optimized operating conditions were determined.

In chapter 2, firstly feasible and simple design with straight channels for the cell-based separation microdevice was made. Glass was selected to be the basic material for the device construction and a highly thin membrane was also selected to be the scaffold for cell culture. Secondly, successful bonding between inorganic glass and organic porous membrane was demonstrated by chemical surface modification at low temperature that is fairly lower than the T_g of membrane. Reliable bonding between glass and membrane *via* formation of silicon-nitrogen chemical bonds was proved by both crack-opening and fluidic tests for burst pressure and long-term durability. This method has extended the use of substrate materials from organic to inorganic such as glass which is more suitable for operation and detection than plastic materials and that the same time provides a possible bonding strategy for more materials but not limited within polymers. Also, the chemical modification by small molecules such as ammonia avoids the clogging to pores when nanoporous membrane is combined with substrates. Flexible changes of membranes due to the simple disassembling of sandwich-structured device allow broad applications in separation of samples, microreactors and cell biological studies, etc.

In chapter 3, successful cell culture was realized on the matrigel modified membrane inside the microchannel. The confluent cell culture and specific protein expressing of tight junction ZO-1 was confirmed for the renal tubule cells on chip. A reliable protocol of renal tubule cell culture on chip was developed for the next demonstration of cellular separating function. The near *in vivo* level of renal tubule separating function consists of reabsorption and clearance was successfully realized on chip in microscale for the first time. The performance of the renal tubule cell on chip which also referred to as a micro-RAD was proved to be much better efficient and selective than the conventional RAD. Moreover, this demonstration firstly introduced the cellular function into the separation unit operation in the microfluidics, which will open a novel research field for the biological study by means of microfluidics.

In chapter 4, a numerical model has been established to describe the mass transfer process of the cell-based microdevice. Reabsorption and clearance were both carried out to test the feasibility of the model. Experimental results showed good agreements to the numerical prediction both for the bare membrane and the cell layer. Cell layer was observed to possess extra driving force to the transfer process than bare membrane by its active transport. Saturation of reabsorption was also confirmed but was lower than that of in vivo. By the first direct physiological measurement of cells in microdevice, performance enhancement by shear stress was verified. Also, the cell layer changed the separation mode to wastes and became a mere barrier to sharply reduce the transport area so as to waken the transmembrane transport of wastes. Similar decline of cell performance was observed in reabsorption and clearance caused by a high shear stress to damage the confluent cell layer. Based on the experimental results, best operation range of flow rate Q_0 was determined and a feeding concentration C_0 near the reabsorption saturation point was recommended for the future practical design of applications.

Conclusively, a novel cell-based separation mode was realized in the society of microfluidics. Biological samples will be able to realize more efficient and selective separation by cells but not only limited within the nephron system. For the cell biological study, the present research supplies a novel and promising platform especially for nephron cells that different with that of conventional cell biological method such as immunostaining. By mimicking a microenvironment

for the cell proliferation and controlling the conditions, one can obtain the direct information of the cell performance variation. Because of the new impacts to the research field mentioned above, this bioartificial renal tubule on chip also plays an import role in the organ-on-a-chip for cell biological studies. For the applications, this device can be developed as a drug screening and in vitro toxication testing platform for a more precise diagnose. In the cell-based therapy, the bioartificial renal tubule on chip as referred to as micro-RAD has a big potential to increase the performance by the numbering up of the microdevices to provide the patients better medical care.

The open problems of the present research are mainly from the following aspects: firstly, more demonstrations of the renal tubule cells should be made on chip such as metabolism and secretion. Secondly, in the present conditions, the renal tubule cells can only maintain the performance (not less than 80% of the best) for 2-3 weeks. How to extend the cell proliferation and performance is always a problem for the cell research. Finally, numerical model is based on simple assumptions and lack of more mathematic descriptions in detail and the membrane parameters such as thickness, pore size and porosity should also be taken into consideration.