報告番号	※ 甲 第 10622 号

主 論 文 の 要 旨

論文題目 Manipulation of Oocytes by Magnetically Driven Microrobot on a Chip（オンチップ磁気駆動マイクロロボットによる卵子操作に関する研究）

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論 文 内 容 の 要 旨

This thesis presents on-chip various manipulations of oocyte, in order to improve the controllability of a single cell in the biotechnology, this thesis introduces four different approaches for a single oocyte manipulation. It includes microbeads loading for potential usage of oocytes loading, oocyte enucleation in high-speed with cutting accuracy control, single oocytes dispensing system, and also, finally to increase the cutting accuracy and investigate the oocyte properties, the orientation control by using Magnetically Driven Microtool (MMT) will also be briefly discussed. These methods make the single cell manipulation much easier and faster, allow the single cell analysis doing in an easier way and could reduce the burden of the operator.

First, cultivating the cells on the surface of microbead has been widely used as temporal scaffolds for cell culture engineering, here, we succeeded in demonstration of the delivery of polystyrene beads (100 & # 181;m) with the flow velocity of from 0.02 ml/h to 0.04 ml/h and MMT frequency from 1 Hz to 6 Hz to adjust the pitch of each micro-bead. The spacing interval of microbeads could be mainly adjusted by changing of MMT frequency and the flow velocity of output stream. This technique shows the potential usage on on-demand delivery of oocytes. As the oocytes is viscoelasticity, which is different from polymer beads, thus further works should be studied on this topic.

Second, since the delivery of the microbeads and oocytes has been studied, next, a microfluidic chip with an MMT for oocyte enucleation is proposed. A microfluidic system was specially designed for enucleation, and the microrobot actively controls the local flow-speed distribution in the microfluidic chip. The microrobot can adjust fluid resistances in a channel and can open or close the channel to control the flow distribution. Analytical modeling was conducted to control the fluid speed distribution using the microrobot, and the model was experimentally validated. The novelties of the developed microfluidic system are as follows: (1) the cutting speed is improved significantly owing to the local fluid flow

control; (2) the cutting volume of the oocyte can be adjusted so that the oocyte undergoes less damage; and (3) the nucleus can be removed properly using the combination of a microrobot and hydrodynamic force. Using this device, a minimally invasive enucleation process has been achieved. The average enucleation time was 2.5 s and the average removal volume ratio was 20 %. The proposed new system has the advantages of faster operation speed, higher cutting precision, and potential for repeatable enucleation.

Third, after the enucleation process, enucleated oocytes are dispensed in sequent from the microfluidic chip. A pair of capacitance sensors has been placed in a microfluidic chip to detect the oocyte, and custom-designed a special buffer zone in the microchannel to decelerate the flow velocity and reduce the hydraulic pressure acting on the oocyte. In the buffer zone, a semicircular bay, formed by equally spaced micropillars, is used to stop the oocyte at the dispensing nozzle hole. Finally, the oocyte is ejected by airflow to the culture array. The novel feature of the developed microfluidic system is that the extraordinary improvement in success rate is accompanied by a lack of change in oocyte survival rate (as assessed by a comparison of survival rates before and after the dispensing procedure). By using this device, we achieved a highly accurate single-oocyte dispensing process with a success rate of 100 %. The oocyte survival rate is approximately 70 %, regardless of whether or not the oocyte is dispensed. The newly proposed system has the advantages of high operation speed and potential usage for two-dimensional micropatterning. In order to achieve continuous oocyte manipulations, all of these modules could be considerably consisted into one microfluidic chip.

Finally, in order to increase the cutting accuracy, and also for a single cell analysis, position of nucleus, or polar body, and so on, the orientation of oocyte is important. However rotation of oocyte in X/Y plane has been achieved, but three-dimensional rotation is still a hard topic, since it effects to the cutting speed and accuracy, therefore, preliminary study on increasing the control accuracy of MMT system and methods for oocyte orientation control also will be discussed at the end of this thesis.