OOCYTE MECHANICAL CARACTERIZATION BY ROBOT INTEGRATED MICROFLUIDIC CHIP FOR HIGH-THROUGHPUT QUALITY EVALUATION
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ABSTRACT
We presents a mechanical characterization of oocyte for high-throughput quality evaluation. In order to achieve the biomedical disposable applications, our characterization system consists of only four parts: a sensing part, an actuation part, a flow-control part, and a robot integrated microfluidic chip (Robochip) as a disposable part. Robochip has advantages of both of micromechanical manipulator and Lab-on-a-Chip devices, which are, briefly, high-accuracy with high-output-force and high-throughput abilities. Robochip contains a pair of a width-tunable-wall and a force sensor.

The mechanical characterization was based on the stress relaxation phenomena. The analytical model of the oocyte was expressed using Maxwell model, we assumed the shape of the deformation follows the Hertz’s contact theory. The mechanical characterization was demonstrated, and the results shows that the elastic and viscoelastic characters were measured. Moreover, experimental and simulated values are good agreement.

KEYWORDS: Oocyte, Mechanical characterization, Robot, Microfluidic chip

INTRODUCTION
Measurement of mechanical characteristics of a single cell have became great interests along the progress of micro/nano technologies. These mechanical characteristics contribute to the evaluations of drug efficiency, and quality evaluations of cell itself. Particularly, the measurement of oocyte mechanical characteristics is expected to predicts pre-implantation rate, bioviability, quality of cryogenically preserved oocytes [1]. Previous characterization systems were based on the micromechanical manipulator, and can be achieve the prices measurement using its high-accurate manipulation ability. However, the high-throughput measurement was difficult due to the technical difficulties caused of its scanning approach. On the other hand, Lab-on-a-Chip devices based on a microfluidic chip has closed environment, and also help to prevent cell contamination as well as restrict the position of cells in 2 dimension in a plane [2, 3]. Moreover, the cost of microfluidic chips are generally low and disposable because they are fabricated by using MEMS processes that can be mass produced. The microfluidics has a lot of advantages for manipulation of small objects. However, the cell manipulations with microfluidics are generally passive way of fluidic force and therefore the noncontact actuation of microrobot in a microfluidic chip is required to active analysis such as mechanical properties measurement. Previously, we have reported the effectiveness of the robot integrated microfluidic chip (robochip) which takes advantages of both of micromechanical manipulator and Lab-on-a-Chip devices [4]. The robochip has a robotic probe and a force sensor, and can be applied to measure the cellular mechanical properties in a closed space of the microfluidic chip. The robochip approach have achieved in the measurement of static cellular stiffness which can be regarded as Young’s modulus, but the dynamic characteristics of a single oocyte, have not yet discussed so far. Herein, we presents the mechanical characterization of oocyte based on the Robochip.

THEORY
Mechanical characterization system
Figure 1 shows the basic concept of the mechanical characterization system. The system composed of a sensing parts, a disposable chip (robochip), an actuation part, and flow control part. Oocytes are injected to the microchannel of a robochip, and transported to the manipulation point in a robochip. The magnetically driven width-tunable-wall whose repetitive positioning accuracy was about 200 nm [2], deforms the oocyte, and cellular reaction force is measured by deformation of opposite force sensor.

Figure 1: Outline of the quality evaluation system
Mechanical characterization of oocyte

Oocytes have elastic and viscoelastic properties, and these mechanical characters are related with the quality itself. Therefore, a oocyte is assumed as a microparticle which has viscoelastic properties, and the microparticle is pushed against the rigid plane of force sensor. In this case, the analytical model of the oocyte can be expressed using Maxwell model, as shown in Fig.2a. The reaction force of oocyte is expressed as follows.

\[
P(t) = \frac{4R_0^{1/2}}{3} \left( 1 - \frac{1}{\nu^2} (E_c + E_v e^{-t/\tau}) \right) \delta(t)^{1/2}
\]

where \( E_c \) is the long term modulus once the material is totally relaxed, \( E_v \) is the constant value of the time dependence parameter, \( \tau \) is the relaxation time defined as the time required until the initial value turn to the value of 1/e. In this model, we assume that the shape of the deformation can be expressed by the Hertz’s contact theory.

Our approach of the cellular force measurement based on the image though the high speed CCD camera, therefore, it is possible to measure the cellular force and the displacement of the deformation as a function of the time. Thus, we can evaluate the relationship between the cellular force and the deformation at each time. Eventually, the characteristics of the oocyte is estimated by the Eq. 2.

\[
\begin{bmatrix}
E_c \\
E_v
\end{bmatrix} = k (B^T B)^{-1} B^T A,
\]

where \( A = \begin{bmatrix}
P(t_1)/\delta(t_1)^{1/2} \\
P(t_2)/\delta(t_2)^{1/2} \\
\vdots \\
P(t_n)/\delta(t_n)^{1/2}
\end{bmatrix} \) and \( B = \begin{bmatrix}
e^{-\delta/\tau} \\
e^{-2\delta/\tau} \\
\vdots \\
e^{-n\delta/\tau}
\end{bmatrix} \).

Sample preparation

Bovine oocytes were obtained from Livestock Improvement Association of Japan Inc. and transported to the laboratory within 24h. The oocytes with cumulus cells were then transferred to a culture dish containing 1% hyaluronidase (Sigma) in Medium 199 (Gibco), and the surrounding cumulus cells were removed enzymatically by pipetting.

RESULTS

Figure 3a shows sequential photographs of the experimental results. The on-chip probe deformed the oocyte, and the reaction force and the displacement of the deformation of the oocyte were measured as a function of the time. The experimental values were measured by the images (200 fps). The reaction force of oocyte decreased with passing the time. The measured cellular force is shown in Fig. 3b. The blue and red line shows the experimental values, the calculated values, respectively. In this case, the value of \( E_c \) and \( E_v \) were calculated as 6.2 kPa and 2.3 kPa. Experimental values and simulated values are good agreement and the results shows that it is possible to adopt the deformation model to analyze the mechanical properties of oocyte.

CONCLUSION

In this study, we proposed the mechanical characterization of oocyte using the robochip. In order to achieve the biomedical disposable applications, our characterization system consists of only four parts: a sensing part, an actuation part, a flow-control part, and a robochip as a disposable part. Robochip has a microchannel which contains a pair of a width-tunable-wall and a force sensor. Oocytes have elastic and viscoelastic properties, and we assumed oocytes as a microparticle which has viscoelastic properties. The mechanical characterization was based on the stress relaxation phenomena. The analytical model of the oocyte was expressed using Maxwell model, we assumed the shape of the
deformation follows the Hertz’s contact theory. The mechanical characterization was demonstrated, and the results shows that the elastic and viscoelastic characters were measured. Moreover, experimental values and simulated values are good agreement and the results shows that it is possible to adopt the deformation model to analyze the mechanical properties of oocyte. This study will contributed to the prediction of pre-implantation rate, bioviability, quality of cryogenically preserved oocytes. In the future, we will develop the automated characterization system for the high-throughput quality evaluation.

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