Development and Reliability Evaluation of Embryo Monitoring System using Time-Lapse Cinematography

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Abstract A simple time-lapse cinematography (TLC) device was developed to enable detection, at the cleavage stages, of embryos that have the potential to develop into high-scoring blastocysts. The TLC device, which consists of an optical microscope system, an illumination system, a PC-controlled camera, and a focus adjustment unit, is encapsulated in a waterproof container to allow operation in a gas-controlled water jacket incubator. Operation of the device in a high humidity environment was ensured by an environmental test in an incubator. By capturing the images of bovine embryos every 10 min for several days, their growing process was precisely recorded. The feature quantities calculated from the temporal difference images were utilized to predict the cleavage timings of the embryos.

Keywords: embryo, time-lapse cinematography, cleavage timing.

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1. Introduction

Assisted reproductive technology (ART) has been drawing attention as a countermeasure to birth rate decrease. In ART, embryos are cultured in a gas-controlled incubator for several days and transferred into the uterus with the intent of establishing pregnancy. Normally, the culture medium is exchanged only once during the culture period, and the state of the embryos is observed with a microscope by taking the embryos out of the incubator. At that time, the embryos suffer stress resulting from the sudden movement, low temperature, and illumination of the microscope, which decreases the number of embryos available for transfer. Hence, reducing such stress for the embryos has become a necessity [1]. On the other hand, good embryonic development has been found to be significantly influenced by the embryonic cleavage timing during the second or third day of culture [2]. Recently, some embryo monitoring systems have been developed and commercialized [3, 4]. However, these systems are expensive and do not satisfy the economic needs of Japanese in vitro fertilization (IVF) clinics, where small incubators are commonly used to store and monitor patients' embryos individually. Commercially available embryo monitoring systems are bulky and do not fit in such small incubators, necessitating the development of a simple and inexpensive device for frequent monitoring of embryo development. Furthermore, observing the embryos through a microscope is

stressful work for the embryologists. The risk of errors such as accidental switching of embryos has also been discussed. In a previous study, a microscope covered with a handcraft chamber [5] was utilized to allow frequent observation. Microscope-based out-of-the-incubator culture systems are useful for laboratory research but are not suitable for ART clinics, owing mainly to economic restrictions. In this study, a simple time-lapse cinematography (TLC) device placed in an incubator was developed (Fig. 1). Our final goal is to identify the embryos that have developed well by constantly observing the embryos in the incubator using this device.

2. Methods

2.1 Design of time-lapse cinematography device

The device was designed to allow constant observation of the embryos being cultured in the incubator. The layout of the components was designed using three-dimensional (3D) computer-aided design (CAD) software (Pro/ENGINEER WF 3.0, PTC). The device consists of an optical microscope system, an illumination system, a PC-controlled camera, and a focus adjustment unit, as shown in Figs. 1 and 2. The optical microscope system utilized an objective (PLN 4X, Olympus) and a specially made infinity-corrected optical system (custom made, Olympus). A USB camera (USB2 uEye LE, IDS) was used to capture the images and manage the light-emitting diode (LED) illumination system via external input–output (IO) ports. The focus of the image was adjusted by an originally designed focusing system, which is driven by a cylinder with a cam groove that converts rotational motion applied by a belt pulley into rectilinear motion of the objective lens (Fig. 2). This system converts a rotation angle of 120 degrees to a vertical displacement of 3 mm. The driving pulley was rotated by a geared stepper motor (SPG20-1000, Copal) that was driven by a motor driver circuit controlled via a USB-IO device [USB-IO 2.0 (AKI), Akizuki Denshi]. The controller PC, USB camera, all other electrical circuits, and the motor were connected via an internal USB hub (USB2-HUB4X, System Talks). The frame, which contains the PCB boards and the USB hub, and the casing of the illumination system were fabricated using a 3D

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Fig. 1 Originally developed TLC device.

Fig. 2 Schematic view of TLC device.

Fig. 3 Control software for TLC device and captured overall image.

printer (Dimension 1200, Stratasys). All the devices, except the illumination unit, were encapsulated in a waterproof aluminum container (191.152.111, ROLEC) to keep them in dry conditions,

Table 1 Settings for embryo observation.	
condition	settings
brightness of illumination	5 of 7 steps
duration	8 days, calculated from start and end times and dates
interval	10 min

because the humidity is almost 100% in the incubator. Because the system can be operated by a single USB connection with an external controller PC, the embryos can be remotely observed using the PC without removing the embryos from the optimal gas and temperature conditions.

2.2 Software for the time-lapse cinematography device

The software used to control the TLC device was developed. The focus of the microscope’s image was adjusted first using an originally coded focusing software (not shown). The photographing interval and sequence were controlled by the software, as shown in Fig. 3. Before starting monitoring of the culture, the conditions for time-lapse photography (illumination intensity, starting date and time, ending date and time, and the interval) were set to the desired values given in Table 1.

3. Experiment

3.1 Environmental test

Operation of the electrical and optical components of the TLC device in a high humidity environment should be avoided. To protect the device from the high humidity environment in the incubator, it was encapsulated in a waterproof container. To ensure proper operation of the device, the external and internal conditions (temperature and humidity) of the device in the incubator were monitored during TLC operation every 10 min for 8 days using a wireless data logging system (RTR53, T&D).

3.2 Time-lapse cinematography observation of bovine embryos in culture

In the development process, embryos follow a series of cleavage divisions, progressing through five stages: the one-cell, two-cell, four-cell, morula, and blastocyst stages. This study was performed according to the principles of the Declaration of Helsinki and was approved by a local ethics committee. In the experiments described below, a single bovine embryo was placed into a poly-dimethylpolysiloxane (PDMS) microwell [6], covered with culture medium, and cultured at 39.0°C in an atmosphere comprising 5% CO₂, 5% O₂, and 90% N₂ with high humidity. The aim of the experiments was to detect, at the cleavage stages, the embryos that have the potential to develop into high-scoring blastocysts from the recording of the developmental kinetics of single bovine embryos cultured in PDMS microwells. The time-lapse images of the embryos were captured by the TLC device six times per hour over 8 days. Nine embryos could be monitored in one batch within the image field of the TLC device (Fig. 3). The feature quantity was defined as the total gray value of 8-bit monochrome image obtained from the temporal image subtraction of sequential time-lapse images of the cultured embryos taken at intervals of 20 min. The time taken for the embryos to grow from

the two-cell stage to the four- and eight-cell stages were measured as the time difference between feature quantity peaks.

4. Results

4.1 Environmental test

Figure 4 shows the results of temperature and humidity measurement on top of and inside the TLC device. These data are also summarized in **Table 2**. On top of the TLC device, the temperature was 38.4°C, and the humidity was 94% at the start of measurement, as shown in **Fig. 4(a)**. From day 1 to day 7, the temperature and humidity were maintained at 39.6°C and 99%, respectively. At the completion of measurements, the temperature was 39.6°C, and the humidity was 94%. Several sudden drops in humidity occurred as a result of the opening and closing of the door of the incubator. As shown in **Fig. 4(b)**, the temperature inside the TLC device was almost stable at 40.1–40.3°C, which was 0.5–0.7°C higher than that on top of the device. By removing the

Fig. 4 Temperature and humidity (a) on top of and (b) inside the TLC device.

Table 2 Temperature and relative humidity (RH) in incubator during the culture period.

	on top of TLC device		inside TLC device	
	TEMP [°C]	RH [%]	TEMP [°C]	RH [%]
door open	38.4	94	35.0	45
day 1	39.6	97	40.1	52
day 2	39.6	99	40.3	53
day 3	39.6	99	40.2	55
day 4	39.6	99	40.2	56
day 5	39.6	99	40.2	57
day 6	39.6	99	40.2	58
day 7	39.6	99	40.3	59
door open	39.6	94	40.1	59

USB hub from the device and slightly lowering the temperature setting of the incubator (by 0.5°C), the average temperature inside and on top of the device could be reduced to 39.0°C for 7 days (data not shown). Conversely, the humidity inside the TLC device was 52%–59%, which was lower than that on top of the device. Under these conditions, the device can fully operate in an incubator.

4.2 Time-lapse cinematography observation of bovine embryos in culture

A typical image taken by the TLC device is shown in **Fig. 3**. As described earlier, the images of nine embryos individually placed in PDMS microwells (diameter: 300 μ m, depth: 200 μ m) were captured every 10 min for 8 days. Images of typical first (one-cell to two-cell) and second (two-cell to four-cell) cleavage sequences are shown in **Fig. 5(a)** and **(b)**, respectively. The cleavage timing was clearly captured in these images. By defining the regions of interest (ROIs) in the microwells as targets, the state of development could be tracked. For a single ROI, the feature quantities were calculated from the temporal difference images (**Fig. 6**). Significant changes in the images before and after cleavage were observed. Hence, the feature quantities calculated from the temporal difference showed some peaks that suggest the cleavage timings. From these data, the timings of the first (one-cell to two-cell), second (two-cell to four-cell), and third (four-cell to eight-cell) cleavages were 12.67 ± 0.17 , 20.83 ± 0.17 , and 28.67 ± 0.17 h, respectively.

Fig. 5 (a) First and (b) second cleavage.

Fig. 6 Cleavage timings predicted using feature quantities of temporal difference images.

5. Discussion

In the environmental test, the humidity in the device was below 59%. However, the humidity increased gradually during the measurement period. To ensure safety of the apparatus for a longer measurement period (more than 8 days), desiccant should be inserted into the TLC device, which would result in a sufficiently low humidity. In the present study, the temperature in the incubator increased from the set value by 0.6°C. This rise is a serious issue for the embryo culture. If the temperature in the incubator is set 0.6°C lower than the desirable temperature, the inner temperature will be maintained at the proper value. As this issue originated from the heat generated by the electrical components of the TLC device after a series of measurements, the internal USB hub was removed from the TLC device, and the temperature on top of the device decreased to a safe level.

This system currently has only one 4× objective lens. Our intention is to simultaneously identify the cleavage timing of several embryos. Thus, the area of inspection should be wider than the conventional observation area commonly used for clinical purposes. To ensure the desired image resolution, we employed a 5 million pixel camera and cropped the region of interest with sufficient resolution for image processing and even decreased the resolution to improve the image processing speed. If the clinical demand requires a more precise image, it is possible to display images with higher resolution.

Use of the PDMS microwells maintains the embryos in constant locations. Images of some cleavages were clearly captured, but some wells located at the edges were partially out of sight, and their images could not be fully captured. Hence, the space between microwells should be reduced to allow the entire image to be captured. The image processing software used to detect the cleavage timing is not robust against the orientation and relative position of blastomeres. To improve this robustness, a better image processing algorithm should be developed.

The application of this system is not restricted to embryo monitoring; we are currently working to apply this system to time-lapse observation of cell taxis on biomaterials.

6. Conclusion

A simple TLC device for monitoring embryo development in an incubator was developed in this study. Using this device, the development of bovine embryos was observed and recorded as time-lapse images. The cleavage timings of bovine embryos were obtained by image processing using the feature quantity calculated from temporal difference images.

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Conflict of interest

We have no conflict of interest relationship with any companies or commercial organizations based on the definition of the Japanese Society of Medical and Biological Engineering.

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