AN AUTOMATED EMBRYO CULTURE SYSTEM USING DYNAMIC MICROARRAY

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ABSTRACT

In this study, we developed an automated embryo culture system, which make an array of embryo, culture them to be blastocyst and collect the blastocyst for the quality control for reproductive technology. A dynamic microarray format has been applied to the culture system for embryo manipulation. The system was examined about the three functions of tapping, culture and release using a mouse embryo as a sample. As a result, the mouse embryos were successfully trapped, cultured and released. We conclude that the system can be applied to fundamental technique of reproductive technology and life science by further development.

KEYWORDS: Embryo culture, Dynamic microarray, Trapping, Collection

INTRODUCTION

Although in vitro culture (IVC) of mammalian embryos is an essential technique in reproductive technology and other related life science disciplines, mammalian embryos are usually cultured manually in a microdroplet of culture medium. The conventional method as using microdroplets needs high procedure of technicians, and a development rate of embryos are often affected by quality of procedure [1]. Moreover, the method cannot manage and observe each embryo individually, even though quality of development profile concerns implantation rate and birthrate [2]. Therefore, automation and observation have been highly desired for IVC of mammalian embryos to stabilize blastocyst development rates and to achieve improved reproducibility. The purpose of this research is to develop an automated embryo culture system, which can manage an array of embryos, culture them to become blastocysts, and collect the blastocysts using the dynamic microarray format that we developed previously [3].

THEORY

The automated embryo culture system consists of a microfluidic device, microsyringe pumps and a time-lapse imaging system in a multigas incubator (Figure 1). The microfluidic device is installed on a microscope. The microfluidic device has three independent dynamic microarray channels which have 60 cases for trapping of embryos. Their inlets and outlets are connected with microsyringe pumps and a waste tank, respectively, via silicone tubes for fluidic operation.

Figure 2 shows the schematic image of trap-and-release mechanism of the dynamic microarray. When the cage is empty, the flow resistance along the straight channel is lower than that of the looped channel, accordingly, an embryo is trapped in the cage (Figure 2(a)). On the contrary, embryos will be bypassed the cage through the looped channel if a trapping-part is occupied by the other embryo. Figure 2(b) shows the release mechanism using an electrolytic bubbling. When voltage is applied to Pt electrodes located at downstream-side of the cage, bubbles generate rapidly by electrolysis in the bubbling chamber. The rapid bubbles generation makes jet flow at the inlet and the outlet of the chamber. The jet flow displaces the trapped embryo into the main flow, and then the embryo is carried to the outlet of the device by the flow.

The time-lapse imaging system is used to obtain growth profiles of each of the embryos, which are managed one-by-one in the dynamic microarray. The microscope of the system has electromotive XYZ stage controlled by a PC for observation of the profiles of every embryos. Evaluation of the growth profiles by the system may contribute to increase the pregnancy rates of the IVC embryos.
Figure 2: Schematic image of trap-and-release mechanism: Trapping and bypassing mechanism (a), release mechanism (b).

EXPERIMENTAL

We examined the three functions of trapping, culture, and release using mouse embryos as a sample. A PDMS chip, which has a part of microchannel, was fabricated by a conventional replica molding method using SU-8 (Microchem). The inlet and outlet ports on the PDMS chip were fabricated by punching holes using trephine (KAI) of 2 mm in diameter. The Pt electrodes were patterned on a glass substrate, then, the substrate was bonded permanently with the PDMS chip by using oxygen plasma.

BDF1 female mice (13 weeks old) and adult male of ICR were used for in vitro fertilization (IVF). IVF was carried out mainly by using the method of Toyoda et al [4]. The zygotes were washed and cultured for 24 h in micropellets. Flow rates 1.0 and 0.2 μL/min were used for trapping and perfusion culture, respectively. The morphologies of embryos were observed by the time-lapse imaging system during the culture. The embryos of the 2 cell stage were used for the culture up to 72 h. Then, the embryos developed into blastocysts were selectively collected from the device using the electrolytic bubbling. Voltage 5.0 V was applied to generate the air bubbles for 300 ms.

In culture experiment, at least five replicated tests were run. The blastocyst rate was calculated using the denominator for the number of cleavage embryos. The student’s t-test was performed for statistical evaluation. Differences at the P < 0.05 level were considered to be statistically significant.

RESULTS AND DISCUSSION

Figure 3 shows a result of trapping operation using the dynamic microarray. As described in theory part, when the cage was empty, an embryo was trapped in the cage. On the other hand, embryos was bypassed the cage, when the cage was occupied by the other embryo. As a result, the embryos were successfully trapped for each cage with the 1.0 μL/min carrier flow rate (Figure 3).

Time-lapsed images of a mouse embryo during culture in the dynamic microarray are shown in Figure 4. The embryo, which became blastocyst normally in the dynamic microarray, has no morphological difference from the one cultured by the conventional microdroplet method. The rate of embryonic development compared with the control experiment by the microdroplet method is shown in Table 1. The system had no significant difference in the culture performance from the conventional method. In our preliminary test, the result suggested that the rate of the embryonic development might be influenced by flow rate and culture medium volume. The system allows the rate of embryonic development to increase by optimizing of the culture conditions in microenvironment [5].

Figure 5 shows the time-lapsed successive images of the blastocyst collection process using the electrolytic bubbling. When the voltage was applied to the electrode which was located at downstream of the cage trapping the blastocyst, air bubble was generated after 100 ms, thereby, the blastocyst was displaced into the main flow by jet flow from inlet of the chamber. The blastocysts could successfully be released from the cages of the dynamic microarray. After release from the microfluidic device, the blastocysts were transferred into the uterus of adult female mouse of ICR as surrogate mother. After 17 days, normal babies were born. As a result, it seems that the blastocysts released from the microfluidic device were no damage by the electrolytic bubbling.

Figure 3: Photograph of trapped embryos in the dynamic microarray. The embryos were successfully trapped for each cage with the flow rate 1.0 μL/min.
Figure 4: Photographs of mouse embryo during culture in the dynamic microarray at 0, 24, 48 and 72 h of the culture. (a) 2 cell stage, (b) Morula, (c) Blastocyst, (d) Expanded blastocyst.

Table 1. Embryonic development rate of ICR × BDF1 mouse embryos cultured in the dynamic microarray compared with the control of microdroplet method. Seven replicated tests were run in the experiment. (*and**: No significant difference by student’s t-test. p<0.05)

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<th>No. (%) developed to</th>
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<tr>
<td>Microdroplet</td>
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<td>Culture system</td>
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<tr>
<td>175</td>
<td>169 (96.6 *)</td>
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<td>323</td>
<td>308 (95.4 *)</td>
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Figure 5: Time-lapsed successive images of a blastocyst collection from the cage by the electrolytic bubbling at 0, 100, 200 and 300 ms of electrolysis.

CONCLUSION

We have proposed the automated embryo culture system for the management of individual mammalian embryos using the dynamic microarray format. The system can manage each embryos in each cages for every culture period. We tested about the three functions of trapping, culture and release using mouse embryos. As a result of our test, we conclude that the system can be applied to the routine embryo culture which is carried out in every life science discipline. If the automated culture system, which can manage the individual embryo, can be realized, it could be applied to not only mass embryo culture but also the improvement of the conception rate which is quite low nowadays. We believe that the microtechnology contributes to mammalian embryo culture involved in medical and life science.

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REFERENCES


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