A HIGH-THROUGHPUT ANTIBODY SCREENING PLATFORM TOWARD EMBRYOLOGY
H. Kimura1,2*, S. Senda1, T. Yoshimura2, Y. Sato3, T. Fujimori3 and T. Fujii2
1Department of Mechanical Engineering, Tokai University, JAPAN, 2Institute of Industrial Science, The University of Tokyo, JAPAN, and 3National Institute for Basic Biology, JAPAN

ABSTRACT
This paper reports a high-throughput antibody screening platform using microfluidic technologies, as a novel tool for immunostaining of mammalian embryos. The technology can not only reduce the consumption amount of sample solutions but also shorten process time by the effect of microenvironment on the immunostaining. As a result of screening test using the device, the available antibodies could be successfully screened from the sample libraries, and the immunostaining process was finished within <2hours. The result shows the proposed method is useful for high-throughput screenings of antibodies toward embryology.

KEYWORDS: High-Throughput Screening, Immunostaining, Antibody, Embryology

INTRODUCTION
Immunostaining with monoclonal antibodies is one of the powerful methods to identify protein expressed in a mammalian embryo in the early stage of its development[1]. However, it is an extremely labor work to find monoclonal antibodies, which can be available for the embryo assays, from huge amount of candidate libraries (>10,000 samples), because the immunostaining of embryos by the conventional method using microdroplets requires tangled procedures. Previously, a microfluidic system has been proposed for high-throughput immunostaining to adherent cells[2]. However, the microfluidic device used in this system is not only impossible to apply to immunostaining of embryos, but also too complex and too expensive to be disposable, because a lot of active switching microvalves for controlling sample selection are integrated. To screen valuable monoclonal antibodies for embryology, high-throughput and easy handling are highly desirable. In this study, we have developed a simple microfluidic device for parallel immunostaining of embryos with different samples at the same time.

Figure 1: Schematic illustration of the high-throughput antibody screening device. The device has 16 individual inlets, reaction chambers and microchannels. An outlet port is connected to a syringe pump to control a flow rate.

Figure 2: The microfluidic device fabricated by the conventional microphotolithography.
EXPERIMENTAL

The microfluidic device consists of 16 inlet ports, 16 reaction chambers and an outlet port connected with a tournament-shaped microchannel each other. The inlet ports are used for introduction of embryos, washing buffers and sample solutions, for immunostaining independently at the each chamber which has a cage to keep embryos (Figure 1). The outlet port is connected to a syringe pump to control flow in the microchannel by sucking. The device made from polydimethylsiloxane (PDMS) was fabricated by conventional micro-photolithography methods. A PDMS chip with a microstructure and a cover glass (thickness: 0.17mm) as bottom of the device were bonded by oxygen plasma bonding method (Figure 2), it is helpful for visualizing embryos by a confocal microscope. The device is placed on the stage of the microscope for observation of immunostained embryos (Figure 3).

For the monoclonal antibody screening, we have evaluated immunostaining reaction effect by microfluidic flow in the device. The flow rate of 1.25 L·min⁻¹·path⁻¹ (20 L·min⁻¹·device⁻¹), that no cross-contamination occurs at the next chambers, was used in the experiment. Mouse embryos pretreated with a fixing solution were introduced into the each reaction chambers from the inlet ports. The solutions for blocking, washing and immunostaining were sequentially introduced into the inlet ports through the procedure shown in Figure 4. The reaction time, the washing time and the sample volumes in the procedure had been optimized in the preliminary experiments. The monoclonal antibody screening tests using five types of antibodies (antigens: E-cadherin, PDGFRα, Nanog, Gata4, Cdx-2) were demonstrated to evaluate the function of the device.

RESULTS AND DISCUSSION

The reaction time of the antibody was investigated by compared with the result of the conventional method. As the result, the reaction time of 12min is enough for immunostaining using the device, despite the conventional method requires over 60min (Figure 5). It might mean that the flow enhances immunostaining reaction. Moreover, although over 50 L solution is needed in the conventional method, only <20 L solution is enough volume in the proposed method using the microfluidic device. These are major advantages by the microfluidic technology for inexpensive immunostaining process. Performance of the proposed method has been examined through immunostaining tests and the screening tests using monoclonal antibodies. The antigens, such as E-cadherin, PDGFRα, Nanog, Gata4 and Cdx-2, were
successfully immunostained without cross contamination on the device (Figure 6). Moreover, the available antibodies could be screened from the sample library, and the immunostaining process was finished within <2hours by using our system setup. These results show the proposed method is useful for high-throughput screening of antibodies toward embryology.

CONCLUSION

In this study, we have proposed a high-throughput immunostaining method using the antibody screening system based on the microfluidic technology. Reduction of the sample volume and reaction time in the embryo immunostaining procedure could be achieved by utilizing the advantages of the microfluidic technology in the proposed method. As a result of the screening test, the available antibodies could be successfully screened from the sample libraries. We believe that the microfluidic technology contributes to embryoology involved in medical and life science.

ACKNOWLEDGEMENTS

This work was supported by JSPS KAKENHI Grants; Grant-in-Aid for Scientific Research on Innovative Areas and Grant-in-Aid for Scientific Research (C). We thank Mr. S. Segawa and Ms. H. Nakamura for assistance in experiments.

REFERENCES


CONTACT

* H. Kimura; phone: +81-463-58-1211; hkimura@tokai-u.jp