

Novel in vitro system for primary culture of hepatocytes toward the study of circadian rhythms of the liver

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Introduction

Recently, a variety of circadian rhythms of various tissues and organs have been revealed to be governed by endogenous genomic oscillators consisting of clock genes. In drug discovery, it is essential for characterizing drug actions to study the clock-genes behaviors leading directly genetic and functional changes in the liver. However, suitable in vitro model culture systems to address these issues have not been proposed, because of both the deterioration of isolated hepatocytes and the desynchronization and damping of circadian rhythm.

Therefore, this study aimed at the development of novel hepatocytes culture system maintaining circadian rhythm. We specifically focused on two points as critical differences between in vivo liver tissue and in vitro culture systems, that is, oxygen supply to hepatocytes and interactions between hepatocytes and nonparenchymal cells.

Materials and methods

A high-oxygen-permeable material, poly-dimethylsiloxane (PDMS) was used for on-site oxygenation. Surface modification of PDMS was conducted to inhibit cells detachment [1]. On the PDMS surfaces amino groups were introduced and reacted with a photo-reactive cross linker by exposing to UV light. Using this cross linker, collagen Type 1-P was covalently immobilized on the PDMS surface (PDMS-UV-CN).

On this PDMS surface, isolated primary rat hepatocytes were inoculated. At 24 hours later, NIH/3T3 (3T3) cells were inoculated on the hepatocytes monolayer (Fig. 1). During 13 days, the culture medium was changed every 24 hours and hepatocytes functions such as albumin secretion and urea synthesis rate were measured. On day 13, histological staining was performed on vertical sections against cell layers. During day 3, RNA was isolated every 8 hours, and gene expression analyses were performed by real-time RT-PCR, focusing on circadian-rhythm-related genes. Hepatocytes homocultured on collagen coated tissue culture polystyrene (TCPS-CN) plates were used as a control (Fig. 1).

Results and discussion

In the coculture on PDMS-UV-CN, various hepatocytes functions were maintained at dramatically higher levels. The histological staining showed that almost complete double-layered

structure of hepatocytes and 3T3 cell monolayers were formed and maintained at least during 13 days. These results demonstrated that long term functional and morphological maintenance were realized by combining the sufficient oxygen supply and the coculture [2].

Then, we focused on the maintenance of gene expression dynamics, especially circadian-rhythm-related genes. As shown in Fig. 2, gene expression dynamisms were lost in the conventional TCPS-CN culture. In contrast, in the PDMS-UV-CN culture, dynamisms of circadian-rhythm-related genes were maintained at remarkably high levels until Day 3 to Day 4. Then, we plan to investigate the mechanism of the maintenance of circadian rhythm focusing on the interaction between hepatocytes and fibroblast cells.

Conclusion

This culture system enables in vitro integration of various parameters that are effective in maintaining circadian rhythm of primary-cultured hepatocytes. Therefore, it can be a promising platform toward the in vitro study of circadian rhythm in the metabolism of the liver.

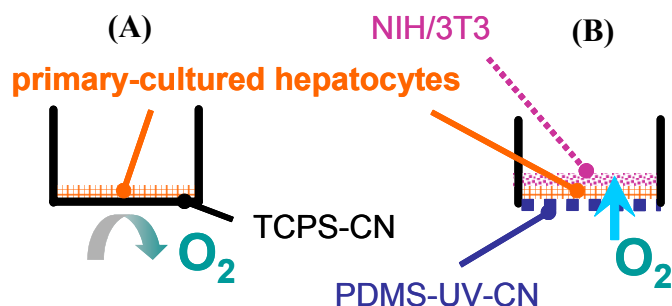


Fig. 1: The images of each well on vertical section of both conventional collagen coated tissue culture polystyrene (TCPS-CN) culture plate (A) and the PDMS culture system (B). The PDMS membranes were stacked and clamped as bottom surfaces between the 24-well poly-carbonate (PC) frames and stainless-steel boards possessing 24 holes not to inhibit oxygen permeation through the PDMS membranes. (A), homoculture of primary-cultured hepatocytes on TCPS-CN plate; (B), coculture of primary-cultured hepatocytes and NIH/3T3 (3T3) cells on PDMS-UV-CN plate.

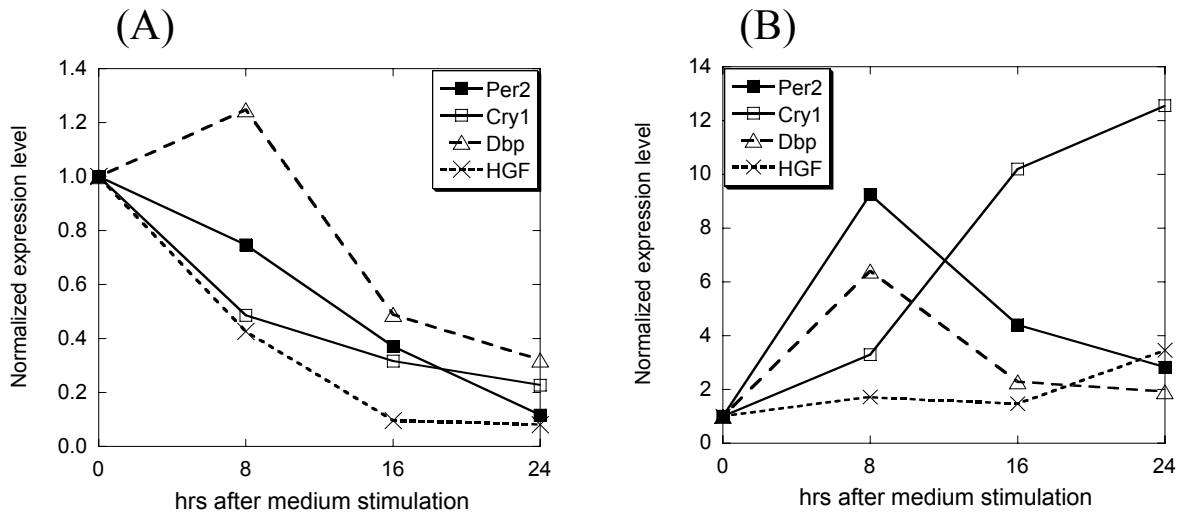


Fig. 2: Temporal expression patterns of circadian-rhythm-related genes after changing medium containing 5% fetal bovine serum and 100 nM dexamethazone. Time 0 corresponded to 48 hours after the inoculation of hepatocytes. The medium was changed twice at time -1 and 0. The cells were collected at indicated time points. The relative mRNA levels of Per2, Cry1, Dbp, and HGF were determined by RT-PCR analysis and were normalized to those of β -actin mRNA at each time point and the value at time 0 of each gene was set to 1. These primers amplified only rat-derived genes specifically, but HGF primers were designed for both rat and mouse genes. (A), homoculture on TCPS-CN; (B), Coculture on PDMS-CN.

References

- [1] Nishikawa, M. et al., *Biotechnol Bioeng*, under revision.
- [2] Nishikawa, M. et al., *J Biotechnol*, in press.