Data Article

Rapid production of engineered human primary hepatocyte/fibroblast sheets

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A B S T R A C T

This data article contains data related to the research article entitled “Vascularized subcutaneous human liver tissue from engineered hepatocyte/fibroblast sheets in mice,” published in Biomaterials [1]. Engineered hepatocyte/fibroblast sheets (EHFSs) are used for construction of vascularized subcutaneous liver tissue without a pre-transplant vascularization procedures. Here, we described a rapid production technique of EHFSs by controlling fibroblast density and coating fetal bovine serum (FBS) onto temperature-responsive culture dishes (TRCDs). The human fibroblast monolayer formed on FBS-coated TRCDs within 1 h when seeded at a high density (at least $1.56 \times 10^5$ cells/cm²). The most rapid EHFS production was achieved soon after the adhesion of human primary hepatocytes onto the fibroblast layer.

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Specifications Table

| Subject area | Biology |
|--------------|---------|
| More specific subject area | Tissue engineering, cell sheet, hepatocyte culture |
| Type of data | Image, graph, figure |
| How data was acquired | Microscope |
| Data format | Raw |

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Experimental factors

Cell sheet, rapid producing technique

Experimental features

Rapid production of engineered human hepatocyte/fibroblast sheet

Data source location

Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Data accessibility

Supplementary data of the article

Value of the data

- FBS served as a good TRCD coating for the rapid preparation of fibroblast monolayers.
- Fibroblast monolayers formed within 1 h by seeding at least $1.56 \times 10^5$ cells/cm$^2$.
- Rapid production of EHFSs was achieved approximately 3 h after the first inoculation of TIG-118 cells.

1. Data and experimental design

1.1. Fibroblast monolayer preparation by controlling cell density and FBS-coating to TRCD

Human fibroblasts (TIG-118 cells) formed a confluent monolayer within 1 h after inoculation with at least $1.56 \times 10^5$ cells/cm$^2$ onto FBS-coated TRCDs (Fig. 1A and B). Fibroblasts seeded at a lower density ($1.04 \times 10^5$ cells/cm$^2$) did not form confluent monolayers. Fibroblasts on uncoated TRCDs were unable to reach confluence despite high-density inoculation and showed non-uniform cell distributions (Fig. 1C and D).

1.2. Human primary hepatocyte density for healthy culture on a FBS-coated TRCD

Human primary hepatocytes on FBS-coated TRCDs were not confluent within 1 day after inoculation under two conditions of hepatocyte densities ($1.04$ and $2.08 \times 10^5$ cells/cm$^2$) (Fig. 2). After

![Fig. 1](image-url)

**Fig. 1.** Phase-contrast micrographs (A, C) and confluency (B, D) of fibroblasts cultured on TRCDs at 2 h after inoculation. Fibroblasts were cultured at 1.04, 1.56, or $2.08 \times 10^5$ cells/cm$^2$ on (A, B) FBS-coated or (C, D) uncoated TRCDs. Scale bar, 100 μm. The dashed lines indicate the confluent.
3 days of culture, the hepatocytes showed a confluent monolayer. Hepatocytes at lower density (1.04 × 10^5 cells/cm^2) were suitable for healthy culture because little dead cells were observed.

1.3. Effects of layer-by-layer procedure for stable, rapid production of EHFSs

Human primary hepatocytes adhered onto the confluent monolayer of fibroblasts for at least 2 h after hepatocyte inoculation. EHFSs were harvested from FBS-coated TRCDs soon after the adhesion of fibroblasts.
hepatocytes by reducing the culture temperature from 37 °C to 20 °C for several minutes (Fig. 3A). Co-suspensions of hepatocytes and fibroblasts formed EHFSs, although the EHFSs were often self-detached from FBS-coated TRCDs without temperature reduction before formation of continuous cell sheet format (Fig. 3B).

2. Materials and methods

2.1. Cell preparation

Human primary hepatocytes were isolated from human liver tissues by perfusing collagenase (130 U/mL, Wako Pure Chemical, Osaka, Japan) [1]. Suspensions with > 80% viable cells were used for this study. Normal human diploid fibroblast TIG-118 cells were purchased from Health Science Research Resources (JCRB0535; Osaka, Japan) [1,2].

2.2. Fibroblast monolayer preparation

To determine the proper conditions for the formation of a confluent monolayer, human fibroblasts were inoculated at 1.04, 1.56, or 2.08 × 10⁵ cells/cm² onto FBS-coated (2 h) or uncoated TRCDs. Minimum Essential Media supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin was used for fibroblast culture (all from Invitrogen, Carlsbad, CA).

At 2 h of culture, the confluency of fibroblasts was measured from phase-contrast micrographs using Win ROOF Version 6.3.0 (Mitani Corp, Fukui, Japan). Data are presented as mean ± standard deviation from 2 independent cell preparations.

2.3. Evaluation of human primary hepatocyte density

To evaluate the better density for human primary hepatocyte culture, hepatocytes were inoculated at 1.04 or 2.08 × 10⁵ cells/cm² onto FBS-coated TRCDs. Hepato-STIM Culture Medium (BD Biosciences, San Jose, CA) supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin was used for hepatocyte culture.

2.4. Rapid production of EHFSs

Human primary hepatocytes were plated at 1.04 × 10⁵ cells/cm² (1.0 × 10⁶ cells/well) onto a confluent layer of TIG-118 fibroblasts plated 1–2 h prior at 1.56 × 10⁵ cells/cm² (1.5 × 10⁶ cells/well) onto FBS-coated TRCDs (Fig. 3A). Co-suspensions of hepatocytes and fibroblasts were also inoculated onto FBS-coated TRCDs (Fig. 3B). Hepato-STIM Culture Medium supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin was used for co-culture.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2015.09.044.

References

[1] Y. Sakai, K. Yamanouchi, K. Ohashi, M. Koike, R. Utoh, H. Hasegawa, et al., Vascularized subcutaneous human liver tissue from engineered hepatocyte/fibroblast sheets in mice, Biomaterials 65 (2015) 66–75.
[2] Y. Sakai, M. Koike, H. Hasegawa, K. Yamanouchi, A. Soyama, M. Takatsuki, et al., Rapid fabricating technique for multi-layered human hepatic cell sheets by forceful contraction of the fibroblast monolayer, PLoS One 8 (2013) e70970.