Data in Brief 5 (2015) 498–501

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

Rapid production of engineered human primary hepatocyte/fibroblast sheets

Yusuke Sakai*, Makiko Koike, Akihiko Soyama, Masaaki Hidaka, Tamotsu Kuroki, Susumu Eguchi

Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan

ARTICLE INFO

Article history:
Received 18 August 2015
Received in revised form
17 September 2015
Accepted 29 September 2015
Available online 9 October 2015

Keywords:
Human primary hepatocyte
Fibroblast
Cell sheet
Tissue engineering

ABSTRACT

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 1.56×10^5 cells/cm^2). The most rapid EHFS production was achieved soon after the adhesion of human primary hepatocytes onto the fibroblast layer.

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*

* Corresponding author.
E-mail address: y.sakai.bioeng@gmail.com (Y. Sakai).

http://dx.doi.org/10.1016/j.dib.2015.09.044

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 1.56×10^5 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 1.56×10^5 cells/cm^2 onto FBS-coated TRCDs (Fig. 1A and B). Fibroblasts seeded at a lower density (1.04×10^5 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 2.08×10^5 cells/cm^2) (Fig. 2). After

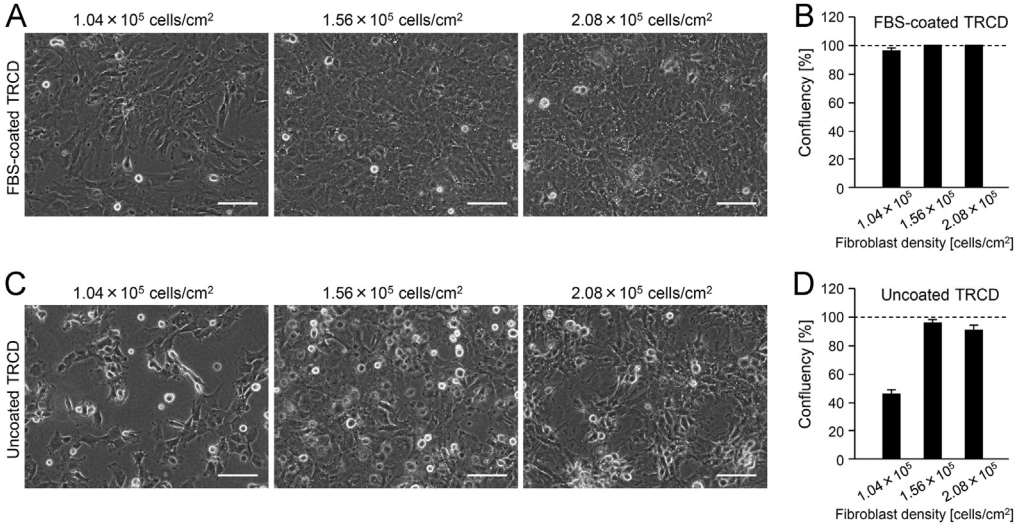

Fig. 1. Phase-contrast micrographs (A, C) and confluency (B, D) of fibroblasts cultured on TRCDs at 2 h after inoculation. Fibroblasts were cultured at 1.04, 1.56, or 2.08×10^5 cells/cm^2 on (A, B) FBS-coated or (C, D) uncoated TRCDs. Scale bar, 100 μm. The dashed lines indicate the confluent.

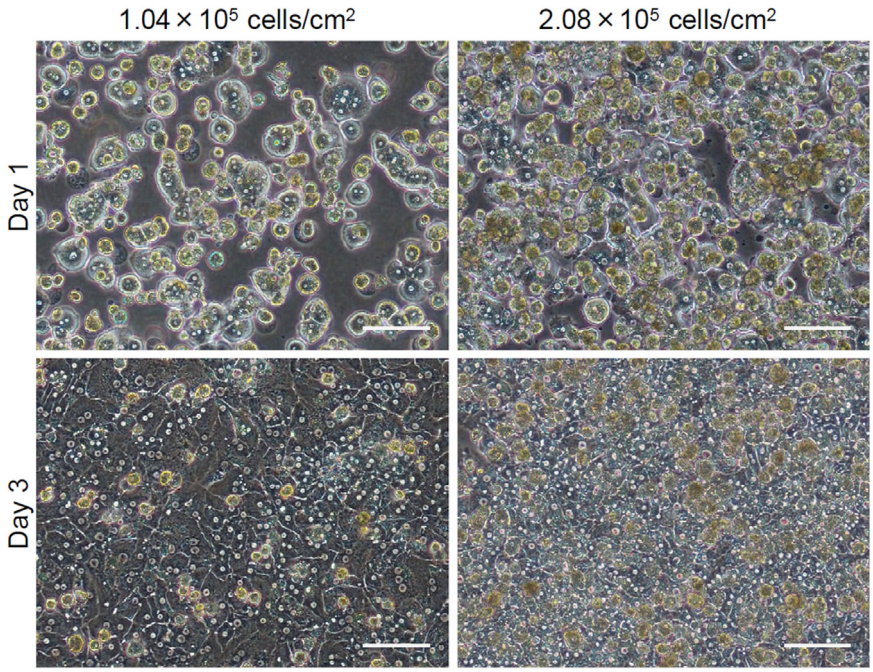

Fig. 2. Phase-contrast micrographs of hepatocytes cultured on FBS-coated TRCDs at 1 and 3 days of culture. Hepatocytes were cultured at 1.04 or 2.08 × 10^5 cells/cm^2. Scale bar, 100 μm.

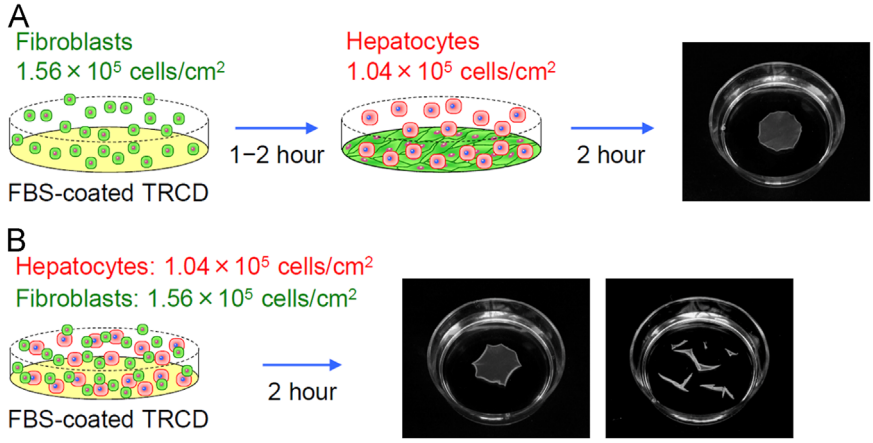

Fig. 3. Rapid production of EHFSs: (A) layer-by-layer procedure and (B) inoculation of co-suspensions.

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

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 2.08×10^5 cells/cm^2 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 2.08×10^5 cells/cm^2 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 1.04×10^5 cells/cm^2 (1.0×10^6 cells/well) onto a confluent layer of TIG-118 fibroblasts plated 1–2 h prior at 1.56×10^5 cells/cm^2 (1.5×10^6 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.

Acknowledgments

This work was supported in part by Takeda Science Foundation to Y. Sakai, Grants-in-Aid for Young Scientists to Y. Sakai (No. 25861161), and Grants-in-Aid for Scientific Research to S. Eguchi (No. 26461916). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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.