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Billion-scale production of hepatocyte-like cells from human induced pluripotent stem cells

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ABSTRACT

Human induced pluripotent stem (iPS) cell-derived hepatocyte-like cells are expected to be utilized in drug screening and regenerative medicine. However, hepatocyte-like cells have not been fully used in such applications because it is difficult to produce such cells on a large scale. In this study, we tried to establish a method to mass produce hepatocyte-like cells using a three-dimensional (3D) cell culture bioreactor called the Rotary Cell Culture System (RCCS). RCCS enabled us to obtain homogenous hepatocyte-like cells on a billion scale (>10^9 cells). The gene expression levels of some hepatocyte markers (*alpha-1 antitrypsin*, *cytochrome* (*CYP*) *1A2*, *CYP2D6*, and *hepatocyte nuclear factor 4alpha*) were higher in 3D-cultured hepatocyte-like cells than in 2D-cultured hepatocyte-like cells. This result suggests that RCCS could provide more suitable conditions for hepatocyte maturation than the conventional 2D cell culture conditions. In addition, more than 90% of hepatocyte-like cells were positive for albumin and could uptake low-density lipoprotein in the culture medium. We succeeded in the large-scale production of homogenous and functional hepatocyte-like cells from human iPS cells. This technology will be useful in drug screening and regenerative medicine, which require enormous numbers of hepatocyte-like cells.

1. Introduction

The liver plays important roles, including drug metabolism and detoxification. In the field of drug screening, human hepatocytes (HHs) are widely used to predict drug metabolism and drug-induced hepatotoxicity. In the field of regenerative medicine, HHs are transplanted into patients with liver disease. For both applications mentioned above, a large number of HHs have to be prepared. In particular, more than one billion (10^9) HHs are required for hepatocyte transplantation [1]. However, it is difficult to prepare HHs on that scale because of a shortage of donors. To overcome this problem, human iPS cell-derived hepatocyte-like cells (human iPS-HLCs) are expected to become alternative cell sources. Several groups, including ours, have developed protocols of efficient hepatocyte differentiation from human iPS cells and have reported that some hepatic functions (such as albumin (ALB) and urea production capacities) of the generated human iPS-HLCs were similar to those of HHs [2–6]. However, human iPS-HLCs resemble fetal hepatocytes rather than adult hepatocytes [4]. Taken together, these results indicate that a novel technology for large-scale production and further maturation of human iPS-HLCs is necessary for their practical use.

Because a cell adhesion surface is unnecessary in three-dimensional (3D) suspension cultures, a 3D suspension culture can provide more cells than conventional two-dimensional (2D) adherent culture. It was reported that a 3D suspension culture method is suitable for the mass production of undifferentiated human ES/iPS cells. Otsuji et al. have shown that the average human ES/iPS cell yield of a 3D sphere culture system (1.4×10^8 cells/

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Abbreviations

2D	two-dimensional
3D	three-dimensional
3D-HLCs	three-dimensional-cultured hepatocyte-like cells
AAT	alpha-1 antitrypsin
AFP	alpha-fetoprotein
ALB	albumin
BMP	bone morphogenetic protein
CK18	cytokeratin 18
CYP	cytochrome P-450
ECM	extracellular matrix
EGF	epidermal growth factor
FGF	fibroblast growth factor
FOXA2	forkhead box A2
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
HCM	Hepatocyte Culture Medium
HGF	hepatocyte growth factor
HHs	human hepatocytes
HNF4α	hepatocyte nuclear factor 4α
human iPS-HLCs	human iPS cells-derived hepatocyte-like cells
iPS cells	induced pluripotent stem cells
LDL	low-density lipoprotein
MEF	mouse embryonic fibroblast
OsM	oncostatin M
PFA	paraformaldehyde
PHHs	primary human hepatocytes
RCCS	Rotary Cell Culture System
TTR	transthyretin

device) was larger than that of a conventional 2D culture (8.3×10^6 cells/100 mm culture dish) [7]. Therefore, it is expected that a 3D suspension culture method would be also suitable for the mass production of human iPS-HLCs. However, to the best of our knowledge, there is no report on a successful large-scale production (more than a billion scales) of human iPS-HLCs. In addition, a 3D culture method is also widely used for the promotion of cellular maturation by providing a 3D microenvironment that can mimic the cellular environment *in vivo*. Various 3D culture devices, such as low-adhesion plates [8], Nanopillar plates [9], and PDMS chips [10,11], have been used for the maturation of human iPS-HLCs. Therefore, a 3D culture device that can be used for the large-scale production and maturation of human iPS-HLCs is needed.

In this study, we decided to use a bioreactor, the Rotary Cell Culture System (RCCS), for large-scale production and maturation. RCCS can perform large-scale (>1000 ml) 3D suspension cultures by rotating cell culture vessels without propeller stirring. This device enables us to perform cell cultures under low shear stress [12]. Because high shear stress adversely affects the viability and function of hepatocytes [13,14], RCCS is expected to be a useful as a bioreactor for the large-scale production and maturation of human iPS-HLCs. In this study, we first tried billion-scale production of human iPS-HLCs by using RCCS. We then examined various hepatic functions of human iPS-HLCs generated by RCCS.

2. Materials and methods

2.1. Human iPS cells

The human iPS cell line, YOW-iPS cells, generated from primary HHs (PHHs) was maintained on a feeder layer of mitomycin C-treated mouse embryonic fibroblast (MEF, Merck Millipore) with ReproStem (ReproCELL) medium supplemented with 10 ng/ml fibroblast growth factor (FGF) 2 (Katayama Kogyo Kagaku). Details related to YOW-iPS cells were previously described in our report [15].

2.2. 2D hepatocyte differentiation

Before the initiation of hepatocyte differentiation, human iPS cells were dissociated into clumps by using dispase (Roche) and plated onto BD Matrigel Basement Membrane Matrix Growth Factor Reduced (BD Biosciences) coated multi-well plate. These cells were cultured in the ReproStem medium for 3 days. The

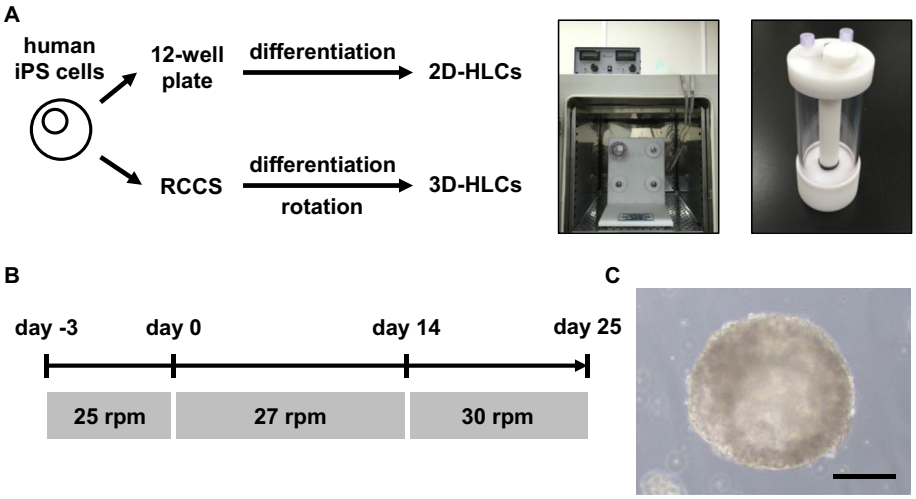

Fig. 1. 3D culture of human iPS cell-derived hepatocyte-like cells using RCCS. (**A**) The procedure for 2D and 3D hepatocyte differentiation is presented schematically. Images of RCCS are shown. Details of the hepatocyte differentiation procedure are described in Materials and Methods. (**B**) Rotation speed of RCCS is shown. Culture vessels rotated at 25, 27, and 30 rpm during pre-culture, hepatoblast differentiation, and hepatocyte maturation, respectively. (**C**) A phase image of 3D-cultured human iPS cell-derived hepatocyte-like cells is shown. The scale bar represents 100 μm.

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