Biochemical and Biophysical Research Communications xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Direct comparison of different coating matrix on the hepatic differentiation from adipose-derived stem cells

Q1 Xuan Zhang, Jiahong Dong *

Hospital and Institute of Hepatobiliary Surgery, Chinese PLA General Hospital, 28th Fu Xing Road, Beijing 100853, People's Republic of China

A R T I C L E I N F O

Article history:
Received 24 October 2014
Available online xxxx

Keywords:
Liver
Decellularized extracellular matrix (DCM)
Adipose-derived mesenchymal stem cells (ADSCs)
Differentiation

A B S T R A C T

Various extracellular matrix components were employed as coating materials to promote hepatic differentiation from ADSCs. However, no consensus was achieved about the optimal coating matrix due to the lack of direct comparison among different coating matrix. In this study, several coating extracellular matrixs were used for hepatic differentiation of ADSCs and direct comparison between them was performed. We demonstrated that liver DCM as coating matrix could significantly enhance the hepatic differentiation from ADSCs compared with collagen, fibronectin and Matrigel both in the presence and absence of GFs, including enhanced hepatocyte-specific genes expression, hepatocyte related protein secretion with improved liver functions. And the differentiated cells also exhibited the characteristics of mature hepatocytes. In conclusion, the study proved an effective hepatic-inducing method and indicated that DCM could promote the differentiation of ADSCs into hepatocyte-like cells, which demonstrates feasibility of liver DCM as a bio-scaffold for liver regenerative medicine and tissue engineering.

1. Introduction

Liver disease is a major cause of mortality in many countries and results in millions deaths annually [1]. For decades, stem cell-based therapies, which hold great promise for the regenerative medicine to repair or reconstitute liver function, have shown the safety, practicability and effectiveness of cell-based tissue regeneration [2–4]. Regarding recent stem cell-based therapies studies, it is critically to develop an efficient way with considerable scale to direct the hepatogenic differentiation from the stem cells. Recently, studies have tried various chemical approaches and factors to direct several stem cell types such as embryonic stem (ES) cells, induced pluripotent stem (iPS) cells and mesenchymal stem cells (MSCs) into functional hepatocyte-like cells [5,6]. Despite the fact that these stem cells have proven their capacity of differentiating into functional hepatocyte-like cells, a highly efficient method for differentiation of stem cells into hepatocytes has not been established yet.

Recently, it has been gradually recognized that stem cell niche, which refers to the cellular microenvironment in which stem cell reside, play a critical role in the fate decisions of cells and could interacts with stem cells themselves to regulate cell fate [7–9]. These cellular micro-environmental factors, such as extracellular space size and shape, matrix elasticity as well as other biochemical and mechanical signals contribute to activity of stem cells, and been shown to regulate the differentiation capacity or destiny of various stem cells [10,11]. The biochemical and mechanical signals that provided from extracellular matrix (ECM) which coordinated interactions with soluble factors and neighboring cells determine the proliferation, survival, migration or proliferation of the cells [7,8,12].

In fact, artificial extracellular matrix (ECM) mimics and natural bio-scaffolds, especially acellular extracellular matrix derived from the decellularized tissue or complex organ, have been proven to successfully support site-appropriate cell attachment or direct the differentiation of several parenchymal and non parenchymal cell types, such as hepatocytes in liver, cardiomyocytes in heart, fibroblasts and endothelial cells for the reason of the preserved "native composition, ultrastructure, and the macroscopic 3D architecture" of native ECM [9,12,13]. What's more, studies also emphasize the important role of tissue-specific biomatrix scaffolds in the lineage restriction of stem cells differentiation. As proven by these studies, decellularized heart matrix could enhanced cardiac lineage differentiation of stem cells [14], and this were also found in acellular natural lung matrix in guiding ESC differentiation toward lung-specific lineage as well as the proliferation and differentiation of ESC in the kidney scaffolds [15,16]. Recently, decellularized liver biomatrix have been used to enhance the differentiation of MSCs into functional hepatocyte-like cells and facilitates treatment of

* Corresponding author.
E-mail address: dongjh301@163.com (J. Dong).

http://dx.doi.org/10.1016/j.bbrc.2014.11.004
0006-291X/© 2014 Published by Elsevier Inc.

liver diseases [17,18]. Various extracellular matrix components could also been employed as coating materials to promote hepatic differentiation from adipose-derived stem cells as well. However, there is no consensus on the optimal coating matrix to induce the hepatic differentiation because of lack of direct comparison among different coating matrix.

The aim of this study was to produce hydrogel scaffold from decellularized liver ECM and to compare the liver DCM as coating matrix for hepatogenic differentiation of ADSCs with several extracellular matrixs, including collagen, fibronectin, Matrigel in the presence and absence of growth factors (GFs). Therefore, the mouse liver was decellularized and processed to form a hepatic gel matrix. ADSCs were then cultivated in the extracellular matrixs coated plates. To determine the differentiation results of the MSCs, RT-PCR, flow cytometric analysis, immune-staining and functional analyses were also performed.

2. Methods

2.1. Isolation and cultivation of AD-MSCs

Mouse adipose-derived mesenchymal stem cells (ADSCs) were prepared as described previously. Briefly, white adipose tissues were isolated from C57BL/6 mice inguinal region and digested with 0.1% type I collagenase + 0.05% trypsin. The single-cell suspended by Alpha Modification of Eagle's Medium (αMEM, HyClone) containing 15% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin was plated into two 25-cm^2 tissue culture flasks. The medium was replaced every 2–3 days and the cells were passaged at a ratio of 1:2. To confirm the immunopheno-type of the cells, surface expression of CD90, CD29, CD45 and CD34 were analyzed at passage 3.

2.2. Preparation and characterization of decellularized rat liver matrix gel

After the Sprague–Dawley (SD) rats were euthanized by 30 mg/kg Pelltobarbitalum Natricum injected intraperitoneally, the liver was fully exposed after across-abdominal incision. The portal vein was then cannulated using an 18-gauge (18G) blunt end needle and injected slowly with about 20 mL heparinized cold PBS (10 IU/mL). The livers were harvested and frozen at −80 °C more than 12 h. The frozen liver was then thawed at 4 °C and loaded into the perfusion system with a perfusion pump to allow the liquids perfusion in 3 mL/min. The livers were decellularized by a modified protocol similar to the whole heart decellularization as previously reported [14,19,20]. Briefly, the liver were perfused with 150 mL heparinized PBS, followed by 0.5% SDS for 12 h, deionized water for 15 min, 1% Triton X-100 for further decellularization and delipidation for 1 h. Finally, the livers were perfused with PBS at least for 72 h to remove detergent residuals.

To determine the residual DNA content in the decellularized matrix, the native liver tissue or decellularized liver matrix were cut into pieces and processed using a TIANamp Genomic DNA assay Kit (TIANGEN, China) following the manufacturer's instructions. The DNA concentration was then determined using NanoDrop 2000C (Thermo Scientific, USA) by standard protocol. The decellularized liver matrix gel was generated as described previously [21,22]. Briefly, the decellularized matrix was lyophilized and ground into a coarse powder, followed by frozen and stored at −80 °C. After that, the aliquots were pepsin-digested in 0.1 M HCl at a concentration of 10 mg ECM per 1 mL HCl. The ECM powder was digested with 1 mg/mL epsin for 2.5–3 days and then added with 1/10 of original digest volume 1 M NaOH and 10× PBS respectively. All processing steps were performed at room temperature. The resulting liquid was diluted with 1× PBS to 5 mg/mL before use.

2.3. Hepatic differentiation of MSCs in vitro

To induce hepatic differentiation, decellularized liver matrix (DLM) gel coated culture plates were used to culture the ADSCs and compared with collagen Type I collagen, fibronectin and Matrigel in the presence or absence of GFs. Briefly, the gels solution were sterilized by exposing to 25 kGy gamma irradiation and diluted with PBS to a final concentration of 0.5 mg/mL Type I collagen (sigma, U.S.A.) solution, 0.5 mg/mL fibronectin (sigma, U.S.A.) solution and 0.5 mg/mL matrigel (sigma, U.S.A.) solution were used as control coating substances. 6-well culture plates were covered with various solutions and incubated overnight at 37 °C. The coated plates were washed three times with phosphate-buffered saline (PBS, Sigma). ADSCs were used at passage 3–6 and seeded at 5×10^3 cells/cm^2 on coated plates and cultured in humidified incubator with 5% CO_2 at 37 °C with or without GFs. As to groups with GFs, cells were cultured for 3 days and replaced with basal medium supplemented with 10 ng/mL basic fibroblast growth factor-4 (bFGF, sigma), 20 ng/mL hepatocyte growth factor (HGF, sigma), 10 ng/mL oncostatin M (OSM, sigma) for 7 days; then replaced the medium with basal medium supplemented with HGF (40 ng/mL), OSM (20 ng/mL), 20 μg/l dexamethasone (Sigma) and 1×insulin-transferrin-selenium premix (ITS +, Lifetechnologies, U.S.A.) for 11 days. As to groups without GFs, cells were treated with no growth factor.

2.4. RT-PCR

Total RNA was extracted using TRIZOL reagent (Invitrogen) after 21 days of differentiation and reverse-transcribed to obtain cDNA. Polymerase chain reaction (PCR) was carried out with the gene specific primers as shown in Table 1, and the glyceraldehydes 3-phosphate dehydrogenase (GAPDH) house keeping gene was used as an endogenous internal control. The amplification protocol included an initial denaturation step at 94 °C for 10 min, followed by 45 cycles of 30 s at 94 °C for denaturation, 30 s at 55 °C for annealing and a final extension at 72 °C for 10 min. The PCR products were analyzed by gel electrophoresis on 1.2% agarose gel stained with ethidium bromide (10 mg/mL, Sigma) and were

Table 1
RT-PCR primers and the expected product sizes.

Gene	Primer		Length
GAPDH	Forward:	CTCTTGCTCTCAGTATCCTTG	372
	Reverse:	GCTCACTGGCATGGCCTTCCG	
Oct4	Forward:	GAAGCAGAAGAGGATCACCTTG	106
	Reverse:	TTCTTAAGGCTGAGCTGCAAG	
AFP	Forward:	CCAGGACCAGGAAGTCTGTT	108
	Reverse:	TAAGCCAAAAGGCTCACACC	
ALB	Forward:	AGACATCCTTATTTCTATGCCC	141
	Reverse:	GACCAATGCTTTCTCCTTCAC	
FOXA1	Forward:	TTCTAAGCTGAGCCAGCTGCA	94
	Reverse:	GCTGAGGTTCTCCGGCTCTTTCAGA	
A1AT	Forward:	CACTATCACCCTGTGGGCAG	84
	Reverse:	CACACTGGCCCCATCATAGAG	
G6PC	Forward:	TCGTTCCCATTCCGCTTC	98
	Reverse:	GGCTTCAGAGAGTCAAAGAGATGC	
CYP3A4	Forward:	TCCTGGCAATCATCCTGGTG	89
	Reverse:	AGGTTTGGGCCCAGGAATC	
CAR	Forward:	TGGGAGGCTGTTAGTGTTCC	102
	Reverse:	GCTATGACCACAACTTCGTGC	

Abbreviations: GAPDH, glyceraldehyde phosphate dehydrogenase; Oct4, octamer-binding transcription factor 4; AFP, alpha-fetoprotein; ALB, album; FOXA1, fork head box A1; A1AT, alpha-1-antitrypsin; G6PC, glucose-6-phosphatase; CYP3A4, cytochrome P450 3A4; CAR, constitutive and rostane receptor. Q4