Biochemical and Biophysical Research Communications 456 (2015) 938–944

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

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ARTICLE INFO

*Article history:*
Received 24 October 2014
Available online 11 November 2014

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

ABSTRACT

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

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http://dx.doi.org/10.1016/j.bbrc.2014.11.004
0006-291X/

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 $5 \times 10^3$ 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.

**Table 2**
DNA contents analysis of decellularized liver compared to normal liver.

| | Fresh liver ($n = 4$) | Decellularized liver matrix ($n = 6$) | $P$-value |
|---|---|---|---|
| DNA (μg/g liver) | 15.4 ± 2.8 | 0.48 ± 0.05 | <0.001 |

visualized and photographed under UV illumination (Uvidoc, UK). The undifferentiated MSCs were used as the negative control (see Table 2).

### 2.5. Flow cytometry analysis

In order to determine the percentage of cells differentiated in the DCM gel scaffold after 21 days of culture in the differentiation medium, the cells were evaluated by flow cytometry analysis. Cells were harvested by digestion and collected by centrifugation, followed by being fixed in 4% paraformaldehyde for 1 h. Finally, the isolated cells were analyzed by FACS with following antibodies: anti-mouse/rat CD90 (1:200, eBiosciences, San Diego, CA), anti-mouse/rat CD29 (1:200, Biolegend, San Diego, CA), anti-mouse CD45 (1:200, Biolegend), anti-mouse CD34 (1:200, Biolegend). The differentiated ADSCs were incubated with primary antibodies (1:200) against alpha fetoprotein (AFP, Santa Cruz Biotechnology, Santa Cruz, CA), albumin (ALB, SantaCruz), cytoketatin 18 (CK18, Santa Cruz), CK19(Santa Cruz), and PE-conjugated anti-rabbit secondary antibody (1:400, Sigma).

### 2.6. Immunofluorescence staining

For the immunofluorescence analysis, the cellular samples were fixed using ice cold 4% paraformaldehyde and rinsed 3 times with PBS. Non-specific sites of the samples were blocked using 5% bovine serum albumin (BSA) for 2 h and were incubated overnight at 4 °C with primary antibody anti-ALB (1:200, Abcam, CA) diluted in antibody dilution solution. After 3 washes with PBS, the samples were incubated for 2 h with the appropriate secondary antibody (1:400) at room temperature for 2 h. DAPI was used to label the nucleus of the cells. The samples were visualized using a confocal microscope (FV-1000, Olympus, Japan).

### 2.7. Hepatocyte-specific function assays

AFP, ALB and urea production were used to analysis the hepatogenic function of differentiated liver cells from MSCs. The conditioned media from the differentiated MSCs cultured with and without exogenous GFs were assayed for AFP and ALB production using quantitative enzyme-linked immunosorbent assay (ELISA) kits (Bethel Laboratories) according to the manufacturer's recommendations. To determine the urea concentrations, the differentiated MSCs were incubated with 1 mL medium containing 5 mM $NH_4Cl$ (Sigma) for 24 h and were measured using a colorimetric assay kit. The level of AFP, ALB and urea was presented in term of pg/cell/day.

## 3. Results

### 3.1. Isolated ADSCs

The isolated cells were cultured in tissue culture dishes and demonstrated spindle-like morphologies at the early stage (Fig. 1). With following cultivation, elongated cells gradually appeared with the time. Flow cytometry was used to further analyze the characteristics of passage cells. The results of cells at passage 3 showed that the expression of CD90 and CD29 (suggested to be one of MSC markers) are 92.13 ± 2.11% and 85.68 ± 2.65% respectively, and also most of the cells were negative for CD45 (3.2% ± 0.3%) and CD34 (3.4% ± 0.4%).

### 3.2. Characterization of DCM gel

We achieved whole-liver decellularization by portal detergent perfusion and obtain a translucent acellular scaffold after

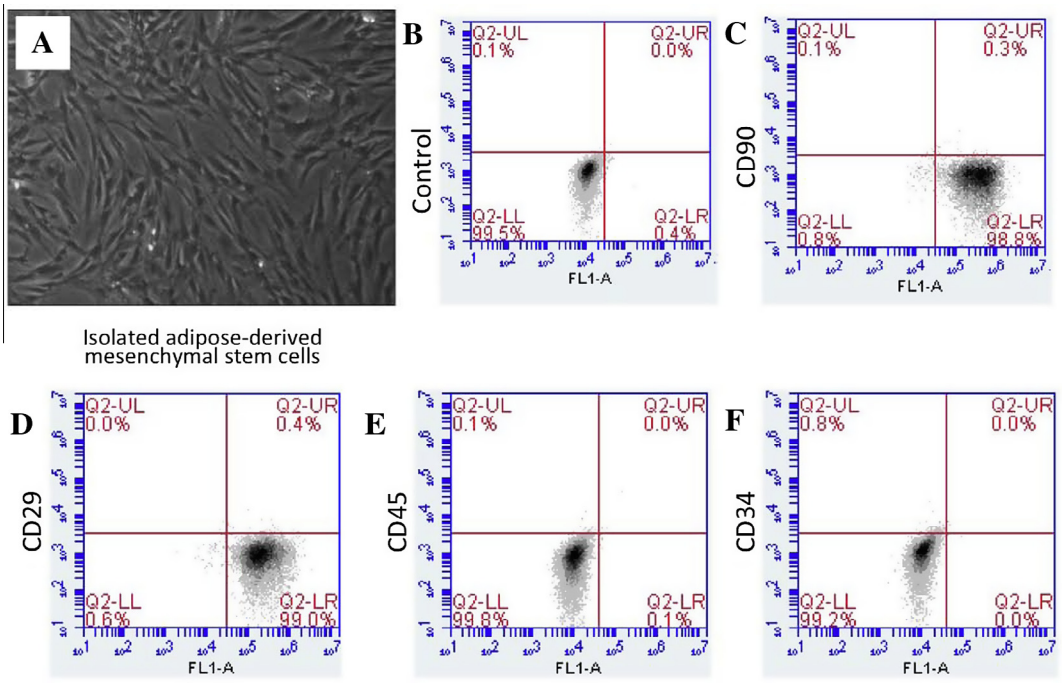


**Fig. 1.** Isolation and characterization of AD-MSCs. (A) The morphology of the ADSCs in the third (P3) passage observed under bright field. (B–F) Surface molecule characterization of the ADSCs at P3 passage analyzed by flow cytometry after incubation with antibodies CD90, CD29, CD45, CD34.

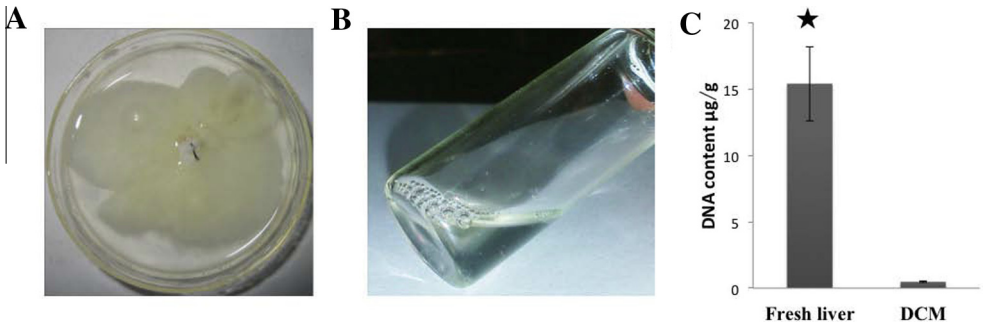


**Fig. 2.** Decellularization and characterization of rat liver matrix gel. (A) General appearance of the rat liver after decellularization and (B) the decellularized liver matrix gel. (C) Residual DNA content in the fresh liver tissue and decellularized liver matrix. *P < 0.05.

decellularization. The perfusion treated scaffold retained the intact three-dimensional geometry and gross shape of liver. Data also shown that decellularized scaffold's residual DNA content was about 3% as compared with the natural liver tissue. After gelation by using pepsin, the solubilized liver ECM gel remained a viscous liquid at a low temperature (at or under room temperature) and become gelation when been induced at 37 °C (Fig. 2).

### 3.3. DCM gel supports hepatogenic differentiation

To evaluate the effects of decellularized liver ECMs with or without GFs on the hepatogenic differentiation of mouse ADSCs, expression levels of hepatocyte genes were examined b RT-PCR analysis. As shown in the Fig. 3A, even through Oct4 mRNA expression in all groups with GFs decreased obviously over 21 days compared to the groups without GFs, whereas Oct4 mRNA expression levels of MSCs cultured in the Matrigel and DCM gel with GFs decreased significantly, which indicated the loss of stemness in MSCs during the differentiation. The mRNA expression of AFP and ALB in the cells cultured in DCM without GFs increased significantly (on day 21s) compared to the others three groups without GFs, and even the expression of AFP and ALB in all four groups with GFs increased, cells cultured in DCM with GFs has the biggest increase. Compared to the ADSCs in other groups with or without GFs, the cells cultured both in Matrigel and DCM with GFs exhibited a dramatically increase in the mRNA expression of FOXA1, A1AT and G6PC. Compared with those of other groups, mRNA expression of CYP3A4 and CAR increase significantly in MSCs in the DCM in the presence of GF on day 21.

To further characterize the differentiated cells, we performed flow cytometry analysis to determine the exact percentage of the ADSCs-derived cells expressing hepatic proteins AFP, ALB and cytoketatins 18 (CK18). In the absence of GFs, AFP percentages in Collagen, Fibronectin, Matrigel and DCM without GFs were 3.9% ± 0.3%, 3.8% ± 0.4%, 5.4% ± 1.0%, 12.2% ± 1.8% respectively; ALB percentages in Collagen, Fibronectin, Matrigel and DCM without GFs were 2.0% ± 0.4%, 2.4% ± 0.3%, 3.4% ± 0.8%, 6.6% ± 1.1% respectively; CK18 percentages in Collagen, Fibronectin, Matrigel and DCM without GFs were 4.0% ± 0.9%, 3.9% ± 0.4%, 6.4% ± 0.8%, 8.4% ± 1.0% respectively. In the presence of GFs, AFP percentages in Collagen, Fibronectin, Matrigel and DCM with GFs were 25.2% ± 4.4%, 22.2% ± 5.1%, 32.4% ± 5.8%, 50.3% ± 8.1% respectively; ALB percentages in Collagen, Fibronectin, Matrigel and DCM with GFs were 10.1% ± 2.3%, 10.8% ± 2.2%, 16.1% ± 2.8%, 26.7% ± 8.2% respectively; CK18 percentages in Collagen, Fibronectin, Matrigel and DCM with GFs were 10.5% ± 3.0%, 9.8% ± 3.3%, 16.5% ± 3.8%, 24.7% ± 4.5% respectively. The data indicated that the percentage of AFP-, ALB- and CK18-positive cells in the DCM with GFs were dramatically higher than in the other groups (Fig. 3C–F).

To confirm the hepatic differentiation of the ADSCs, the expression of AFP (the definitive marker of the hepatic endoderm or early hepatic differentiation) was analyzed using immunofluorescence staining as well (Fig. 3B). The increases in the protein expression of AFP was detected in cells cultured on the DCM scaffold coated plates in the presence of GFs as compare with the others groups.

### 3.4. Hepatic functionality of differentiated ADSCs

To evaluate whether these hepatocyte-like cells derived from ADSCs also have typical functional hepatic features, AFP and ALB secretion, ammonia metabolism activity were analyzed. Both AFP and ALB proteins were detected at lower levels in the cells cultured in the Collage and Fibronectin without GFs groups as compared with the Matrigel and DCM without GFs groups. The secretion of AFP and ALB in four groups with GFs increased while compare with the other four groups without GFs, whereas the production of AFP and ALB in the cells cultured in DCM with GFs were significantly higher than the cells of three other groups with GFs. Moreover, the urea production was significantly higher in the DCM cultured cells than in the collage, fibronectin and Matrigel cultured cells without GF (Fig. 4). Compared with the groups without GFs, the urea production of four groups all rise several times in the presence of GF respectively but cells cultured in DCM with GFs still has a significant high levels than cells cultured under the other three conditions with GFs (Fig. 4).

## 4. Discussion

Recently, it was reported that decellularized liver 3D matrix scaffold with complex and varied environmental factors support to facilitate the activity and function of the hepatic or stem cell [17,18,21]. In this study, we tried to direct compare the hepatogenic-differentiation-inducing effect of a natural 3D matrix gel from the decellularized liver ECM and several extracellular matrixs, including collagen, fibronectin, Matrigel for used as coating matrix. The results clearly demonstrated that decellularized liver ECM gel, either on its own or in the presence of GFs, performed better in enhancing the hepatogenic differentiation of mouse ADSCs, which induced the lineage-specific differentiation of ADSCs into functional hepatocyte-like cells than the others.

Extracellular matrixs (ECMs) are secreted extracellular molecules that consist primarily of a diverse variety of growth factors, glycosaminoglycans (GAGs) and fibrous proteins such as a variety of collagen types, fibronectin and laminin. It is ECM that provides the most important dynamic and complex structural or biochemical support in regulating the functions of the surrounding cells and tissues. Under these conditions, cells and ECM contribute to the development of complex tissue, which eventually lead to the for-

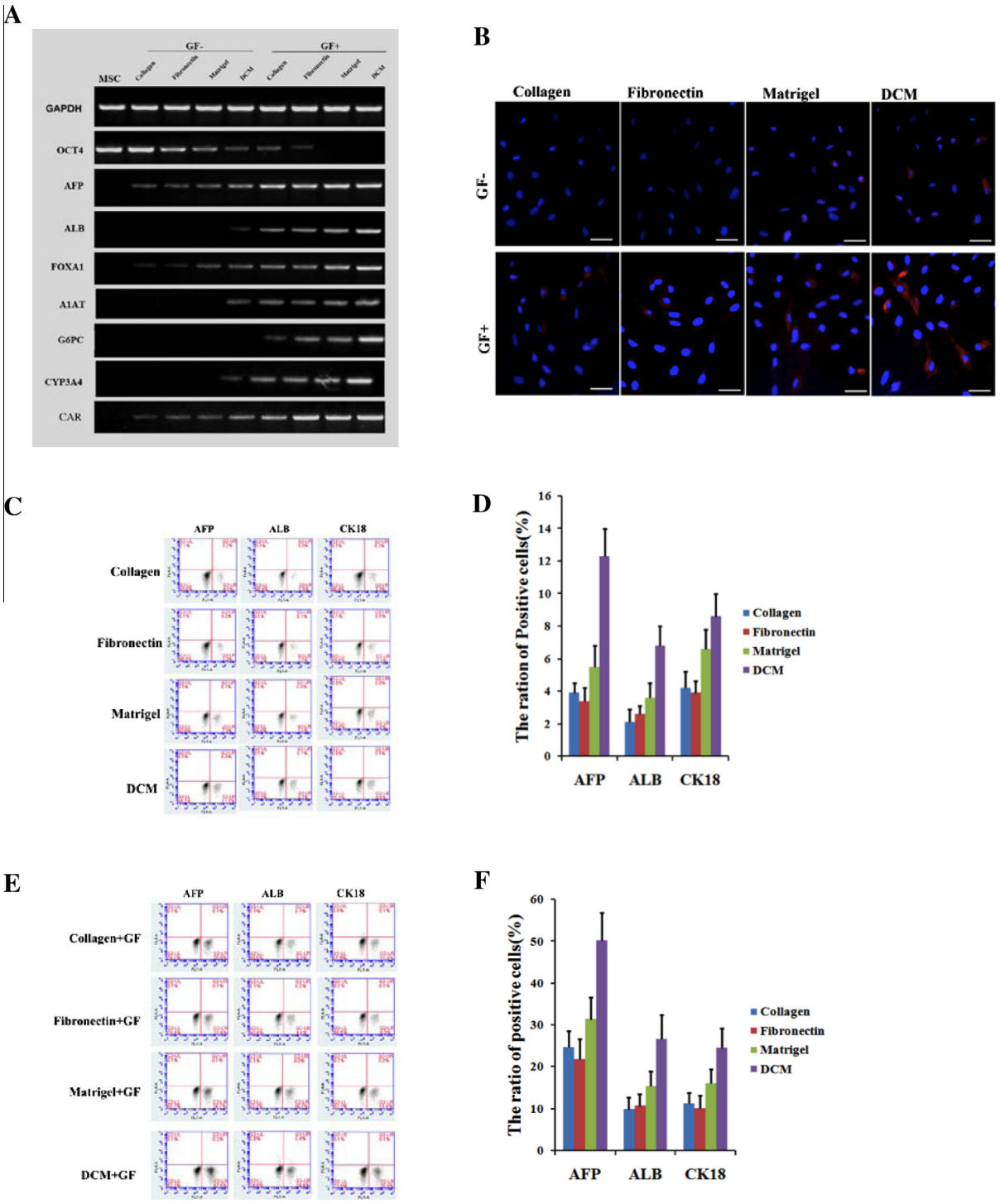


**Fig. 3.** Analysis of hepatic differentiation from adipose-derived stem cells. (A) Expression levels of liver-specific gene expression by ADSCs-derived cells after 21 days of cultures in different cultured scaffold with or without factors (GF). The undifferentiated MSCs were used as controls. (B) Immunofluorescence staining AFP expression of ADSCs-derived cells after 21 days of cultures in different cultured scaffold with or without factors (GFs). (C–F) Flow cytometry analysis of differentiated cells: (C, D) the exact percentage of the ADSCs-derived cells expressing hepatic proteins AFP, ALB and cytoketatins 18 (CK18) in the absence of GF. (E, F) The exact percentage of the ADSCs-derived cells expressing hepatic proteins AFP, ALB and CK18 in the absence of GF. 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.

mation functional organs [9,23]. Therefore, the renewal and differentiation of cells are coordinated with the multiple changing spatial and temporal factors, and this is difficult to achieve under normal 2D conditions. Besides, the artificial 3D culture systems that were thought to mimic the micro-environment of the native tissue were only to a limited extent due to the extremely complicated features of ECM [24,25].

Recently, it is reported that whole organ-derived ECM scaffolds prepared by techniques that involve perfusion-based decellularization of donor organs could largely preserved the macroscopic 3D

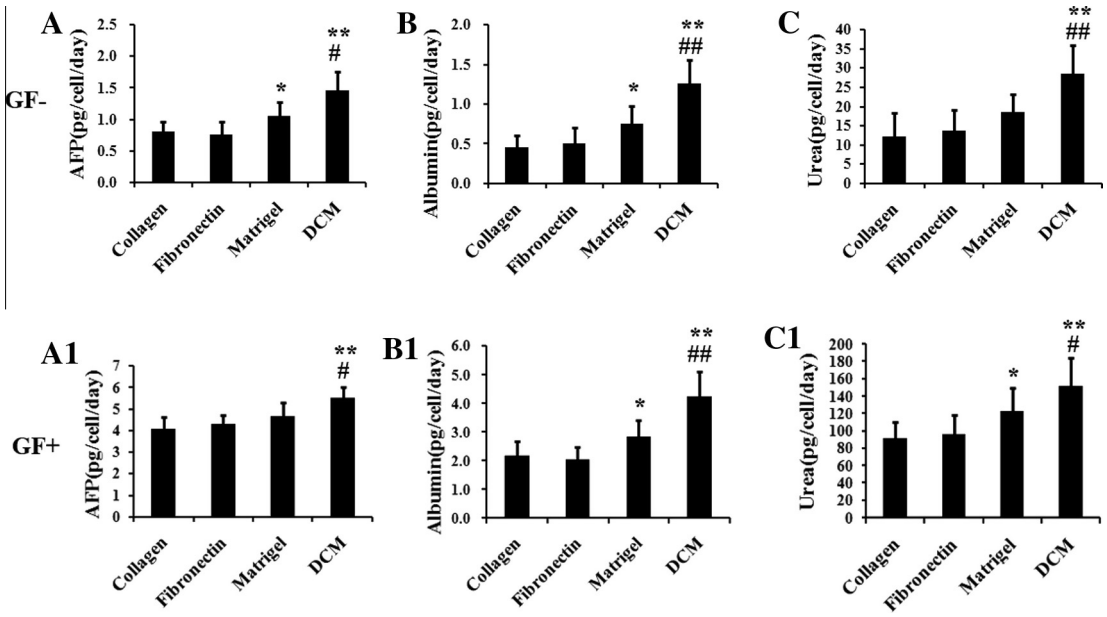


**Fig. 4.** Hepatic functionality of differentiated ADSCs. (A–C) Functional tests of ADSC-derived cells after 21 days of cultures in different cultured scaffold without factors (GF). (A) Alpha-fetoprotein (AFP), (B) Albumin (ALB) and (C) Urea production by the ADSCs-derived cells was analyzed after 21 days of cultures. (A1-C1) Functional tests of ADSC-derived cells after 21 days of cultures in different cultured scaffold with factors (GF). (A1) AFP, (B1) ALB and (C1) Urea production by the ADSCs-derived cells was analyzed after 21 days of cultures. "★" stands for $P < 0.05$, "★" with"#"stands for $P < 0.001$.

architecture, the native composition and ultrastructure of tissues and organs [23,26]. The decellularized 3-D scafflods derived from donor organs such as heart [19], liver [20] and lung [27] could be seeded with either functional parenchymal cells or selected stem cell populations [28,29].

In this study, the decellularized liver matrix was prepared by a modified common protocol based on SDS perfusion combined with chemical and physical techniques as previously reported [14,19, 20,28]. A translucent matrix with intact 3D liver geometry and most ECM compositions was obtained within a within a short period. The amount of residual dsDNA retained in the decellularized liver was less than 4%.

Since ECM is the secretion of the resident cell populations, it is definitely that decellularized organ-specific ECM scaffolds are different in the component molecules composition as well as its ultrastructural organization, which depend on the source tissue/organs the most [23]. Therefore, the organ-specific differentiation-inducing effect of the decellularized ECM-organ-specific 3D scaffold matrix could enhance certain lineage-specific differentiation of stem cells. The previously studies had proven it in the 3D matrix obtained from the heart, kidney, lung and liver [14,16,17]. Consider these, to understand the effects of ECM gel prepared from decellularized live matrix on cell growth and hepatic cell differentiation, we cultured the ADSCs into the biomatrix scaffold under GFs conditions and compare it with some regular ECM compositions such as collagen, fibronectin and matrigel. The results of hepatic genes DNA analysis revealed that the liver matrix gel directed differentiation of the stem/progenitor cells into functional hepatic lineage. The flow cytometry analysis and AFP immunofluorescence staining confirmed the presence of differentiated hepatic cells, and the hepatic functionality analysis indicated the liver-specific metabolic function of it as well.

However, it's important to note that the hepatogenic differentiation of ADSCs cultured under the conditions of liver ECM gel in presence of GFs is better than the ADSCs cultured in liver ECM gel without GFs. One reason may be that the decellularization process resulted in a certain extent loss of ECM compositions, especially the GFs binding to GAGs or the inactivation of the GFs [23].

## 5. Conclusions

The results showed that the potential of a 3D gelling scaffold derived from decellularized whole-liver matrix for liver regenerative medicine or tissue engineering use. We successfully demonstrated solubilized liver matrix could form a gel in vitro, and the liver organ-specific bio-matrix scaffold could providing better biomimetic environment and enhance the hepatogenic differentiation of mouse ADSCs with or without hepatic GFs than other ECM components (collagen, fibronectin and Matrigel in this study). The present study clearly proved the feasibility of a liver matrix gel based strategy for the production of functional hepatocytes and may have considerable potential in the future for liver cell-based therapy or tissue engineering.

## Conflict of interest statement

The authors have no conflict of interest to disclose.

## Acknowledgments

*Funding sources*: This research was supported in part by the National Key Technology R&D Program of China through Grant No. 2012BAI06B01 and National S&T Major Project for Infectious Diseases of China through Grant No. 2012ZX10002-017.

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