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Differentiation of Human Embryonic Stem Cells to Hepatocytes Using Deleted Variant of HGF and Poly-amino-urethane-Coated Nonwoven Polytetrafluoroethylene Fabric

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Human embryonic stem (hES) cells have recently been studied as an attractive source for the development of a bioartificial liver (BAL). Here we evaluate the differentiation capacity of hES cells into hepatocytes. hES cells were subjected to suspension culture for 5 days, and then cultured onto poly-amino-urethane (PAU)-coated, nonwoven polytetrafluoroethylene (PTFE) fabric in the presence of fibroblast growth factor-2 (bFGF) (100 ng/ml) for 3 days, then with deleted variant of hepatocyte growth factor (dHGF) (100 ng/ml) and 1% dimethyl sulfoxide (DMSO) for 8 days, and finally with dexamethasone (10^{-7} M) for 3 days. The hES cells showed gene expression of albumin in a time-dependent manner of the hepatic differentiation process. The resultant hES-derived hepatocytes metabolized the loaded ammonia and lidocaine at 7.8% and 23.6%, respectively. A million of such hepatocytes produced albumin and urea at 351.2 ng and urea at 7.0 μg. Scanning electron microscopy showed good attachment of the cells on the surface of the PTFE fabric and well-developed glycogen rosettes and Gap junction. In the present work we have demonstrated the efficient differentiation of hES cells to functional hepatocytes. The findings are useful to develop a BAL.

Key words: Human ES cells; Hepatocyte; Hepatocyte growth factor; Differentiation

INTRODUCTION

Acute liver failure (ALF) is a clinical syndrome that is caused by liver damage by a large number of noxious agents and is associated with a high mortality rate. Orthotopic liver transplantation can improve the survival of patients with ALF and is currently the treatment of choice for patients with signs of poor prognostic (14,27). However, the shortage of donor livers, high costs, and life-long requirement for immunosuppression limit the use of this from of therapy (27). Several approaches have been investigated to provide short-term hepatic support until the damaged liver spontaneously recovers or a donor liver becomes available for transplantation. A combination of continuous hemodiafiltration and plasma exchange (1) or albumin dialysis (21,24) is currently used as a temporary support for many patients with failing liver function, but such treatments contribute to some improvements in blood parameters and unfortunately many patients do not survive.

Therefore, more effective therapies, such as bioartificial livers (BALs), should be explored (7). BAL devices containing functionally active hepatocytes are capable of removing toxins from the blood and also of providing physiologically active substances into the patient's circulation. Most of the currently available BALs utilize porcine hepatocytes due to severe shortage of human livers for hepatocyte isolation. Bioincompatibility of humans and pigs and fear of transmission of unknown viruses are major hurdles (17,19,25). Thus, researchers have been exploring alternative sources of human hepatocytes, such as immortalized human hepatocytes (10) and embryonic stem (ES) cell-derived hepatocytes (2,9, 12,23). One of the unique advantages of ES cells over immortalized hepatocytes is that ES cells can be grown without any genetic manipulation and, when provided an appropriate environment of differentiation, the cells can differentiate into any type of mature functional cells. We have currently developed a new type of bioartificial pancreas that allows a three-dimensional cell culture us-

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ing a poly-amino-urethan (PAU)-coated, nonwoven polytetrafluoroethylene (PTFE) fabric and controlled blood glucose levels in diabetic pigs (8).

In this study, we have applied PAU-coated PTEF cloth for a scaffold of human ES (hES) cells in the process of hepatic differentiation. As a potent hepatic differentiation inducing grow factor, we have focused on the use of deleted variant of heaptocyte grow factor (HGF), dHGF, based on our previous work on functional culture of primary human hepatocytes.

Here we report the efficient differentiation of hES cells to functional hepatocytes using PAU-coated PTEF cloth and dHGF in a total of 14 days.

MATERIALS AND METHODS

Culture of hES Cells

The hES cell line KhES-1 (kindly provided by Dr. N. Nakatsuji, Kyoto University) was used for the present studies. The cells were cultured with mouse embryonic fibroblast (MEF) conditioned medium containing 80% knockout DMEM, 2 mM Gluta MAX, 0.1 mM β-mercaptoethanol, and 4 ng/ml basic fibroblast growth factor (bFGF) (R&D Systems, Inc.) in six-well plates, the surface of which was coated with Matrigel (Becton Dickinson), according to a feeder-free method (28). For passage, the cells were dissociated with the use of collagenase IV solution (200 U/ml) (Gibico BRL/Invitrogen, Co.). The cell culture was conducted in a 5% CO_2/air mixture at 37°C.

Embryonic Body (EB) Formation of hES Cells

To form embryonic bodies (22), hES cells (2.5×10^5 cells/ml) were suspended in Dulbecco's Eagle medium Nutrient Mixture F-12 (DMEM/F12) (Ham) 1:1 (Gibico BRL/Invitrogen, Co.), 10% fetal bovine serum (Cell Culture Technology, Co.), 100 μg/ml penicillin, and 100 μg/ml streptomycin (Sigma). hES cells were incubated by suspension culture method for 5 days to develop into EBs (Fig. 1).

Hepatic Differentiation of hES Cells

The resultant EBs formed by hES cells were inoculated in each well of a 12-well plate that contained a 10 × 10-mm piece of PTFE fabric coated with PAU, which has cellular adhesive property, and allowed to attach and spread on the PTFE as a platform of three-dimensional culture condition for all process of differentiation (8). The EBs were treated for 3 days with 100 ng/ml basic fibroblast growth factor (bFGF2) (PreproTech EC, UK), with 100 ng/ml deleted variant of hepatocyte growth factor (dHGF) and 1% dimethyl sulfoxide (DMSO) for 8 days, and finally with 10^{-7} M dexamethasone (DEX) (Sigma) for 3 days. As a control, the EBs were similarly cultured in the wells of 12-well-plates coated with Matrigel instead of the use of PAU-coated PTFE fabric.

Morphological Assessment

During the time of culture, morphological appearance of hES cells was observed using a phase contrast microscope (Olympus CK40-SL Japan). On days 1 and 14, the cells inoculated onto unwoven PTFE cloth coated with PAU were subjected to microstructural analysis using scanning electron microscope (SEM) (Hitachi S-2300, Hitachi Co. Ltd., Tokyo, Japan) and transitional electron microscope (TEM) (Hitachi H-7100, Hitachi Co. Ltd.). For SEM, the samples were washed with PBS followed by fixation with 2% glutaraldehyde for 2 h at 37°C, and gently washed with PBS. The samples were then postfixed with osmium tetraoxide for 2 h and dehydration was accomplished using a graded series of ethanol (50%, 60%, 70%, 80%, 90%, and 99%). The samples were then dried at critical point for 2 h in absolute alcohol and mounted on an aluminum stub and sputter-coated with gold before viewing under SEM. For TEM, the cells were fixed, first in 2.5% glutaraldehyde in 0.1 M PB, and then in 1.0% OsO_4 in 0.1 M PB (pH 7.2). The samples were dehydrated through graded concentrations of ethanol and embedded in Epon, as previously reported (11,18). Ultrathin sections of the samples were double stained with uranyl and observed under TEM. Ten different areas were randomly chosen and examined.

Gene Expression of Albumin in hES Cells

To evaluate the gene expression of albumin at the mRNA level in hES cells harvested, we performed a reverse transcription-polymerase chain reaction (RT-PCR) analysis, as previously reported (5,18). For PCR, 35 cycles were applied. Undifferentiated hES cells and normal human hepatocytes were used as a negative and a positive control, respectively. The human β-actin gene, housekeeping gene, served as an internal control. Primers used were as follows: albumin [576 base pairs (bp)], sense, 5′-AAACCTCTTGTGGAAGAGCC-3′, antisense, 5′-CAAAGCAGGTCTCCTTATCG-3′; β-actin (610 bp), sense, 5′-TGACGGGGTCACCCACACTGTGCCCATCTA-3′, antisense, 5′-CTAGAAGCATTTGCGGTGGACGATGGACGG-3′.

Using RNAs obtained for RT-PCR analysis mentioned above, real-time RT-PCR assay for the gene expression of albumin was performed, as previously reported (18). Briefly, 0.1 μg of total RNA from each sample was prepared for reverse transcription. mRNA copy numbers of albumin and β-actin, the housekeeping gene (HKG), were determined by real-time quantitative RT-PCR using a LightCycler instrument, a LightCycler

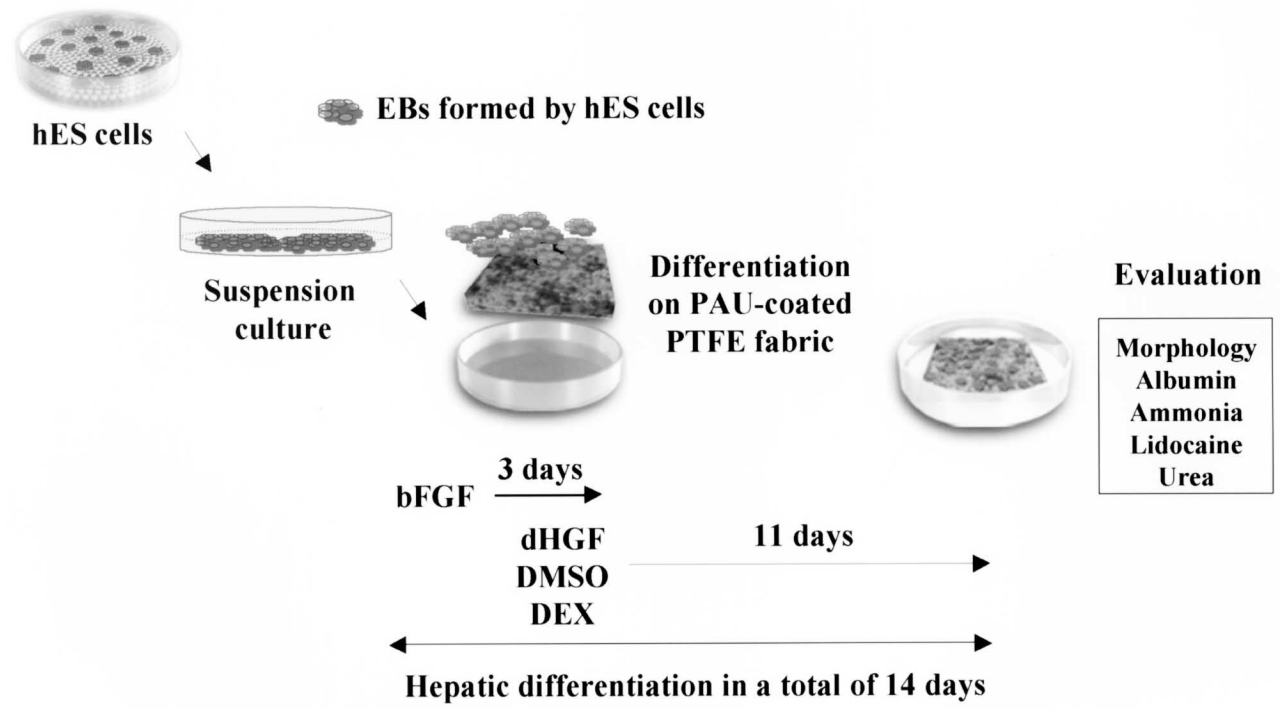

Figure 1. Schematic representation of the strategy for differentiation of hES cells to hepatocytes. Human embryonic stem (hES) cells were cultured in suspension method for 5 days to form embryoid bodies (22). The resulting EBs were transferred onto nonwoven polytetrafluoroethylene (PTFE) fabric coated with PAU and treated with fibroblast growth factor (FGF)-2 (100 ng/ml) for 3 days, then with the deleted variant of hepatocyte growth factor (dHGF; 100 ng/ml)) and 1% dimethyl sulfoxide (DMSO) for 8 days, and at the end stage with dexamethasone (DEX; 10^{-7} M) for 3 days. As a control, EBs were cultured with Matrigel instead of PTFE cloth.

DNA Master SYBR Green I kit, and LightCycler Control Kit DNA (Roche Diagnostics GmbH, Mannheim, Germany). Copy numbers of mRNA were calculated from serially diluted standard curves generated from a cDNA template, which represented in vitro samples and confirmed bands with conventional PCR. Data were analyzed by using LightCycler Software (Roche Molecular Biochemicals). All expression levels were normalized to β-actin in each well.

Evaluation of Metabolic and Synthetic Capacities of hES Cells

At the end of hepatic differentiation of 14 days, hES cells cultured with Matrigel or unwoven PTFE were subjected to metabolic and synthetic tests. Ammonium sulfate (0.56 mM) and lidocaine (1 mg/ml) were added to individual wells of 12-well plates and the amount of each substrate remaining in the media after culture for 4 h was measured. The ammonia concentration was determined using a Fuji Dri-Chem slide (Fuji Co., Tokyo, Japan) and concentration of lidocaine was measured by SRL (Tokyo, Japan). Four hours after ammonia loading, 10 μm of culture medium was collected for urea synthesis, as previously reported (5). Albumin secretion into the culture medium for 24 h was measured by an albumin enzyme-linked immunosorbent assay kit (ALBU WELL II; Exocell, Philadelphia, PA). These functional parameters were compared per microgram cell.

Statistical Analyses

Mean values are presented with SDs. A Student's *t*-test was used to calculate the significance of difference in mean values. A value of $p < 0.05$ was considered statistically significant.

RESULTS

EBs Attached Favorably on PAU-Coated Nonwoven PTFE Fabric and Spread Out During Hepatic Differentiation

hES cells were incubated by suspension culture method for 5 days and formed EBs. The resultant EBs attached favorably on the surface of nonwoven PTFE fabric coated with PAU that has cellular adhesive prop-

erties. The EBs were uniformly distributed over the PTFE cloth (Fig. 1A). At day 14 of hepatic differentiation process, hES cells were uniformly distributed over the PTFE cloth (Fig. 2B). Such hES-derived hepatocytes revealed polygonal shape with two nuclei (Fig. 2C), which is compatible with the characteristics of normal human hepatocytes in culture. Notably, TEM showed that such cells demonstrated well-developed glycogen rosettes, indicated by arrows in Fig. 2E and Gap junction between the cells (Fig. 2D).

hES Cell-Derived Hepatocytes Showed Albumin Gene Expression and Albumin Secretion

The expression of albumin was detected in the time-dependent manner of the process of hepatic differentiation of 14 days. hES cells cultured on PAU-coated PTFE fabric showed a more intense band for albumin than Matrigel-treated ones (Fig. 3). No albumin expression was detected in either undifferentiated hES cells or EBs on day 5 (Fig. 3). Such expression profile was also confirmed by a real-time PCR. The data are representative from three independent experiments.

The amount of albumin produced by hepatocytes induced by hES cells on PTFE fabric at day 14 of the differentiation process for 24 h (351.2 ng/ml) was better than that produced by hepatocytes on Matrigel (286.2 ng/ml), as shown in Figure 4A. The value was compared per microgram cell. No albumin production was detected in undifferentiated human ES cells. Data are means ± SD. The data are representative from three independent experiments.

hES-Derived Heaptocytes Metabolized Ammonia and Lidocaine and Produced Urea

At day 14 of the hepatic differentiation process, hES cell-derived hepatocytes induced on PAU-coated PTFE fabric metabolized 7.8% of the loaded ammonia and 23.6% of the loaded lidocaine, respectively (Fig. 4B, C). Such metabolic activities were not observed in undifferentiated hES cells. The use of PAU-coated PTFE fabric significantly enhanced hepatic function of hES-drived hepatocytes in terms of albumin (Fig. 4A) and urea synthesis (Fig. 4D) and lidocaine metabolism compared to that of Matrigel. The values were compared per micro-

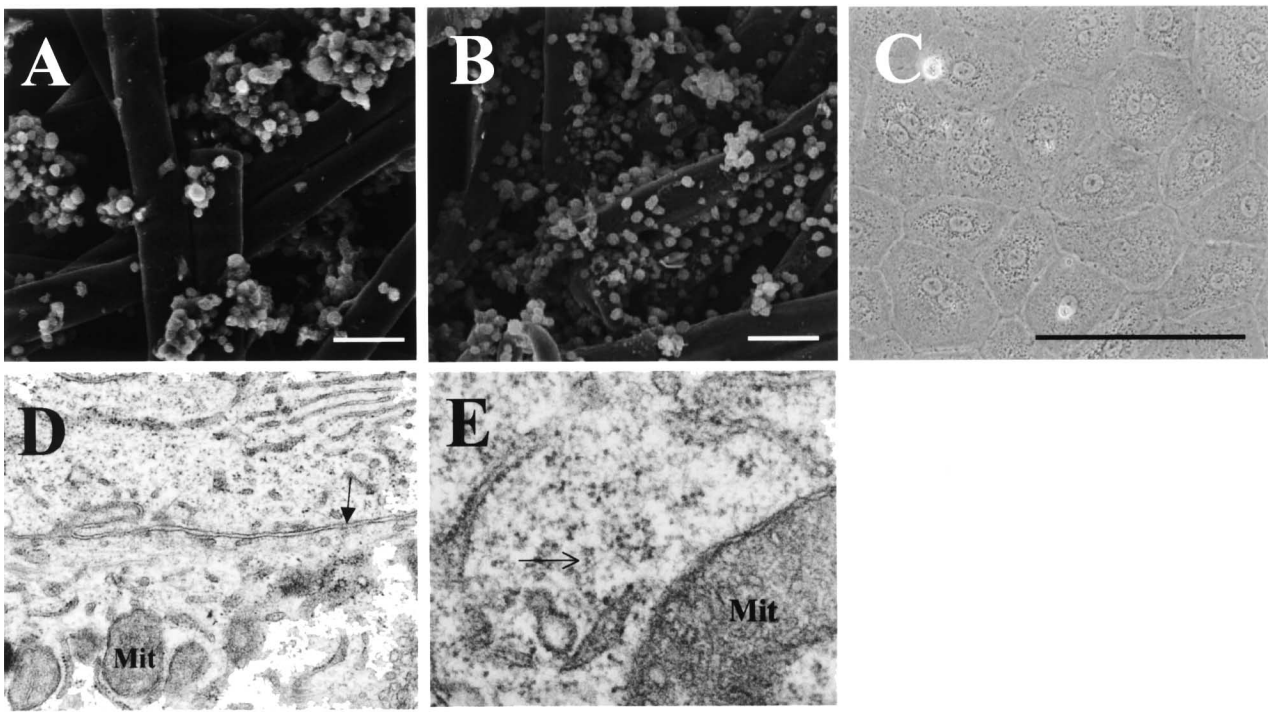

Figure 2. Morphology of hES cell-derived hepatocytes. (A) The EBs formed by hES cells attached favorably on the surface of nonwoven PTFE fabric coated with PAU. The EBs were uniformly distributed over the PTFE cloth. Scale bar: 100 μm. (B) At day 14 of hepatic differentiation process, hES cells were uniformly distributed over the PTFE cloth. Scale bar: 100 μm. (C) The cells morphologically revealed polygonal shape with two nuclei and enriched cytoplasmic granules, which is compatible with the characteristics of normal human hepatocytes in culture (original magnification ×200). Scale bar: 50 μm. TEM showed that such hES cell-derived hapatocytes demonstrated well-developed glycogen rosettes (E) and Gap junction between the cells (D) (original magnification ×15,000).

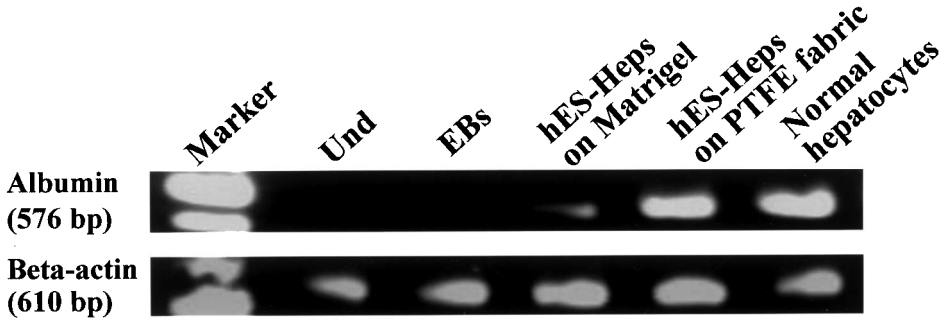

Figure 3. Gene expression profile of albumin of hES cell-derived hepatocytes. At 14 days during the process of differentiating hES cells into hepatocytes, the expression profile of albumin was analyzed by RT-PCR. Normal human hepatocytes served as positive control, and undifferentiated hES cells served as negative control. Such profile was confirmed by real-time RT-PCR analysis. Und, undifferentiated hES cells; EBs, embryonic bodies formed by hES cells on day 5; hES-Heps on Matrigel, hES-derived hepatocytes cultured on Matrigel on day 14 of hepatic differentiation; hES-Heps on PTFE fabric, hES-derived heapatocytes cultured on PAU-coated nonwoven PTFE fabric on day 14 of hepatic differentiation.

gram cell. Data are means ± SD. The data are representative from three independent experiments.

DISCUSSION

Normal human hepatocytes are an ideal source for BAL development; however, the shortage of donor livers severely limits their use of normal human hepatocytes in BAL. In addition, once human hepatocytes are available from discarded livers that are not suitable for organ transplantation, primary isolated hepatocytes do not proliferate in vitro. To overcome these problems, pluripotent stem cells have been investigated as a potential alternative to hepatocytes (2,9,12,23) because they can unlimitedly replicate in the undifferentiated state and produce progenitors of various tissue-specific cells. Although methods for hepatic differentiation of human ES cells have been reported, none of them have described drug- or ammonia-metabolizing activities (13, 20). In the present study, our hES cell-derived hepatocytes metabolized 23.6% of the loaded lidocaine in a total of 14 days of differentiation process, which was half that of normal human hepatocytes. Because hyperammonemia is one of the causes of hepatic encepaphalopathy (4,14), detoxification of ammonia is an extremely important function of hES cell-derived hepatocytes. Our hES cell-derived hepatocytes metabolized 7.8% of the loaded ammonia, which was only one fifth that of normal human hepatocytes, but it is encouraging, because our data are the first report of ammonia metabolism in in vitro culture of human ES cells.

To enhance the functions of hES cell-derived hepatocytes, such as ammonia-metabolizing activity, we have now considered the following two strategies: 1) coculture of hES cells with nonparenchymal human liver cells, and 2) application of activin-A for global endodermal induction of hES cells in an earlier stage of hepatic differentiation (6). Heterotypic cell interactions have been recognized to be central to the differentiation and function of many organ systems (3). In both the developing and mature adult liver, cell-to-cell interactions are important for coordinating the sophisticated liver functions (3). Previously our laboratory has developed human liver endothelial cell line TMNK-1 (16), human liver stellate cell line TWNT-1 (26), and human cholangiocyte cell line MMNK-1 (15). Thus, coculture of hES cells with these cell lines would be attractive.

Researchers have often used collagen or Matrigel for hepatic differentiation of hES cells as a scaffold, but we have to take it for granted that such a material is animal derived. Considering the clinical application of hES-derived hepatocytes, we have to explore nonanimal-derived materials. PAU-coated nonwoven PTEF fabric is animal free and is chemically synthetic. We have already confirmed that the material is biocompatible when used as a component of an extracorporeal bioartificial pancreas in diabetic pigs. In fact, the use of PTEF fabric coated with PAU allowed the hES cells to form three-dimentional structure and cell–cell interactions.

Another important issue is purification of the hepatic population from the whole hES cells after hepatic differentiation. The use of hepatocyte-specific promoter-based cell sorting or specific receptor-mediated magnetic cell sorting would be a useful means to purify such populations.

The present study has clearly demonstrated that in vitro hepatic differentiation of hES cells is practically feasible. Further efforts will be required to generate more mature hepatocytes whose functions are compati-

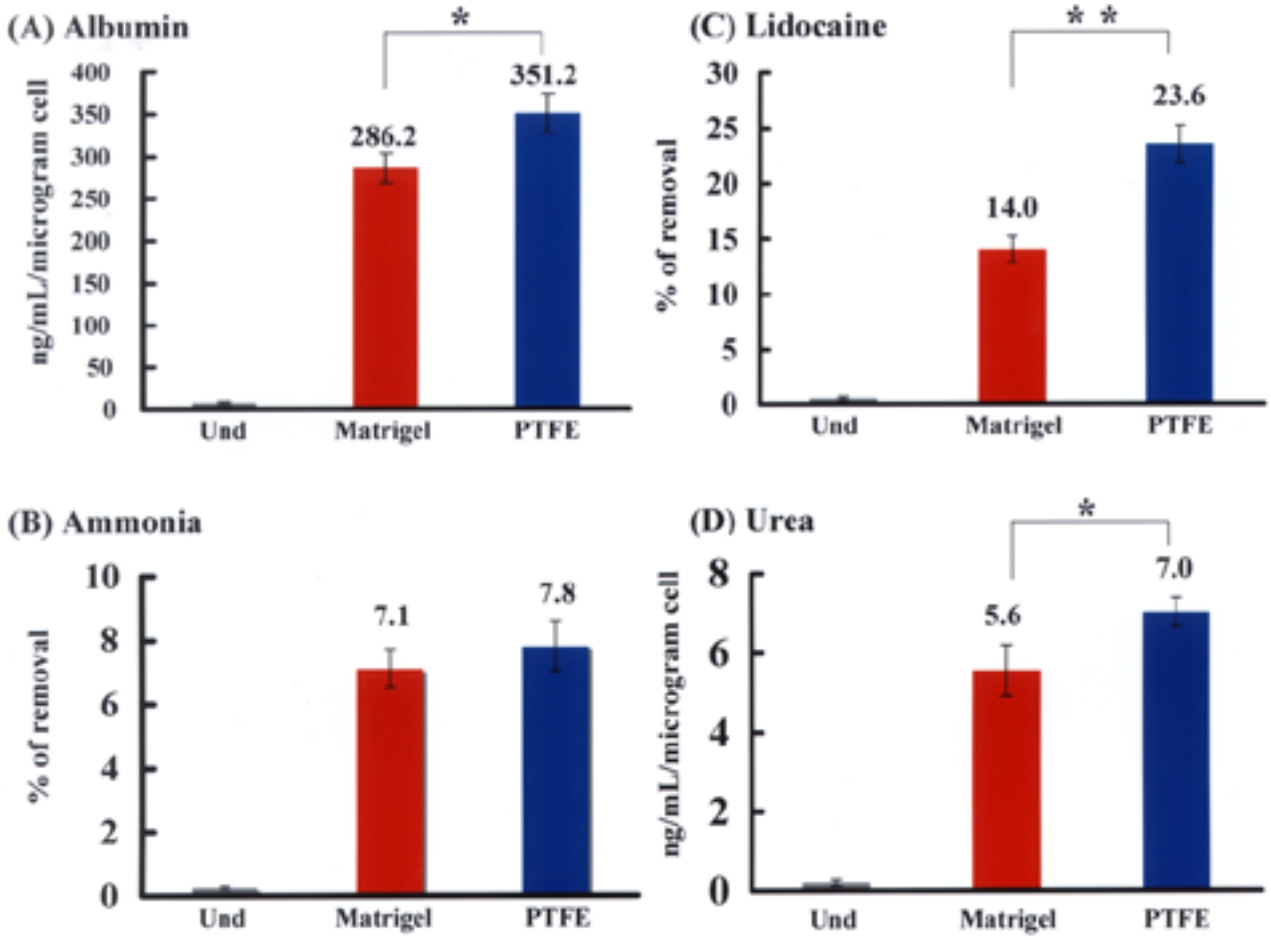

Figure 4. Functional capacities of hES cell-derived hepatocytes. Application of PTFE fabric coated with PAU in hepatic differentiation significantly enhanced albumin production (A), lidocaine metabolism (C), and urea synthesis (D) of hES cell-derived hepatocytes. (B) The use of PAU-coated PTFE fabric increased ammonia-metabolizing activity of hES-derived hepatocytes, but not significant compared to Matrigel. $*p < 0.05$ and $**p < 0.01$. These parameters were compared per microgram cell. Data are means ± SD. The data are representative from three independent experiments.

ble to normal human hepatocytes. We believe that this work would be an important step toward the potential application of hES cells to treat patients suffering from ALF with BAL.

REFERENCES

1. Abe, T.; Kobata, H.; Hanba, Y.; Kitabata, Y.; Narukawa, N.; Hasegawa, H.; Fukagawa, M. Study of plasma exchange for liver failure: Beneficial and harmful effects. Ther. Apher. Dial. 8:180–184; 2004.
2. Alison, M. R.; Poulsom, R.; Jeffery, R.; Dhillon, A. P.; Quaglia, A.; Jacob, J.; Novelli, M.; Prentice, G.; Williamson, J.; Wright, N. A. Hepatocytes from non-hepatic adult stem cells. Nature 406:257; 2000.
3. Bhatia, S. N.; Balis, U. J.; Yarmush, M. L.; Toner, M. Effect of cell–cell interactions in preservation of cellular phenotype: Cocultivation of hepatocytes and nonparenchymal cells. FASEB J. 13:1883–1900; 1999.
4. Chen, S. C.; Mullon, C.; Kahaku, E.; Watanabe, F.; Hewitt, W.; Eguchi, S.; Middleton, Y.; Arkadopoulos, N.; Rozga, J.; Solomon, B.; Demetriou, A. A. Treatment of severe liver failure with a bioartificial liver. Ann. NY Acad. Sci. 831:350–360; 1997.
5. Chen, Y.; Kobayashi, N.; Suzuki, S.; Soto-Gutierrez, A.; Rivas-Carrillo, J. D.; Tanaka, K.; Navarro-Alvarez, N.; Fukazawa, T.; Narushima, M.; Miki, A.; Okitsu, T.; Amemiya, H.; Tanaka, N. Transplantation of human hepatocytes cultured with deleted variant of hepatocyte growth factor prolongs the survival of mice with acute liver failure. Transplantation 79:1378–1385; 2005.
6. D'Amour, K. A.; Agulnick, A. D.; Eliazer, S.; Kelly, O. G.; Kroon, E.; Baetge, E. E. Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat. Biotechnol. 23:1534–1541; 2005.
7. Demetriou, A. A.; Brown, Jr., R. S.; Busuttil, R. W.; Fair, J.; McGuire, B. M.; Rosenthal, P.; Am Esch, 2nd, J. S.; Lerut, J.; Nyberg, S. L.; Salizzoni, M.; Fagan, E. A.; de Hemptinne, B.; Broelsch, C. E.; Muraca, M.; Salmeron, J. M.; Rabkin, J. M.; Metselaar, H. J.; Pratt, D.; De La Mata, M.; McChesney, L. P.; Everson, G. T.; Lavin,

P. T.; Stevens, A. C.; Pitkin, Z.; Solomon, B. A. Prospective, randomized, multicenter, controlled trial of a bioartificial liver in treating acute liver failure. Ann. Surg. 239: 660–670; 2004.
8. Ikeda, H.; Kobayashi, N.; Tanaka, Y.; Nakaji, S.; Yong, C.; Okitsu, T.; Oshita, M.; Matsumoto, S.; Noguchi, H.; Narushima, M.; Tanaka, K.; Miki, A.; Rivas-Carrillo, J. D.; Soto-Gutierrez, A.; Navarro-Alvarez, N.; Tanaka, K.; Jun, H-S.; Tanaka, N.; Yoon, J. W. A newly developed bioartificial pancreas successfully controls blood glucose in totally pancreatectomized diabetic pigs. Tissue Eng. (in press).
9. Jang, Y. Y.; Collector, M. I.; Baylin, S. B.; Diehl, A. M.; Sharkis, S. J. Hematopoietic stem cells convert into liver cells within days without fusion. Nat. Cell Biol. 6:532–539; 2004.
10. Kobayashi, N.; Fujiwara, T.; Westerman, K. A.; Inoue, Y.; Sakaguchi, M.; Noguchi, H.; Miyazaki, M.; Cai, J.; Tanaka, N.; Fox, I. J.; Leboulch, P. Prevention of acute liver failure in rats with reversibly immortalized human hepatocytes. Science 287:1258–1262; 2000.
11. Kobayashi, N.; Taguchi, T.; Noguchi, H.; Okitsu, T.; Totsugawa, T.; Watanabe, T.; Matsumura, T.; Fujiwara, T.; Urata, H.; Kishimoto, N.; Hayashi, N.; Nakaji, S.; Murakami, T.; Tanaka, N. Rapidly functional immobilization of immortalized human hepatocytes using cell adhesive GRGDS peptide-carrying cellulose microspheres. Cell Transplant. 10:387–392; 2001.
12. Lagasse, E.; Connors, H.; Al-Dhalimy, M.; Reitsma, M.; Dohse, M.; Osborne, L.; Wang, X.; Finegold, M.; Weissman, I. L.; Grompe, M. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat. Med. 6: 1229–1234; 2000.
13. Lavon, N.; Yanuka, O.; Benvenisty, N. Differentiation and isolation of hepatic-like cells from human embryonic stem cells. Differentiation 72:230–238; 2004.
14. Lee, W. M. Acute liver failure. N. Engl. J. Med. 329: 1862–1872; 1993.
15. Maruyama, M.; Kobayashi, N.; Westerman, K. A.; Sakaguchi, M.; Allain, J. E.; Totsugawa, T.; Okitsu, T.; Fukazawa, T.; Weber, A.; Stolz, D. B.; Leboulch, P.; Tanaka, N. Establishment of a highly differentiated immortalized human cholangiocyte cell line with SV40T and hTERT. Transplantation 77:446–451; 2004.
16. Matsumura, T.; Takesue, M.; Westerman, K. A.; Okitsu, T.; Sakaguchi, M.; Fukazawa, T.; Totsugawa, T.; Noguchi, H.; Yamamoto, S.; Stolz, D. B.; Tanaka, N.; Leboulch, P.; Kobayashi, N. Establishment of an immortalized human-liver endothelial cell line with SV40T and hTERT. Transplantation 77:1357–1365; 2004.
17. Nagata, H.; Ito, M.; Cai, J.; Edge, A. S.; Platt, J. L.; Fox, I. J. Treatment of cirrhosis and liver failure in rats by hepatocyte xenotransplantation. Gastroenterology 124:422–431; 2003.
18. Narushima, M.; Kobayashi, N.; Okitsu, T.; Tanaka, Y.; Li, S. A.; Chen, Y.; Miki, A.; Tanaka, K.; Nakaji, S.; Takei, K.; Gutierrez, A. S.; Rivas-Carrillo, J. D.; Navarro-Alvarez, N.; Jun, H. S.; Westerman, K. A.; Noguchi, H.; Lakey, J. R.; Leboulch, P.; Tanaka, N.; Yoon, J. W. A human beta-cell line for transplantation therapy to control type 1 diabetes. Nat. Biotechnol. 23:1274–1282; 2005.
19. Platt, J. L. Xenotransplanting hepatocytes: The triumph of a cup half full. Nat. Med. 3:26–27; 1997.
20. Rambhatla, L.; Chiu, C. P.; Kundu, P.; Peng, Y.; Carpenter, M. K. Generation of hepatocyte-like cells from human embryonic stem cells. Cell Transplant. 12:1–11; 2003.
21. Rifai, K.; Ernst, T.; Kretschmer, U.; Bahr, M. J.; Schneider, A.; Hafer, C.; Haller, H.; Manns, M. P.; Fliser, D. Prometheus—a new extracorporeal system for the treatment of liver failure. J. Hepatol. 39:984–990; 2003.
22. Schleger, C.; Krebsfaenger, N.; Kalkuhl, A.; Bader, R.; Singer, T. Innovative cell culture methods in drug development. Altex 18:5–8; 2001.
23. Schwartz, R. E.; Reyes, M.; Koodie, L.; Jiang, Y.; Blackstad, M.; Lund, T.; Lenvik, T.; Johnson, S.; Hu, W. S.; Verfaillie, C. M. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J. Clin. Invest. 109:1291–1302; 2002.
24. Sen, S.; Davies, N. A.; Mookerjee, R. P.; Cheshire, L. M.; Hodges, S. J.; Williams, R.; Jalan, R. Pathophysiological effects of albumin dialysis in acute-on-chronic liver failure: A randomized controlled study. Liver Transpl. 10: 1109–1119; 2004.
25. van de Kerkhove, M. P.; Germans, M. R.; Deurholt, T.; Hoekstra, R.; Joziasse, D. H.; van Wijk, A. C.; van Gulik, T. M.; Chamuleau, R. A.; Roos, A. Evidence for Gal-alpha(1-3)Gal expression on primary porcine hepatocytes: Implications for bioartificial liver systems. J. Hepatol. 42: 541–547; 2005.
26. Watanabe, T.; Shibata, N.; Westerman, K. A.; Okitsu, T.; Allain, J. E.; Sakaguchi, M.; Totsugawa, T.; Maruyama, M.; Matsumura, T.; Noguchi, H.; Yamamoto, S.; Hikida, M.; Ohmori, A.; Reth, M.; Weber, A.; Tanaka, N.; Leboulch, P.; Kobayashi, N. Establishment of immortalized human hepatic stellate scavenger cells to develop bioartificial livers. Transplantation 75:1873–1880; 2003.
27. Williams, R.; Wendon, J. Indications for orthotopic liver transplantation in fulminant liver failure. Hepatology 20: S5–S10; 1994.
28. Xu, C.; Inokuma, M. S.; Denham, J.; Golds, K.; Kundu, P.; Gold, J. D.; Carpenter, M. K. Feeder-free growth of undifferentiated human embryonic stem cells. Nat. Biotechnol. 19:971–974; 2001.