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Coculture With Mesenchymal Stem Cells Results in Improved Viability and Function of Human Hepatocytes

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Hepatocyte transplantation is becoming an accepted therapy for acute liver failure, either as a bridge to liver regeneration or to organ transplantation. Hepatocytes provide liver function in place of the failing organ. The maintenance of sufficient viability and function of the transplanted hepatocytes is a concern. There is a lot of recent interest in mesenchymal stem cells (MSCs) for the provision of structural and trophic support to hepatocytes, but few studies currently use primary human hepatocytes. The aim of this study was to investigate if coculture of human MSCs with cryopreserved human hepatocytes may improve their function and viability, thus with potential for cellular therapy of liver disease. MSCs were isolated from human umbilical cord or adipose tissue. Hepatocytes were isolated from donor organs unsuitable for transplantation. MSCs and hepatocytes were cocultured in both direct and indirect contact. Conditioned medium (CM) from cocultured MSCs and hepatocytes was also used on hepatocytes. Viability and liver-specific function were compared between test and controls. Human hepatocytes that were cocultured directly with MSCs demonstrated improved production of albumin from day 5 to day 25 of culture. This effect was most prominent at day 15. Likewise, urea production was improved in coculture from day 5 to 25. Indirect coculture demonstrated improved albumin production by day 4 (1,107 ng/ml) versus hepatocyte monoculture (940 ng/ml). Hepatocytes in CM demonstrated a nonsignificant improvement in function. The viability of cocultured hepatocytes was superior to that of monocultured cells with up to a 16% improvement. Thus, coculture of human hepatocytes with MSCs demonstrates both improved function and viability. The effect is seen mainly with direct coculture but can also be seen in indirect culture and with CM. Such coculture conditions may convey major advantages in hepatocyte survival and function for cell transplantation.

Key words: Mesenchymal stem cells (MSCs); Hepatocyte transplantation; Acute liver failure

INTRODUCTION

Hepatocyte transplantation is making its transition from bench to bedside for both liver-based metabolic disorders and acute liver failure. Over 80 patients have now been transplanted worldwide, and the safety of the procedure together with medium-term success has been established (10). A major limiting factor in the field is the availability of good-quality cells, as hepatocytes are generally derived from grafts that are deemed unsuitable for transplantation. There is also a need to develop techniques that will improve the engraftment, survival, and function of transplanted hepatocytes (8). A major advantage of hepatocyte transplantation is the ability to cryopreserve cells once they are isolated from donor livers. The cells can then be stored and thawed for use whenever required, which is important in the treatment of acute liver failure. Cryopreservation, however, can have deleterious effects on cell viability and on the attachment and thus possibly the engraftment of cells (30).

Recent advances have directed attention toward the clinical potential of using stem cells in cell transplantation. In particular, mesenchymal stem cells (MSCs) have great promise in this respect. MSCs are multipotent, adherently growing cells, which provide support for hematopoietic cells within the bone marrow (9). They are a readily available source of stem cells that can also be isolated from umbilical cord blood and matrix, placental tissue, adipose tissue, and other sources (2,7). MSCs play a major role in tissue repair, both through localized immune-suppressive effects and through the release of

soluble trophic factors to affect neighboring cells, properties that make them excellent candidates for improving the survival of transplanted cells (22–24). MSCs also have antiapoptotic, proregenerative effects in the setting of myocardial infarction, kidney failure, and stroke (12,18).

MSCs have been shown to differentiate into hepatocytes in appropriate conditions in vitro and in vivo (1,5). In addition, they can modulate liver injury and promote native liver regeneration (3,23) with improved survival in animal models of liver disease and in pilot clinical studies of liver failure (13,16,17,27). It is not known whether this therapeutic effect is due to the potential of MSCs to differentiate into hepatocytes in the appropriate microenvironment or to their stimulatory/trophic effects. A major limitation of previous coculture studies has been the use of nonhuman (usually rodent) cells that do not necessarily behave in the same way as human cells. Understanding the behavior of human cells in this context is particularly important prior to translation to the clinical setting. The hypothesis of this study is that MSCs will promote human hepatocyte viability and function. This study focuses on the in vitro coculture of different sources of MSCs (adipose derived and umbilical cord matrix derived) with cryopreserved human hepatocytes. Both umbilical cord and adipose tissue are readily available sources of MSCs and were considered practical for potential translation to clinical practice.

MATERIALS AND METHODS

Isolation and Cryopreservation of Human Hepatocytes

Human liver tissue was obtained from donor tissue that was rejected or unused for orthotopic liver transplantation or from liver resections at King's College Hospital (London, UK). Consent was obtained to use all the tissue for research in accordance with the Research Ethics Committee of King's College Hospital. Tissue was anonymized at the source. Isolation of human hepatocytes was carried out using a modified collagenase perfusion technique (20). The isolated hepatocytes were cryopreserved in University of Wisconsin solution (Bristol-Myers Squibb Pharma Ltd., Uxbridge, Middlesex, UK)/10% dimethyl sulfoxide (DMSO; Sigma-Aldrich, Poole, Dorset, UK) (v/v)/5% glucose (Sigma-Aldrich) using a controlled rate freezer and stored at −140°C for later use (31).

Isolation and Expansion of Human Mesenchymal Stem Cells From Umbilical Cord Matrix (UC-MSCs)

MSCs were isolated from human umbilical cord matrix (4). The umbilical cord was transferred in 0.9% NaCl sterile solution on ice and processed immediately. The cords were incised along their length to expose underlying Wharton's jelly, and the umbilical vein and the arteries were pulled away. The remaining mesenchymal tissue was scraped off with a sterile scalpel and centrifuged at $250 \times g$ for 5 min at room temperature. The pellet was resuspended in Hank's balanced salt solution (HBSS) (Lonza, Castleford, UK) containing 1 mg/ml collagenase type I (Sigma-Aldrich) and 100 U/ml penicillin/100 µg/ml streptomycin (Invitrogen, Paisley, UK). The tissue was transferred to a T75 flask (Thermo-Scientific Nunc, Hemel Hempstead, UK) with a ventilated cap and digested at 37°C in a humidified atmosphere containing 5% CO_2 overnight. The homogenate was diluted in phosphate-buffered saline (PBS), triturated with a glass Pasteur pipette, and centrifuged at $400 \times g$ for 10 min. The cell pellet was resuspended in Dulbecco's modified Eagle medium (DMEM; Invitrogen) with 10% (v/v) fetal bovine serum (FBS; Invitrogen) and 100 U/ml penicillin/100 µg/ml streptomycin. Trypan blue exclusion assay (Invitrogen) was used to count the viable cells, and the cells were plated in a T75 flask and incubated at 37°C in a humidified atmosphere containing 5% CO_2. The medium was changed after 24 h to remove the nonadherent cells. When cultures reached confluence, the cells were trypsinized using trypsin (Gibco, Paisley, UK) and subcultured in fresh flasks.

Isolation of Adipose-Derived MSCs (AD-MSCs)

AD-MSCs were also used for experiments and were purchased from Invitrogen. These cells had been isolated using mechanical and enzymatic digestion following harvesting from lipo-adipose suction. The AD-MSCs had been purified following labeling with antibodies [cluster of differentiation 29 (CD29), CD44, CD73, CD90, CD105, CD166] and flow cytometry sorting. The cells were cryopreserved and, following thawing, were cultured as above. The ninth through the 12th passages were used for further experiments.

Immunophenotyping of UC-MSCs

Undifferentiated UC-MSCs were trypsinized and suspended in PBS/1% FBS and then incubated with the following fluorescein isothiocynate (FITC)-, phycoerythrin (PE)-, or allophycocyanine (APC)-conjugated antibodies: CD13 (1 ∞g/test), CD105 (0.25 ∞g/test), CD90 (1 ∞g/test), CD44 (0.5 ∞g/test), CD117 (0.25 ∞g/test), CD31 (0.125 ∞g/test) (all eBioscience, Hatfield, UK), CD73 (0.5 ∞g/test), and human leukocyte antigen (HLA)-DR (20 ∞l/test) (both BD Pharmingen, Oxford, UK) for 30 min at 4°C. The cells were then washed and resuspended in 0.5 ml PBS. Cells were analyzed on a FACSCanto II flow cytometer (BD Biosciences), and at least 10,000 events were acquired for each sample.

Adipogenic and Osteogenic Differentiation of UC-MSCs

Adipogenic differentiation was induced by cultivation of confluent cultures in DMEM containing 20% (v/v) FBS,

2.5 µg/ml insulin, 100 µmol/L indomethacin, 5 µmol/L rosiglitazone, and 10 nmol/L dexamethasone (all from Sigma-Aldrich) for 4 weeks with medium changes twice a week. Differentiated cells were analyzed by staining lipid vesicles with Oil Red O (Sigma-Aldrich).

For osteogenic differentiation, the cells were cultured in DMEM containing 10% (v/v) FBS, 10 mmol/L -glycerophosphate (Sigma-Aldrich), 5 µg/ml ascorbic acid (Sigma-Aldrich), and 10 nmol/L dexamethasone for 4 weeks with medium changes twice a week. Calcium deposition was evaluated by Alizarin Red S staining (Sigma-Aldrich).

Coculture of Human Hepatocytes With MSCs

Cryopreserved human hepatocytes were used for experiments. Batches that did not reach 70% viability after Percoll (GE Healthcare, Little Chalfont, UK) following thawing were not used. Cells were seeded into each collagen IV-coated culture vessel (collagen IV from Sigma-Aldrich) at a fixed density of $1.5 \times 10^5/cm^2$ live cells (to standardize). UC-MSCs or AD-MSCs were added once hepatocytes (HCs) were attached using HCs to MSCs ratios of 3:1, 6:1, and 10:1. Experiments compared coculture to HC monoculture with equal numbers of HCs. The cells were cocultured in William's medium E (Sigma-Aldrich) supplemented with 10% FBS, 10 nM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; Cambrex, Invitrogen), 2 mM glutamine (Invitrogen), 0.5 µg/ml (10^{-7} M) insulin (Sigma-Aldrich), and 10^{-7} M dexamethasone. This is standard HC culture medium. The medium was changed the following day and 24 h later. The supernatant was collected and stored at $-80°C$ for later analysis. The media were then collected every other day afterward with the media changed 24 h before. Indirect coculture was undertaken using a porous Transwell membrane, pore size 0.4 ∝m (BD Biosciences). HCs were first seeded at a density of $1.5 \times 10^5/cm^2$ on the collagen-coated plate. Once attached, the MSCs were seeded onto the Transwell membrane inserts at a density of 1.5×10^4. Again, the medium was changed 24 h later and then collected at days 1, 2, and 4 with medium change 24 h prior to each time point.

Hepatocytes were also cultured using conditioned medium, which was collected from cocultures of HCs/UC-MSCs. This was to elicit the possible effects on HCs of paracrine factors secreted by UC-MSCs when in contact with HCs. The HCs and UC-MSCs were cultured in standard culture medium for 24 h before the conditioned medium was collected. The conditioned medium was then diluted with the same amount of fresh standard culture medium to achieve HCs/UC-MSCs equivalent ratios of 3:1 and applied to the HC monoculture. Human HCs cultured in standard medium only, medium conditioned from UC-MSC culture alone, and standard HC culture

medium only and HCs in direct coculture with UC-MSCs were used as controls.

Albumin Secretion ELISA

Albumin concentration in the medium was measured using human albumin enzyme-linked immunosorbent assay (ELISA) quantification kit (Bethyl Laboratories, Montgomery, TX, USA) according to the manufacturer's instructions.

Urea Production

Urea concentration in the medium was measured using QuantiChrome Urea Assay Kit (BioAssay Systems, Cambridge, UK) according to the manufacturer's instructions.

Identification of Liver-Specific Genes in MSCs Following Coculture Using Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from both HCs and MSCs grown in both indirect coculture and in monoculture (controls) using TRIzol® and reverse transcribed to cDNA using Omniscript® Reverse Transcriptase kit (Qiagen, Crawley, West Sussex, UK) according to the manufacturers' protocols. PCR amplification was undertaken using primers (Eurofins MWG Operon, Ebersburg, Germany) for human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (fw: 5 -cggagtcaacggatttggtcgtat-3 , rv: 5 -agccttctccatggtggt gaagac-3), albumin (fw: 5 -ccttggtgttgattgcctttgctc-3 , rv: 5 -catcacatcaacctctggtctcacc-3), and cytokeratin 18 (fw: 5 -ccatgcgccagtctgtggag-3 , rv: 5 -agcagattgaggagagcaccac-3). PCR products were amplified using Thermo Scientific Px2 Thermal Cycler and then analyzed using 2% agarose gel electrophoresis (Sigma-Aldrich).

Viability Studies

Viability of HCs was assessed using trypan blue, Annexin-V/propidium iodide staining (Promega, Southampton, UK) with flow cytometry, the M30 CytoDeath ELISA assay (PEVIVA, distributed by Bioaxxess, Tewkesbury, UK, which measures cleavage of cytokeratin 18) on the cell lysate and supernatant as well as RT-PCR for caspase 3 expression. Real-time PCR was undertaken using RNA isolated from HCs in indirect coculture as above, and following reverse transcription, relative quantification real-time PCR was undertaken using a gene expression assay (probe) for caspase 3, TaqMan® Universal Master Mix, and ABI Prism7000 Sequence detection system (Applied Biosystems, Warrington, UK).

Data Analysis

Values are expressed as means ± standard deviation (SD). The statistical significance of differences between groups was tested using one-way analysis of variance (ANOVA,

with Bonferroni correction) or Student's *t* test. A level of p 0.05 was considered to be statistically significant.

RESULTS

Isolation of Human MSCs From Human Umbilical Cord Matrix

Spindle-shaped cells (Fig. 1A) expressed MSC-specific markers CD13, CD73, CD105, CD44, and CD90, but not CD31, CD117, or HLA-DR (Fig. 1B). In vitro differentiation to adipogenic and osteogenic cells was also demonstrated (data not shown). These results suggested that the cells isolated were UC-MSCs fulfilling the criteria of the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (9).

In Vitro Coculture of Human HCs and Human AD-MSCs

There was increased albumin production in the coculture system from day 3 onward as shown in Figure 2. This effect was seen across all ratios of HCs to AD-MSCs. Albumin secretion of the HCs in monoculture decreased from day 3, and by day 9 it was undetectable. On the other hand, there was continuous production of albumin in all cocultured cells. There was a gradual increase of albumin production in cocultured cells that were in 6:1 and 3:1 ratios. From these results, it seems

that AD-MSCs can help maintain HC function in vitro via direct contact coculture of these two types of cells.

Long-Term In Vitro Coculture of Human HCs With UC-MSCs

Human HCs cocultured with UC-MSCs also showed improved albumin production (Fig. 3) as those cocultured with AD-MSCs. At day 1, human HCs cultured under the different conditions secreted similar amounts of albumin followed by a fall over the first 5 days. However, after day 5, albumin secretion from the HCs cocultured with UC-MSCs continuously increased until day 15, while that from the HCs in monoculture decreased. UC-MSCs induced a significant increase in albumin secretion by HCs into the culture medium, approximately 15-fold at day 15 ($2,190 \pm 170$ ng/24 h/well) at 3:1 ratio compared to day 1 (140 ± 5 ng/24 h/well, $p < 0.001$). In both cococulture systems, MSCs were also cultured alone as a control. Neither AD-MSCs nor UC-MSCs secreted albumin in monoculture.

Hepatocytes cocultured in different ratios with UC-MSCs using different coculture ratios produced similar amounts of urea, which was maintained at day 3 but fell by day 5 (Fig. 4). However, after day 5, urea production from the HCs cocultured with UC-MSCs continuously increased until day 13, while that from the HCs in
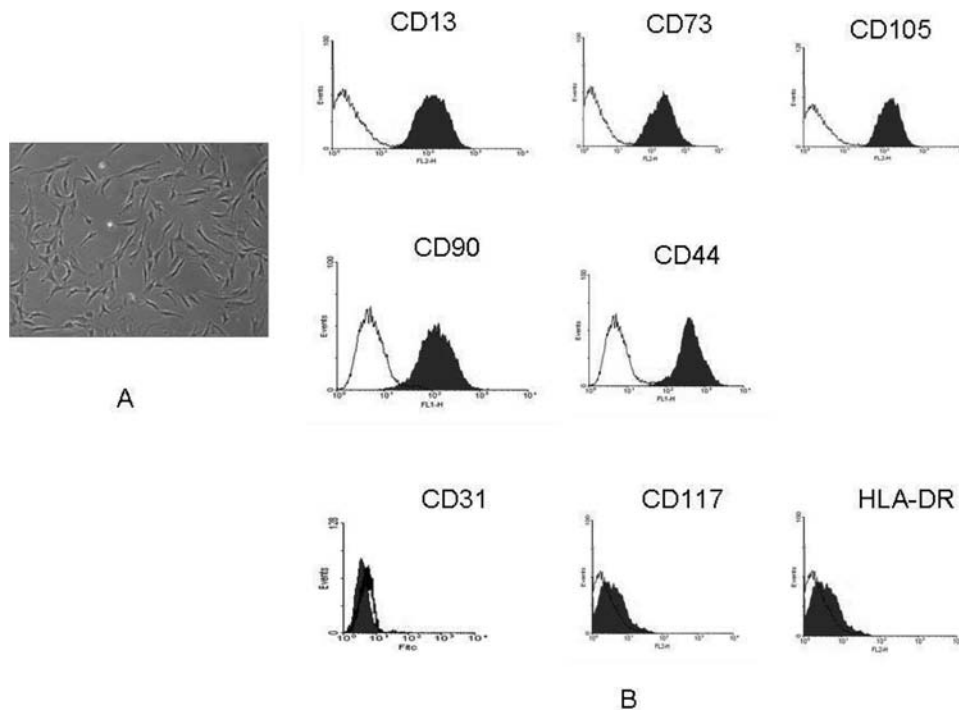

Figure 1. Characterization of isolated MSCs from umbilical cord matrix. (A) Morphology of UC-MSCs using phase-contrast light microscope 100× magnification. Cells are spindle-shaped and fibroblast-like. (B) Flow cytometric analysis of human UC-MSCs. UC-MSCs were positive for cluster of differentiation 13 (CD13), CD73, CD105, CD90, and CD44 and negative for CD31, CD117, and human leukocyte antigen (HLA)-DR.
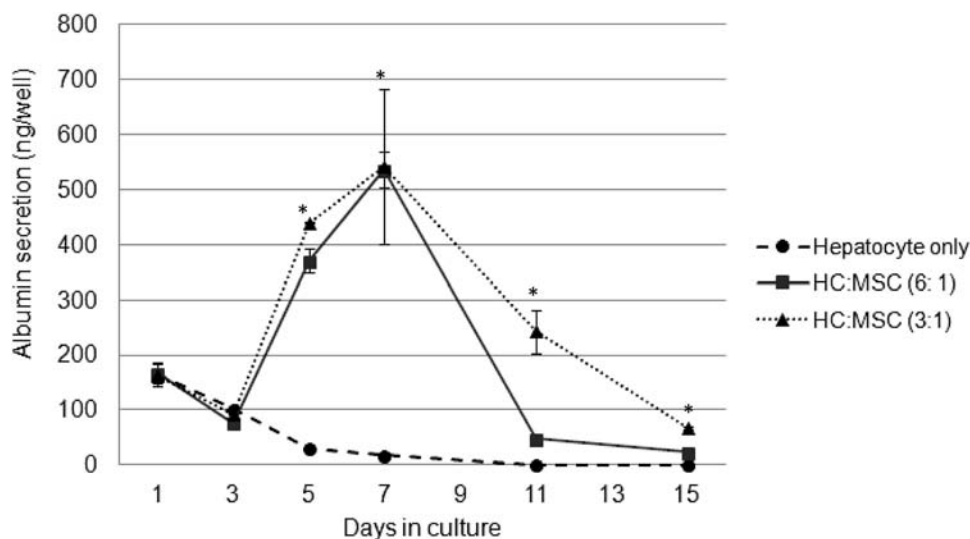
Figure 2. Albumin secretion in cryopreserved HCs monocultured and cocultured with AD-MSCs at ratios of HCs to MSCs of 3:1 and 6:1. Monoculture or coculture was undertaken for 2 weeks and albumin expression measured using ELISA. Albumin production was significantly increased in coculture from day 5 up to day 15 at both 3:1 and 6:1 ratios ($n = 3$; *$p < 0.001$ compared to HC monoculture).

monoculture continued to decrease and was undetectable from day 13. Even at day 25, urea production in the cocultured HCs (3:1 ratio to UC-MSCs) was still higher than day 1. UC-MSCs cultured alone did not produce urea.

Indirect Coculture of AD-MSCs and HCs

Indirect coculture was undertaken through a porous Transwell membrane in order to determine if paracrine effects may be responsible for the improved function of HCs as seen in direct coculture. By day 4, albumin
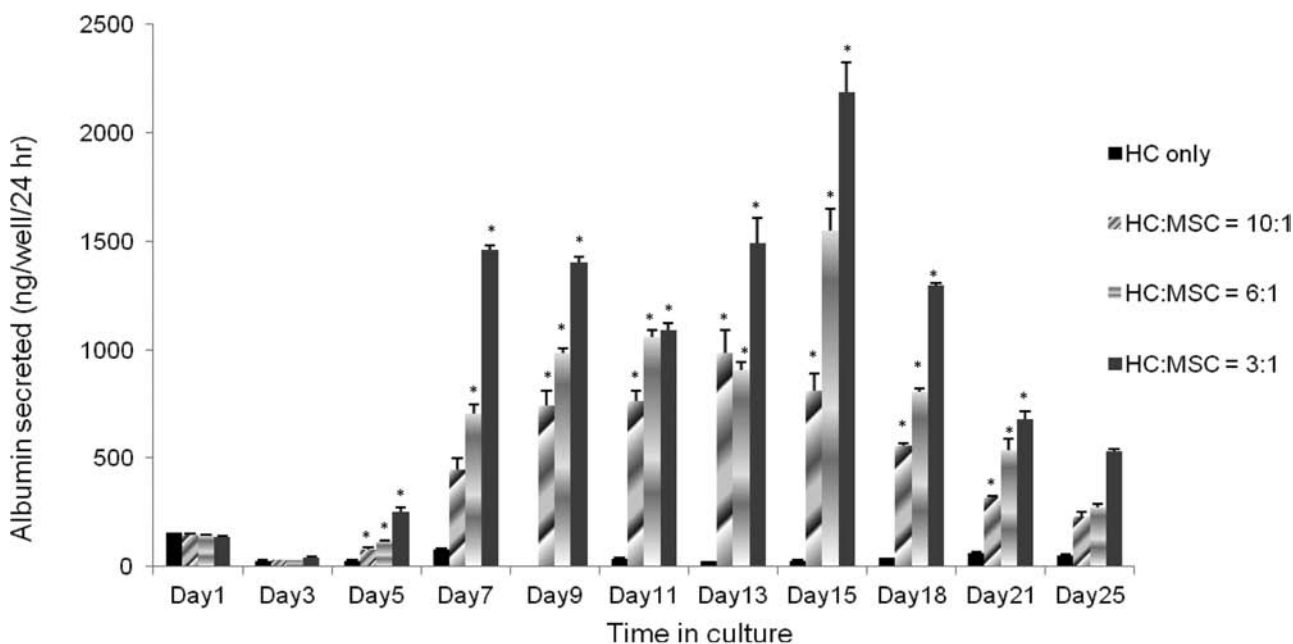

Figure 3. Albumin secretion from cryopreserved human HCs cocultured with UC-MSCs compared with HC monoculture. Conditions were maintained over 25 days. There was a significant increase in albumin production from cocultured cells from day 5 until day 25. This was seen at all ratios of HCs to MSCs but was best at 3:1 ratio ($n = 3$; *$p < 0.001$ compared to HC monoculture).
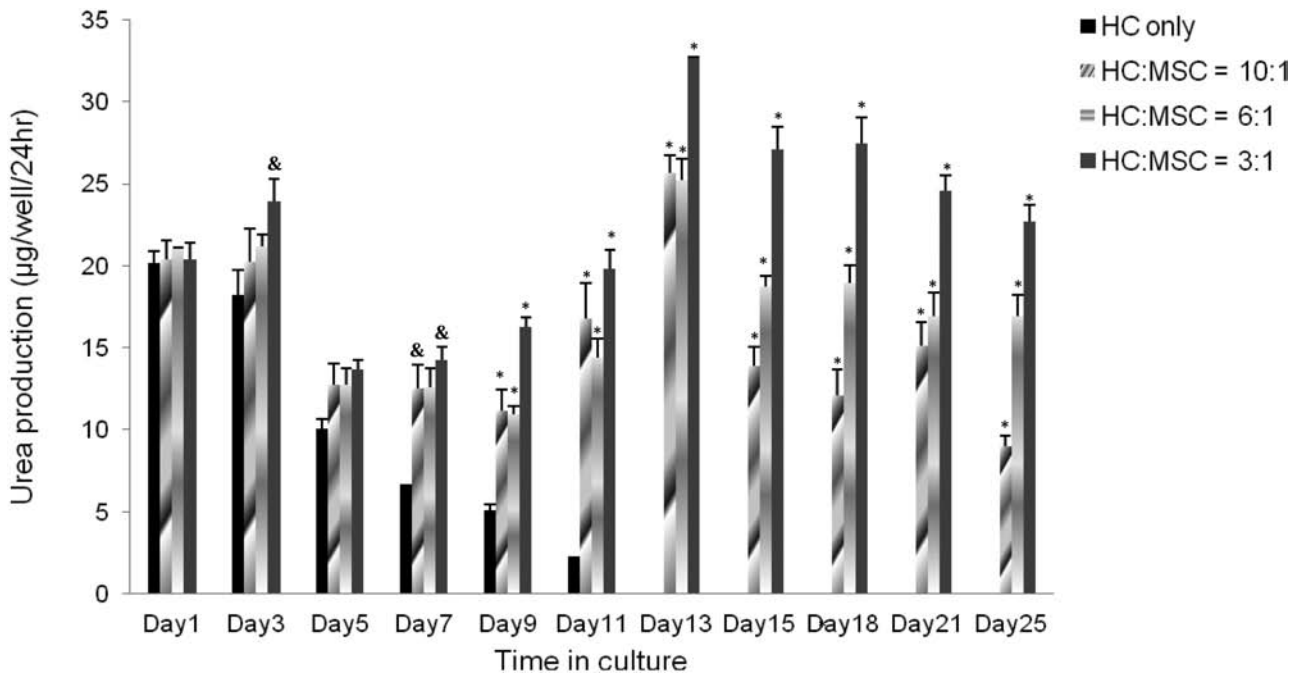
Figure 4. Urea production from cryopreserved human HCs cocultured with UC-MSCs at three different ratios (10:1, 6:1, and 3:1) versus HC monoculture over 25 days in culture. Urea production was significantly improved in coculture from day 5 to day 25. This was highest at day 13 but was maintained through to day 25. All ratios of HCs to MSCs demonstrated this effect, but the 3:1 ratio demonstrated the optimal effect ($n = 3$; $*p < 0.001$ or $\&p < 0.01$ compared to HC monoculture).

secretion by HCs in coculture was $1{,}107 \pm 70$ ng/ml/24 h versus 941 ± 17 ng/ml/24 h in HCs cultured alone. However, this did not meet statistical significance.

The Effect of UC-MSC Conditioned Medium (CM) on Human HC In Vitro Culture

Figure 5 shows albumin secretion from HCs cultured using either CM from cocultures of HCs and UC-MSCs in 3:1 or 6:1 ratios or HCs alone (control). Though there was no significant effect in the first few days of culture, by day 5 there was a significantly greater production of albumin by HCs cultured in medium that was conditioned by HCs and UC-MSCs in coculture. Overall, HCs cultured in CM showed less effect on HC function demonstrated by albumin secretion compared to that from the direct contact coculture, but it was still considerably higher than that from the cells cultured in regular HC medium. HCs cultured in medium conditioned by UC-MSCs alone did not show any increase in albumin production versus standard culture medium.

Morphology and Viability of Cocultured Cells

The morphology of the HCs in monoculture and cocultured with UC-MSCs in 6:1 ratio is shown in Figure 6. At day 1, there was no visible difference in the morphology of HCs cultured in these two conditions (Fig. 6A, B). The cells showed typical morphology of HCs cultured in vitro. They were polygonal, some of which had two nuclei. However, at day 7, monocultured HCs (Fig. 6C) lost their morphological integrity, while the cells cocultured with UC-MSCs (Fig. 6D) were still joined together and growing well. By day 13, the majority of the cells in monoculture (Fig. 6E) were dead while HCs cocultured with UC-MSCs in 6:1 ratio were still healthy and growing in a sheet-like fashion where HCs exhibited stereotypical polygonal morphology with distinct nuclei and nucleoli and well-demarcated cell–cell borders.

Trypan blue staining confirmed this finding with a viability of $40.4 \pm 7.6\%$ in cocultured HCs versus $23.5 \pm 3.5\%$ in monocultured cells.

RT-PCR was used to detect expression of caspase 3 in HCs that were cocultured versus monocultured hepatocytes. There was a demonstrable decrease in expression of the marker in cocultured cells (data not shown). The M30 CytoDeath ELISA kit was also used to detect apoptosis in cultured HCs as this marker will only measure HC rather than MSC death. Measurement of cleavage of cytokeratin 18 (CK18) showed that the CK18 fragment level in HCs cocultured with UC-MSCs at 3:1 ratio (0.0048 units) was much lower than that for HCs in
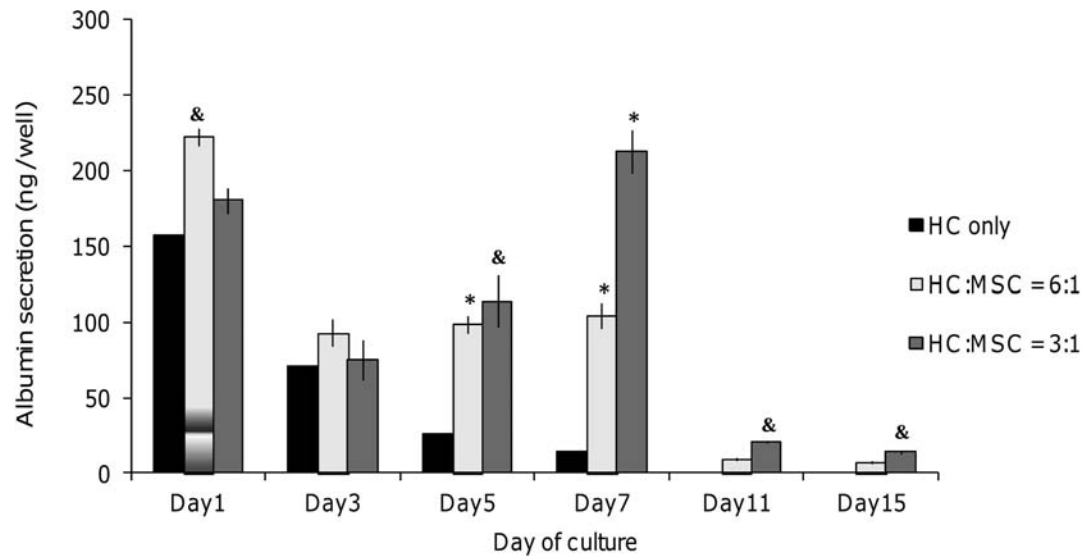
Figure 5. Albumin secretion from HCs cultured in either HC culture medium (control) or CM from cocultures of HCs/MSCs, which were cultured at a HC/MSC ratio of 6:1 and 3:1. Concentration of albumin in the CM that was added was measured separately and subtracted from the result from the corresponding well. This demonstrates improved albumin production in HCs that were cultured in medium from previous cocultures of HCs and MSCs ($n = 3$; *$p < 0.001$ or &$p < 0.01$ compared to HC conditioned medium alone).

monoculture (0.3157 units) at day 15 and through 1 month culture (Fig. 7).

Transdifferentiation of MSCs May Partly, but Not Completely, Explain the Improved HC Functionality in Coculture

AD-MSCs from indirect cocultures were harvested and underwent PCR for albumin and CK18 expression. Though albumin was demonstrably expressed following 72 h of coculture, differentiation was only partial as demonstrated by there being no expression of CK18 expression (data not shown).

DISCUSSION

Coculture of human HCs and AD-MSCs or UC-MSCs was demonstrated to help human HCs maintain their function long term in vitro culture compared to HC monoculture. This effect was best seen in direct coculture systems but also seen using conditioned medium from coculture. The latter suggests the possible contribution of paracrine factors secreted by HC-stimulated MSCs. These data also demonstrate that total cell death (and specifically HC apoptosis) was decreased in both direct and indirect cocultures. It is likely that increased function is due at least in part to improved survival.

Previous studies have demonstrated that bone marrow-derived stem cells (BMSCs) have a protective effect on rodent HCs in vitro and in vivo. Isoda et al. demonstrated

that BMSCs supported HC function when in coculture. They hypothesized that interleukin 6 (IL-6) was responsible for the improved production of urea, though not of albumin (14). Coculture and cotransplantation of BMSCs in Nagase analbuminemia rats and HCs demonstrated improved function in vitro and in vivo (29). Liu and Chang demonstrated coencapsulation and transplantation of BMSCs, and HCs resulted in improved lowering of bilirubin in Gunn rats (19). Recently, MSCs have become a focus for attention in this context (6,24). Coculture of rat MSCs with HCs in a liver-assist device (LAD) demonstrated survival benefits over LAD with HCs alone in a rat model of acute liver failure (32). Mohajerani et al. transplanted human HCs detached from cocultures with BMSCs into mice and found improved engraftment (21). MSCs are known to provide structural support for cells in the body and have antiapoptotic, immunomodulatory effects. Gomez-Aristizabal et al. explored the effects of bone marrow-derived mesenchymal stem cells (BM-MSCs) and umbilical cord perivascular cells cocultured with rat HCs and human lymphocytes and found a profunction effect on the HCs and an anti-inflammatory effect when cocultured with lymphocytes (11). These benefits are likely mediated by a combination of both cell-to-cell contact and through soluble factors (i.e., growth factors, cytokines, extracellular matrix glycoproteins) (3).

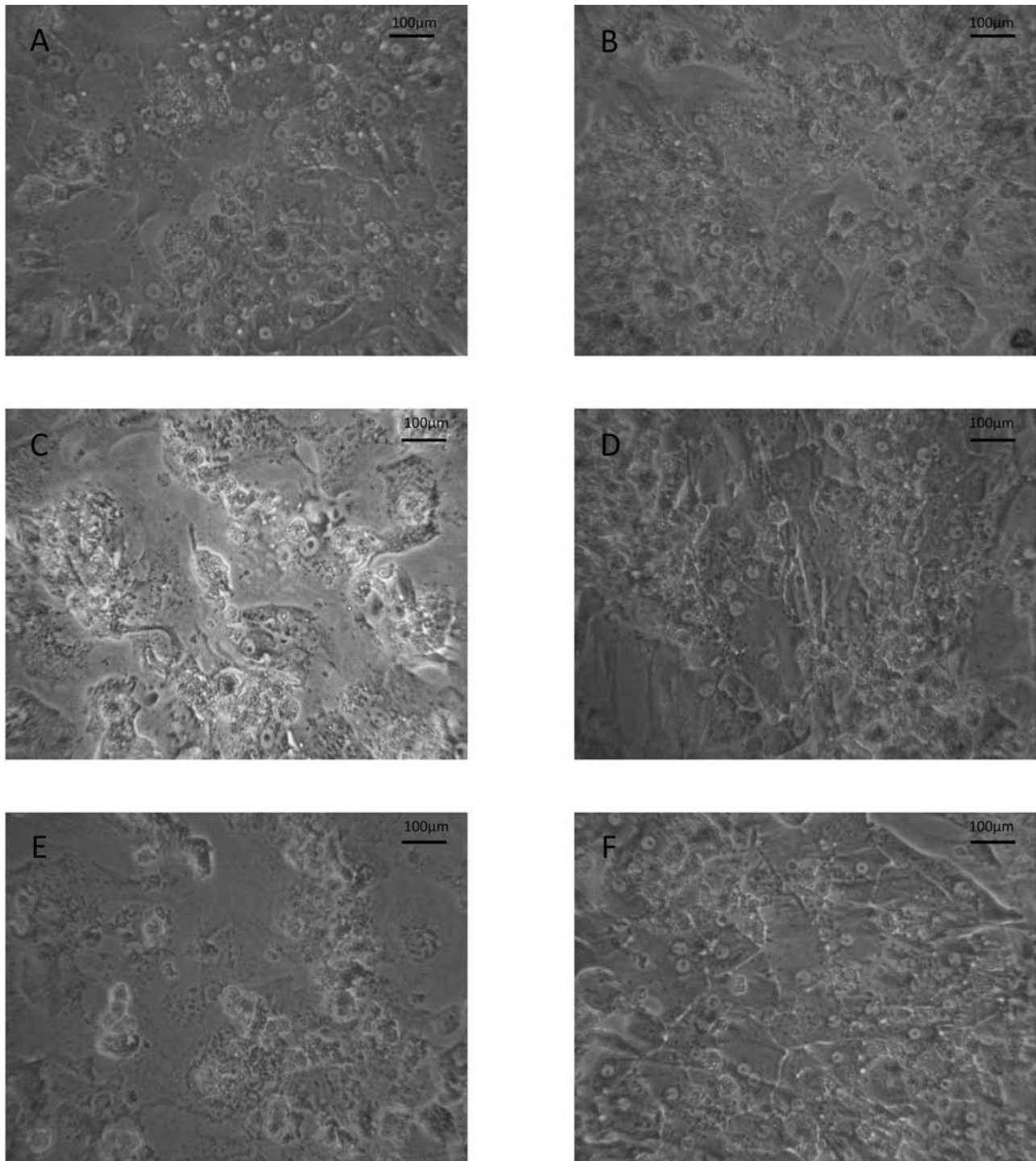Improved production of albumin, possibly secondary to transdifferentiation of MSCs into HC-like cells, was

Figure 6. Morphology of the HCs in monoculture (A, C, E) and cocultured with UC-MSCs in 6:1 ratio (B, D, F) at day 1, 7, and 13, respectively, under the phase-contrast light microscope (200×). This demonstrates fewer surviving HCs over the period in monoculture versus coculture.

seen in a pig model of fulminant liver failure with intraportal transplantation of BM-MSCs (17). We have demonstrated the expression of albumin at a gene, but not at a protein level, in MSCs, which have been in indirect coculture with HCs. It is more likely that injured HCs induce the production of cytokines/growth factors by MSCs, which, in turn, improves viability and function of HCs, for example, IL-6 or hepatocyte growth factor (HGF) (15).

We show that culture of HCs in medium previously conditioned by cocultured HCs and MSCs demonstrated
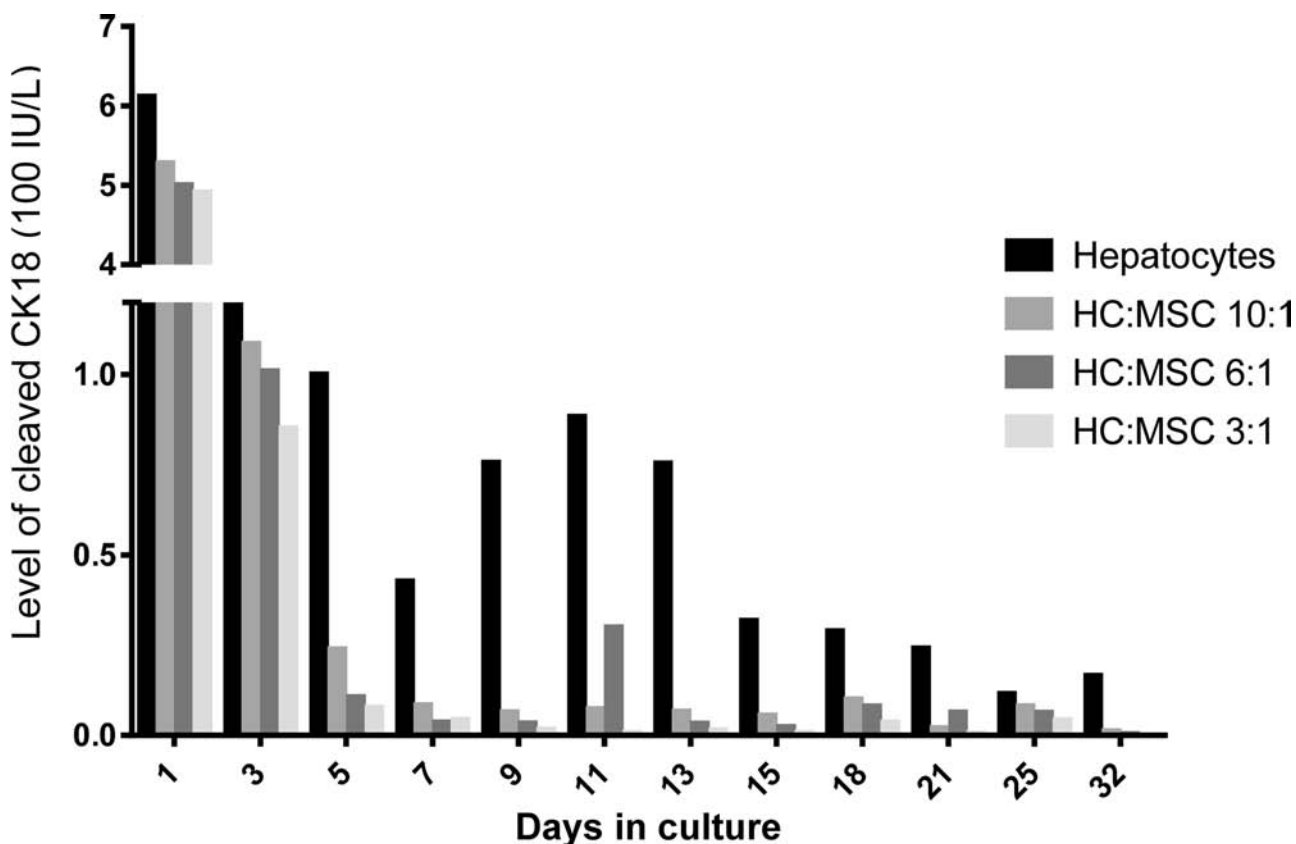
Figure 7. Level of cleaved cytokeratin 18 (CK18Asp396) under different conditions. This illustrates increased specific HC cell death in monoculture versus coculture.

improved function, whereas HCs cultured in medium conditioned by MSCs alone did not show an effect. This may suggest that the presence of HCs is needed to stimulate MSCs to produce the relevant factors/cytokines. Cell-to-cell contact may also have a role in provision of structural support for growing HCs; of note, HC function in direct coculture was superior to that in indirect coculture.

This supportive role of MSCs is particularly promising in the context of cell transplantation for acute liver failure (ALF). Hepatocyte transplantation alone may provide a bridge to either regeneration of the native liver or to transplantation, allowing sufficient time for an organ to become available (25,26,28). MSCs are a potential alternative to HC transplantation for ALF, as discussed. Though they may hasten native liver recovery, MSCs will not necessarily provide the missing liver function. Transplantation of both MSCs and HCs in ALF may provide the optimal combination of liver support with anti-inflammatory effects (11,20).

In summary, direct coculture with UC- and AD-MSCs appears to improve viability and function of HCs. This effect was not seen with CM from MSCs alone, but there was an effect seen with CM from cocultures of HCs and MSCs. This could suggest that the presence of HCs is

necessary to stimulate the MSCs to secrete prosurvival factors. The combination of transplanted HCs and MSCs may have great promise in the cellular therapy of ALF, in particular.

REFERENCES

1. Aurich, I.; Mueller, L. P.; Aurich, H.; Luetzkendorf, J.; Tisljar, K.; Dollinger, M. M.; Schormann, W.; Walldorf, J.; Hengstler, J. G.; Fleig, W. E.; Christ, B. Functional integration of hepatocytes derived from human mesenchymal stem cells into mouse livers. Gut 56:405–415; 2007.

2. Baksh, D.; Yao, R.; Tuan, R. S. Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. Stem Cells 25:1384–1392; 2007.

3. Banas, A.; Teratani, T.; Yamamoto, Y.; Tokuhara, M.; Takeshita, F.; Osaki, M.; Kawamata, M.; Kato, T.; Okochi, H.; Ochiya, T. IFATS collection: In vivo therapeutic potential of human adipose tissue mesenchymal stem cells after transplantation into mice with liver injury. Stem Cells 26: 2705–2712; 2008.

4. Campard, D.; Lysy, P. A.; Najimi, M.; Sokal, E. M. Native umbilical cord matrix stem cells express hepatic markers and differentiate into hepatocyte-like cells. Gastroenterology 134:833–848; 2008.

5. Chamberlain, J.; Yamagami, T.; Colletti, E.; Theise, N. D.; Desai, J.; Frias, A.; Pixley, J.; Zanjani, E. D.; Porada, C. D.; Almeida-Porada, G. Efficient generation of human hepatocytes by the intrahepatic delivery of clonal human mesenchymal stem cells in fetal sheep. Hepatology 46:1935–1945; 2007.

6. Chang Liu, Z.; Chang, T. M. Coencapsulation of hepatocytes and bone marrow cells: In vitro and in vivo studies. Biotechnol. Annu. Rev. 12:137–151; 2006.

7. Chien, C. C.; Yen, B. L.; Lee, F. K.; Lai, T. H.; Chen, Y. C.; Chan, S. H.; Huang, H. I. In vitro differentiation of human placenta-derived multipotent cells into hepatocyte-like cells. Stem Cells 24:1759–1768; 2006.

8. Dhawan, A.; Puppi, J.; Hughes, R. D.; Mitry, R. R. Human hepatocyte transplantation: Current experience and future challenges. Nat. Rev. Gastroenterol. Hepatol. 7:288–298; 2010.

9. Dominici, M.; Le Blanc, K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.; Krause, D.; Deans, R.; Keating, A.; Prockop, D.; Horwitz, E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8: 315–317; 2006.

10. Fitzpatrick, E.; Mitry, R. R.; Dhawan, A. Human hepatocyte transplantation: State of the art. J. Intern. Med. 266: 339–357; 2009.

11. Gomez-Aristizabal, A.; Ng, C.; Ng, J.; Davies, J. E. Effects of two mesenchymal cell populations on hepatocytes and lymphocytes. Liver Transpl 18:1384–1394; 2012.

12. Grinnemo, K. H.; Mansson, A.; Dellgren, G.; Klingberg, D.; Wardell, E.; Drvota, V.; Tammik, C.; Holgersson, J.; Ringden, O.; Sylven, C.; Le Blanc, K. Xenoreactivity and engraftment of human mesenchymal stem cells transplanted into infarcted rat myocardium. J. Thorac. Cardiovasc. Surg. 127:1293–1300; 2004.

13. Houlihan, D. D.; Newsome, P. N. Critical review of clinical trials of bone marrow stem cells in liver disease. Gastroenterology 135:438–450; 2008.

14. Isoda, K.; Kojima, M.; Takeda, M.; Higashiyama, S.; Kawase, M.; Yagi, K. Maintenance of hepatocyte functions by coculture with bone marrow stromal cells. J. Biosci. Bioeng. 97:343–346; 2004.

15. Klein, C.; Wustefeld, T.; Assmus, U.; Roskams, T.; Rose-John, S.; Muller, M.; Manns, M. P.; Ernst, M.; Trautwein, C. The IL-6-gp130-STAT3 pathway in hepatocytes triggers liver protection in T cell-mediated liver injury. J. Clin. Invest. 115:860–869; 2005.

16. Kuo, T. K.; Hung, S. P.; Chuang, C. H.; Chen, C. T.; Shih, Y. R.; Fang, S. C.; Yang, V. W.; Lee, O. K. Stem cell therapy for liver disease: Parameters governing the success of using bone marrow mesenchymal stem cells. Gastroenterology 134:2111–2121, 2121 e2111–2113; 2008.

17. Li, J.; Zhang, L.; Xin, J.; Jiang, L.; Li, J.; Zhang, T.; Jin, L.; Li, J.; Zhou, P.; Hao, S.; Cao, H.; Li, L. Immediate intraportal transplantation of human bone marrow mesenchymal stem cells prevents death from fulminant hepatic failure in pigs. Hepatology 56:1044–1052; 2012.

18. Li, Y.; Chen, J.; Chen, X. G.; Wang, L.; Gautam, S. C.; Xu, Y. X.; Katakowski, M.; Zhang, L. J.; Lu, M.; Janakiraman, N.; Chopp, M. Human marrow stromal cell therapy for stroke in rat: Neurotrophins and functional recovery. Neurology 59:514–523; 2002.

19. Liu, Z. C.; Chang, T. M. Coencapsulation of hepatocytes and bone marrow stem cells: In vitro conversion of ammonia and in vivo lowering of bilirubin in hyperbilirubemia Gunn rats. Int. J. Artif. Organs 26:491–497; 2003.

20. Mitry, R. R.; Hughes, R. D.; Dhawan, A. Progress in human hepatocytes: Isolation, culture and cryopreservation. Semin. Cell Dev. Biol. 13:463–467; 2002.

21. Mohajerani, S. A.; Nourbakhsh, M.; Cadili, A.; Lakey, J. R.; Kneteman, N. M. Transplant of primary human hepatocytes cocultured with bone marrow stromal cells to SCID Alb-uPAmice. Cell Med. 1:81–92; 2010.

22. Parekkadan, B.; van Poll, D.; Megeed, Z.; Kobayashi, N.; Tilles, A. W.; Berthiaume, F.; Yarmush, M. L. Immunomodulation of activated hepatic stellate cells by mesenchymal stem cells. Biochem. Biophys. Res. Commun. 363: 247–252; 2007.

23. Parekkadan, B.; van Poll, D.; Suganuma, K.; Carter, E. A.; Berthiaume, F.; Tilles, A. W.; Yarmush, M. L. Mesenchymal stem cell-derived molecules reverse fulminant hepatic failure. PLoS One 2:e941; 2007.

24. Puglisi, M. A.; Tesori, V.; Lattanzi, W.; Piscaglia, A. C.; Gasbarrini, G. B.; D'Ugo, D. M.; Gasbarrini, A. Therapeutic implications of mesenchymal stem cells in liver injury. J. Biomed. Biotechnol. 2011:860578; 2011.

25. Sarkis, R.; Benoist, S.; Honiger, J.; Baudrimont, M.; Delelo, R.; Balladur, P.; Capeau, J.; Nordlinger, B. Transplanted cryopreserved encapsulated porcine hepatocytes are as effective as fresh hepatocytes in preventing death from acute liver failure in rats. Transplantation 70:58–64; 2000.

26. Schneider, A.; Attaran, M.; Meier, P. N.; Strassburg, C.; Manns, M. P.; Ott, M.; Barthold, M.; Arseniev, L.; Becker, T.; Panning, B. Hepatocyte transplantation in an acute liver failure due to mushroom poisoning. Transplantation 82: 1115–1116; 2006.

27. Shi, M.; Zhang, Z.; Xu, R.; Lin, H.; Fu, J.; Zou, Z.; Zhang, A.; Shi, J.; Chen, L.; Lv, S.; He, W.; Geng, H.; Jin, L.; Liu, Z.; Wang, F. S. Human mesenchymal stem cell transfusion is safe and improves liver function in acute-on-chronic liver failure patients. Stem Cells Transl. Med. 1: 725–731; 2012.

28. Strom, S. C.; Fisher, R. A.; Thompson, M. T.; Sanyal, A. J.; Cole, P. E.; Ham, J. M.; Posner, M. P. Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. Transplantation 63:559–569; 1997.

29. Takeda, M.; Yamamoto, M.; Isoda, K.; Higashiyama, S.; Hirose, M.; Ohgushi, H.; Kawase, M.; Yagi, K. Availability of bone marrow stromal cells in three-dimensional coculture with hepatocytes and transplantation into liver-damaged mice. J. Biosci. Bioeng. 100:77–81; 2005.

30. Terry, C.; Bailey, M.; Mitry, R. R.; Lehec, S. C.; Dhawan, A.; Hughes, R. D. Analysis of the effects of cryopreservation on

rat hepatocytes using SELDI-TOF mass spectrometry. Cell Transplant. 15:35–43; 2006.

31. Terry, C.; Dhawan, A.; Mitry, R. R.; Hughes, R. D. Cryo-preservation of isolated human hepatocytes for transplantation: State of the art. Cryobiology 53:149–159; 2006.

32. Yagi, H.; Parekkadan, B.; Suganuma, K.; Soto-Gutierrez, A.; Tompkins, R. G.; Tilles, A. W.; Yarmush, M. L. Long-term superior performance of a stem cell/hepatocyte device for the treatment of acute liver failure. Tissue Eng. Part A 15:3377–3388; 2009.