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藤井 美緒

長崎大学 博士 医学

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Title: *In vivo* construction of liver tissue by implantation of a hepatic non-parenchymal/adipose-derived stem cell sheet

Short title: Subcutaneous construction of liver tissue by cell sheet implantation

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Abstract

Subcutaneous hepatocyte sheet implantation is an attractive therapeutic option for various liver diseases. However, this technique is limited by the availability of hepatocytes. Thus, the use of hepatic non-parenchymal cells (NPCs) containing small hepatocytes, which have the ability to proliferate more rapidly than mature hepatocytes, for transplantation has been suggested. The aim of our study was to construct liver tissue subcutaneously in rats by implanting NPC sheets co-cultivated with adipose-derived stem cells (ADSCs), which produce certain angiogenic factors. We crafted NPC-ADSC sheets on temperature-responsive culture dishes. NPCs formed functioning bile canaliculi and stored glycogen. In addition, their ability to produce albumin was not inferior to that of hepatocytes. Albumin production increased in time co-cultivated with ADSCs. We then implanted the co-cultivated cell sheets subcutaneously. The co-cultivated sheets retained glycogen, formed bile canaliculi, showed signs of vascularization and survived subcutaneously without pre-vascularization. These results suggest that NPCs can be a viable option in cell therapy for liver diseases. This technique using co-cultivated cell sheets may be useful in the field of regenerative medicine.

Keywords: cell sheet implantation, co-cultivated sheet, hepatic non-parenchymal cell, small hepatocyte, adipose-derived stem cell, cell transplantation
1. Introduction

Liver transplantation is an established therapeutic procedure for various liver diseases. However, it has drawbacks, including a high invasiveness and a lack of easily available donor organs. Hepatocyte transplantation has therefore been performed as an alternative to liver transplantation. Generally, primary or cryopreserved hepatocytes are infused into the portal vein (Underhill et al., 2006; Rust and Gores, 2000). However, the therapeutic effects with this method have been insufficient and temporary in most cases, mainly because most of the transplanted cells die from hypoxia or anoikis (Yamanouchi et al., 2009; Smets et al., 2002). Therefore, a method able to utilize transplanted cells effectively with low invasiveness is desired.

To this end, being able to easily craft cell sheets on temperature-responsive culture dishes (TRCDs) (CellSeed Inc., Japan) is quite attractive; after the cells obtain confluence, we cool them to 20 °C and harvest them as sheets with an undisturbed formation and extracellular matrix (Yang et al., 2005; Ohashi et al., 2007). Since no chemical treatments are needed to harvest the sheets, the native cellular function is maintained (Yang et al., 2005; Ohashi et al., 2007; Sakai et al., 2013). These sheets can then be immediately engrafted into biotissue (Ohashi et al., 2007; Sakai et al., 2015). Such cell sheet engineering is useful for treating a range of liver diseases due to its low invasiveness via subcutaneous tissue.
We believe that hepatocyte sheets will prove extremely useful for tissue engineering in clinical settings, but issues such as difficulty making three-dimensional liver tissue structures and the limited availability of hepatocytes may prove bothersome. To resolve these issues, we focused on the utility of hepatic non-parenchymal cells (NPCs). The liver tissue is comprised of 70% hepatocytes and 30% NPCs. The NPC fraction contains vessel endothelial cells, Kupffer cells, stellate cells, small hepatocytes, bile duct epithelium cells and others, as shown in Table 1 (Ogawa et al., 2004). Small hepatocytes have the ability to proliferate more rapidly than mature hepatocytes (Mitaka et al., 1999; Tateno and Yoshizato, 1996; Tateno et al., 2000; Ogawa et al., 2004) and have been reported to differentiate into mature hepatocytes and cholangiocytes (Tateno and Yoshizato, 1996). We therefore believe that using NPCs can help form tissue consisting of not only hepatocytes but also other bile duct structures by cultivation. Furthermore, although NPCs have not been considered as resources for cell therapy thus far, their utilization might help resolve the cell shortage plaguing such therapies.

The long-term survival rates for hepatocyte sheets under the skin are reportedly low, due to hypoxic conditions (Ohashi et al., 2007). We previously found that implanted human hepatocytes/fibroblasts co-cultivated sheets under the skin of mice survived for at least 8 weeks, with vascularization induced (Sakai et al., 2015; Baimakhanov et al., 2016). We concluded that humoral factors produced by fibroblasts contributed to the induction of
vascularization around and in the transplanted cell sheets, resulting in improved oxygen transport. In the present study, we decided to use adipose derived stem cells (ADSCs), which are expected to produce larger amounts of humoral factors (Banas et al., 2008; Efimenko et al., 2011) than fibroblasts, as support cells.

The aim of our study was to create co-cultivated cell sheets consisting of NPCs and ADSCs, which may be useful for cell therapy, and to construct liver tissues subcutaneously in rats.

2. Materials and Methods

2.1. Animals

We used male Fischer 344 rats obtained from Japan SLC, Inc., Japan. All animal care, treatments and procedures were approved by the Biomedical Research Center and Committee of Nagasaki University. The donors were 7 weeks old, and the recipients were 4 to 5 weeks old.
2.2. *Isolation of ADSCs*

ADSCs were isolated as reported previously (Tholpady et al., 2003) with some modification. The donor rats were anesthetized with isoflurane (Wako Pure Chemical Industries, Japan). A lower abdominal midline incision was made to expose the inguinal adipose tissue. The inguinal adipose tissue was carefully dissected, rinsed with cold PBS (Wako Pure Chemical Industries) and minced into small fragments with a blade. The fragments were digested with 0.1% collagenase type I (Thermo Fischer Scientific K. K., Japan) in PBS for 60 min at 37 °C with vigorous shaking. The top lipid layer was removed, and the remaining digest was filtered through a 70-μm Cell Strainer (Corning Incorporated, USA) and centrifuged at 220 g for 10 min. The pellet was treated with 160 mM NH₄Cl for 10 min to lyse the red blood cells. The remaining cells were suspended in Dulbecco’s modified Eagle’s medium (Wako Pure Chemical Industries) supplemented with 10% fetal bovine serum (Life Technologies Japan, Ltd., Japan) and plated at a density of 1×10⁶ cells in 90-mm cell culture dishes (Thermo Fischer Scientific K. K., Japan). The culture media was changed thrice weekly. The cells were maintained at subconfluent levels and passaged using trypsin/EDTA (Life Technologies Japan, Ltd.). Passage 2 ADSCs were used for our experiments.
2.3. Isolation of the NPCs and hepatocytes

NPCs and hepatocytes were isolated using a collagenase perfusion method in accordance with previous reports (Ogawa et al., 2004) with some modification (Figure 1). The rats were anesthetized with isoflurane. After an upper abdominal midline incision, the incision line was extended to under the costal arch to improve the field of view. The portal vein was then cannulated with an 18-gauge catheter (Terumo Corporation, Japan), and the intrathoracic inferior vena cava was incised to reflux. Perfusion was performed with Hanks' Balanced Salt Solution with NaHCO₃ without calcium chloride and magnesium sulfate (Sigma-Aldrich, USA) supplemented with 0.1 M EGTA (DOJINDO LABORATORIES, Japan), Hepes (DOJINDO LABORATORIES) and 0.05% collagenase (Wako Pure Chemical Industries, Ltd.) solution for digestion. After adequate digestion, the liver was excised and chopped finely in Dulbecco’s modified Eagle’s medium low glucose (Wako Pure Chemical Industries) supplemented with 3.5g/L glucose, 10mM Hepes, 10% fetal bovine serum (Life Technologies Japan, Ltd., Japan) and 1% Penicillin-Streptomycin-Glutamine (Thermo Fischer Scientific K. K.). After filtration through a gauze wetted with medium, the cell suspension was centrifuged twice at 50 g for 1 min. The pellet was obtained as hepatocytes and purified by 45% Percoll density gradient centrifugation. The supernatant was collected and centrifuged 3 times at 150 g for 5 min. We obtained a pellet including NPCs, and the pellet was resuspended in the medium. The cell number and viability of both hepatocytes and NPCs were counted using the
trypan blue exclusion test, and cells with > 80% viability were used for the experiment.

2.4. FACS analysis

Flow cytometry was performed using the following mouse anti-rat monoclonal antibodies: anti-CD45 FITC, anti-CD90 FITC and anti-CD31 FITC (all from LifeSpan BioSciences, Inc., USA). ADSCs in passage 2 were incubated with the respective antibody for 15 min at 4 °C and then washed with PBS and analyzed with a FACSCanto II (Becton, Dickinson and Company, USA).

2.5. Creating and implanting co-cultivated cell sheets

The procedure for crafting the sheets is shown in Figure 2. We disseminated $1.0 \times 10^5$ ADSCs on 35-mm TRCDs. After reaching confluence, $1.0 \times 10^6$ NPCs or hepatocytes in 2 ml medium were seeded on top of confluent ADSCs. Two days later, the cultured cells were harvested as a sheet by cooling to 20 °C and prepared for implantation. We then transplanted these sheets into the subcutaneous tissue of the recipient rats (Baimakhanov et al., 2016). Briefly, after being anesthetized with isoflurane, the recipient rats received a belly skin mid-line incision, and subcutaneous pockets were made on either side of the incision line. Three co-cultivated sheets per side were implanted. Seven days after implantation of the sheets, we performed 70% hepatectomy to stimulate the small hepatocytes within the sheets to grow. At 28 days
after implantation, the animals were sacrificed to obtain samples of the abdominal walls with the cell sheets.

2.6. **Determination of vascular endothelial growth factor and hepatocyte growth factor concentrations**

To evaluate the production of angiogenic factors, we cultured ADSCs alone on the TRCDs. The medium was changed every other day and collected at Day 10. The vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) secretion into the medium from ADSCs over a 48-h period were measured using a VEGF Rat ELISA kit (Abcam plc, UK) and RAT HGF EIA (Institute of Immunology Co. LTD., Japan), respectively. As a control, we also cultured fibroblasts (normal rat dermis of P1 neonates; Cell Applications, INC., USA) on the dishes.

2.7. **Appearances of the cultured cells**

We evaluated the cultured cells 0, 1, 3, 5 and 7 days after seeding via microscopy.
2.8. Ability to produce albumin of NPCs or hepatocytes

We used two types of dishes in the present study: 35-mm non-coated tissue culture dishes (AGC TECHNO GLASS CO., LTD., Japan) on which ADSCs were cultured confluently beforehand, and 35-mm collagen-coated dishes (AGC TECHNO GLASS CO., LTD.). We divided the cultured cells into four groups based on the culture conditions as follows: NPCs on collagen-coated dishes (N), hepatocytes on collagen-coated dishes (H), NPCs on confluent ADSCs (AN) and hepatocytes on ADSCs (AH). A 2-ml suspension of NPCs or hepatocytes at a density of 0.5×10^6 cells/ml was seeded onto each dish. The dishes were then incubated at 37 °C in 5% carbon dioxide. We collected the medium 1, 3, 5, and 7 days after cell dissemination and cryopreserved it at -30 °C. The albumin concentrations in these samples were measured using an enzyme-linked immunosorbent assay. A 96-well microwell plate (Thermo Fischer Scientific K. K.) was coated with goat IgG fraction to rat albumin (COSMO BIO Co., LTD., Japan) for 1 h. After blocking nonspecific responses, each sample was seeded onto the plate and left to rest for 1 h, followed by another 1-h rest with peroxidase-conjugated sheep IgG fraction to rat albumin antibody (COSMO BIO Co., LTD.). Finally, the substrate was added, and the extinction was measured using a microplate reader (Bio-Rad Laboratories, Inc., USA) at 405 nm.
2.9. Evaluation of bile canaliculus structure

The cells were cultivated in 35-mm glass-based dished (AGC TECHNO GLASS CO., LTD.) for 3 days. We used choly-llysyl-fluorescein (Corning Life Sciences, USA), which is a fluorescein-labeled reagent in choly glycine, a base component of bile acid, in order to visualize the bile acid excretion process of the bile canaliculus structures formed between cells as a fluorescence signal.

2.10. Histological evaluations of co-cultivated cell sheets

We evaluated the co-cultivated cell sheets three days after seeding of NPCs via microscopy. The harvested sheets were fixed in 4% paraformaldehyde phosphate buffer solution (Wako Pure Chemical Industries, Ltd.) and embedded in paraffin for subsequent histological evaluations. To confirm that sheets had the ability to store glycogen, Periodic Acid-Schiff (PAS) staining with PAS staining kit (Merck KGaA, Germany) was performed.

Immunohistochemical staining using the avidin-biotin peroxidase complex (ABC) method was performed. The primary antibodies were anti-CD26 antibody (Santa Cruz Biotechnology, USA) and anti-Ki-67 antibody (Thermo Fischer Scientific K. K.). Five-micrometer-thick paraffin sections were deparaffinized. We used the LSAB+ Kit, HRP (Dako, Japan), which is a technique based on the labelled-streptavidin-biotin method. Endogenous peroxidase activity was quenched by first incubating the specimens for 5 min in
3% hydrogen peroxide. We then applied the above primary antibodies or negative control reagent to the specimens and incubated them for 30 min. We did sequential incubations with biotinylated link antibody and peroxidase-labelled streptavidin. Staining was completed after incubation with substrate-chromogen solution and counterstaining by hematoxylin.

2.11. Histological evaluation of implanted cell sheet

The samples were examined histologically. The resected specimens were fixed in paraformaldehyde phosphate buffer solution and embedded in paraffin for the subsequent histological evaluation. To confirm the ability to retain glycogen, PAS staining was done via the procedure mentioned above, and the samples were observed using a microscope. We performed immunohistochemical staining via the ABC method. The primary antibodies were anti-rat albumin antibody (Bethyl Laboratories, USA), anti-CD26 antibody (Santa Cruz Biotechnology), anti-PECAM-1 (CD31) antibody (M-20) (Santa Cruz Biotechnology), anti-cytokeratin 19 (CK19) antibody (Leica Biosystems, Germany), anti-Ki-67 antibody (Thermo Fischer Scientific K. K.), anti-cytokeratin 8 (CK8) antibody (Abcam plc.) and anti-phosphoenolpyruvate carboxykinase-1 (PCK-1) antibody (Abcam plc.).
2.12. Statistical analyses

The data were analyzed with the Prism 4 software program (GraphPad Software, Inc., USA) and expressed as the average ± standard deviation. Mann-Whitney’s U test was used for the comparison of two groups. The data were analyzed using the analysis of variance (ANOVA) for multiple comparisons. For p values < 0.05, the Tukey-Kramer test was applied for multiple comparisons.

Results

2.13. Characterization of ADSCs

Flow cytometry revealed that the population of ADSCs was positive for CD90 and negative for CD45 and CD31 (Figure 3a), findings which are concordant with those of previous reports (Yoshimura et al., 2007; Ishimura et al., 2008).

2.14. Secretion of angiogenic factors

To evaluate the function of ADSCs, we measured the amounts of VEGF and HGF secreted in the media over 48 h at Day 10 (Figure 3b). ADSCs produced significantly larger amounts of VEGF than fibroblasts (847.2 vs. 78.3 ng/ml/10^6 p < 0.01). With regard to HGF, secretion was detected from ADSCs (42.0 ng/ml/10^6), the amount from fibroblasts could not be determined.
2.15. **Appearances of the cultured cells**

Figure 4a shows ADSCs that reached confluence. The ADSCs were attached to the surface of the dish and exhibited a slim, fusiform morphology. Figure 4b shows NPCs co-cultivated with ADSCs at Day 3. Polygonal hepatocyte-like cells with one to three nucleoli, potentially derived from small hepatocytes, spread over the ADSCs.

2.16. **Ability of NPCs or hepatocytes to produce albumin**

The concentration of albumin 1, 3, 5, and 7 days after seeding was measured for each group (Figure 4b). The highest albumin production was observed in Group AN on Day 7. In Group AN, the albumin concentrations on Day 7 differed significantly from the values on other measured days (Day 1: 7586.9; 3: 23567.0; 5: 49946.6; 7: 78596.1 μg/ml, p < 0.01). In Group AH, the albumin concentration on Day 7 was the largest, similar to group AN (Day 1: 8932.0; 3: 25445.0; 5: 35186.0; 7: 59320.9 μg/ml, p < 0.01). The albumin concentration continued to increase until Day 7 in the co-cultivated groups. In addition, the albumin production in Groups AN and AH on Day 7 were greater than those in Groups N and H (AN Day 7: 78596.1; N: 14999.5; AH: 59320.9; and H: 22949.9 μg/ml, p < 0.01). Although there were no significant differences, Group AN tended to produce greater amounts of albumin than Group AH 7 days after seeding.
2.17. Evaluation of bile canaliculus structures

The formation of a functional bile canaliculus was demonstrated using choly-l-lysyl-fluorescein dye. Figure 5a showed the excretion of the fluorochrome between cells at 3 days after seeding, indicating the formation of a functional bile canaliculus.

2.18. Histological evaluations of co-cultivated cell sheets

After harvesting, the cell sheets shrunk to approximately 20-25 mm in diameter. Under microscopy, the cell sheets showed single-layered columnar or cuboidal-shaped cells aligned in a line (Figure 5b). Positive PAS staining revealed glycogen accumulation in the cytoplasm (Figure 5c). Immunohistochemical staining demonstrated the formation of bile canaliculi with CD26 positivity (Figure 5d) and DNA synthesis by Ki-67 positivity (Figure 5e).

2.19. Histological evaluations of implanted cell sheets

NPC-ADSC co-cultivated sheets survived for at least 4 weeks. After 4 weeks, the implanted subcutaneous cell sheets consisted of a linear array of polygonal cells with eosinophilic cytoplasm and round nuclei, resembling hepatocytes (Figure 6a). These cells showed a positive reaction on PAS staining, indicating glycogen storage (Figure 6b). The cells were also positive for both albumin (Figure 6c) and CK8 (Figure 6d), suggesting that they expressed characteristics of hepatocytes. Furthermore, the cells were positive for PCK1,
important factor in the metabolic pathway of gluconeogenesis (Figure 6e). CD31, a marker for the vascular endothelium, was also positively expressed within and around the cell aggregates, suggesting neovascularization (Figure 6f). In addition, the cell junctions showed CD26 positivity, indicating bile canaliculi formation (Figure 6g).

3. Discussion

In the present study, we subcutaneously implanted NPC-ADSC co-cultivated sheets in rats and demonstrated that the sheets survived for at least 4 weeks. The cells not only retained the normal structures and mutual junctions of hepatocytes, as shown by CK8 and CD26 positivity, they also exhibited the functional characteristics of hepatocytes, such as glycogen storage and the expression of PCK-1. The existence of glycogen and an enzyme involved in the gluconeogenesis pathway indicated that normal glucose metabolism of the liver could indeed function with subcutaneous implantation. In addition, neovascularization appeared to exist, given the positivity of CD31, a marker of vascular endothelium, within and around the cell aggregates. Furthermore, humoral factors such as VEGF and HGF, which were excreted by ADSCs, may have induced new blood vessel formation, ensuring long-term cell survival provided the oxygen and nutrition supply remained adequate.
NPCs have not received much attention in the hepatocyte transplantation field as sources of cell therapy. We therefore focused on small hepatocytes in the fraction of NPCs in the present study. Small hepatocytes were initially reported in 1992 as hepatocyte progenitor cells in the liver with a higher proliferative ability than mature hepatocytes (Mitaka et al., 1992). Small hepatocytes express hepatocyte markers such as albumin or cytokeratin 8/18, show hepatocellular morphological characteristics and can differentiate into mature hepatocytes \textit{in vitro} (Mitaka et al., 1992; Thorgeirsson, 1993). In our study, NPCs showed the ability to produce albumin, an ability likely mainly derived from small hepatocytes (Tateno and Yoshizato, 1999), and showed comparable characteristics to hepatocytes \textit{in vitro}. These findings suggested that NPCs, which are typically discarded after isolation of hepatocytes, may be an additional option for cell therapy for liver diseases.

Both NPCs and hepatocytes co-cultivated with ADSCs demonstrated continuous and increasing production of albumin compared with the respective mono-cultures. The VEGF and HGF produced by ADSCs might have improved the oxygen transport to NPCs and maintained the viability and function of these cells both \textit{in vitro} and \textit{in vivo} (Nakagami et al., 2005). After implantation, in addition to these effects, the humoral factors might have improved or maintained the function of hepatic cells by vascularization or even stimulated proliferation of the hepatic cells, allowing the NPCs to survive subcutaneously without...
pre-vascularization (Sakai et al., 2015; Baimakhanov et al., 2016).

In the liver, unconjugated bilirubin is taken up into hepatocytes, converted into hydrosoluble conjugated bilirubin by the enzyme UGT1A1 and secreted into the bile canaliculi formed between hepatocytes (Adachi and Morooka, 2009). The secreted bilirubin flows into the bile ducts, consisting of bile duct epithelial cells, to the duodenum. In the present study, we observed the formation of bile canaliculus structures between hepatocytes both in vitro and in vivo by immunohistochemistry. Furthermore, the excretion of fluorochrome was demonstrated in these structures in vitro, suggesting that the cells in the sheets were able to metabolize and excrete bilirubin. However, we were unable to observe bile duct epithelial cells that expressed CK19 (data not shown) or mature bile duct structures in vitro or in vivo.

Given that bile duct epithelial cells should be contained in the NPC fraction initially (Ogawa et al., 2004), we anticipated that transplanted cell sheets would show differentiated bile duct structures. However, despite the presence of humoral factors from ADSCs or other cells in the NPC fraction, the formation of bile duct structures was not possible merely by the presence of bile duct epithelial cells. Although we did not evaluate the existence of bile duct epithelia over time, these cells might have disappeared while being cultured. Future studies should therefore determine the proportion of cell types included in NPC fraction and the optimal culture conditions for both hepatocytes and bile duct epithelial cells. Additionally,
other techniques, such as culturing EpCAM-positive bile duct epithelial cells in the liver, isolating small hepatocytes with FACS (Dollé et al., 2015) or adding TGF-β to the culture medium, may be considered for bile duct differentiation (Clotman et al., 2005). Our findings confirmed that NPCs include cells that retain hepatocytic functions, such as small hepatocytes. Therefore, in liver regenerative medicine, NPCs can be used as an additional cell source.

We will next attempt to construct subcutaneous liver tissue with three-dimensional (3D) structures. Because the number of cells implanted subcutaneously is limited, we carried out hepatectomy in order to provide hepatotrophic stimulation and encourage the growth of the implanted cell sheets. However, we were unable to demonstrate remarkable growth of the implanted cells due to the stimulation being only temporary. Several strategies for generating liver tissue with 3D structures (i.e. continuous hepatotrophic stimulation), such as the addition of thyroxine (Moro et al., 2004) or HGF (Guha et al., 2005) to promote hepatocyte growth or stacking cell sheets in vitro (Haraguchi et al., 2012), have been suggested. We previously reported that, in a rat model of radiation-induced liver damage combined with partial hepatectomy, implanted multilayered hepatocyte sheets were able to survive subcutaneously with continuous proliferative activity, thereby maintaining the liver function in vivo for at least 2 months and providing metabolic support for rats (Baimakhanov et al., 2016).
We will further investigate the application of this technique in other experimental liver disease models, such as the no-albumin rat, Gunn rat (Crigler-Najjar syndrome model) and the chronic liver failure model (liver fibrosis with CCl₄), and examine the improvement in the liver function and the survival rate.

In conclusion, NPCs showed identical functions to hepatocytes in vitro. The NPC-ADSC co-cultivated sheets were able to survive subcutaneously for 4 weeks without pre-vascularization. These results suggest that NPCs may represent additional resources for cell therapy for liver disease, and this technique using co-cultivated cell sheets may prove useful for cell transplantation.

**Conflicts of interest**

The authors declare no conflicts of interest in association with this study.
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Table 1. Types of hepatic cells

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<td>Parenchymal cell</td>
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<td>Non-parenchymal cell (NPC)</td>
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<td>Vascular endothelial cell</td>
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<td>Monocyte-derived macrophage</td>
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<td>Others</td>
<td>Small hepatocyte</td>
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<td>Bile duct epithelial cell</td>
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Figure 1. The procedures used to isolate hepatocytes and hepatic non-parenchymal cells.
Figure 2. A schematic diagram of the overall study.
Figure 3. The expression of cell surface markers on adipose-derived stem cells (ADSCs) evaluated by flow cytometry (a). Amounts of vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) secreted in the media over 48 h at Day 10 (n=5 each) (b). The values are expressed as the means ± SD.
Figure 4. Cultured cells under microscopy. Slim fusiform ADSCs at 3 days after seeding (a), and NPCs co-cultured with ADSCs at Day 3 (b) (original magnification 100×). Albumin concentrations in the culture medium (c). N: NPCs, AN: ADSCs + NPCs (co-cultivated), H: hepatocytes, AH: ADSCs + hepatocytes (co-cultivated) (n=7 each). The values are expressed as the means ± SD. * p < 0.01
Figure 5. The excretion of fluorochrome between NPCs (a). Photomicrographs of the co-cultivated sheets. H&E (b), PAS (c), CD26 (d) and Ki-67 (e).
Figure 6. Photomicrographs of the transplanted sheets. H&E (a), PAS (b), albumin (c), CK8 (d), PCK1 (e), CD26 (f) and CD31 (g).