Hepatocyte Transplantation
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Methods and Protocols

Edited by

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Preface

Cellular therapy using human hepatocytes is being evaluated worldwide as an alternative to organ transplantation in patients with liver-based metabolic disease and acute liver failure. The basis for clinical use has come from the demonstration of efficacy in animal models of acute and chronic liver disease.

Protocols have been developed for the isolation of hepatocytes from liver tissue under GMP conditions and also for improved methods of cryopreservation, so hepatocytes can be stored for later clinical use. Assays are used to assess the quality and function of the hepatocytes prior to transplantation. There are clinical protocols for administration of cells directly into the patient’s liver.

The engraftment of donor cells in the recipient liver can be detected by DNA techniques or functional proteins in the case of genetic liver disorders. In vivo methods are needed to track the fate of hepatocytes after transplantation.

Due to the shortage of donor organs, the future of hepatocyte transplantation will be alternative sources of liver cells such as foetal hepatoblasts or stem cell-derived hepatocytes. Methods for culture and in vitro proliferation of stem cells will be important for their application.

It is hoped that this volume from the experts in the field provides the reader with the practical protocols to enable them to perform and investigate hepatocyte transplantation. Needless to say this is a rapidly developing field, and new and improved techniques are being developed all the time.

Anil Dhawan & Robin D. Hughes
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Color Plates

Color Plate 1: Apoptotic nuclei and bodies observed in mouse primary hepatocyte cultures after staurosporine treatment (white arrows). Freshly isolated mouse hepatocytes were plated for 24 h on a collagen type I-coated coverslips in well plates and treated for 4 h with 1 μM staurosporine. Cells were thereafter fixed with 4% of formaldehyde for 20 min at room temperature, stained with DAPI for 30 min and analyzed using a fluorescence microscopy. (see discussion on p. 63)

Color Plate 2: Condensation of chromatin at the periphery of the nucleus in apoptotic mouse hepatocytes (black arrows). (A) Primary mouse hepatocytes were plated for 24 h in a coated collagen type I well plates and treated for 4 h with 1 μM staurosporine. Cells were thereafter fixed with 4% formaldehyde for 20 min at room temperature and stained with HE for 10 min. (B) slice of mouse liver prefixed with formaldehyde, paraffin-embedded and HE-stained. (see discussion on p. 65)

Color Plate 3: Transplantation of autologous hepatocytes into *Macaca mulatta* after retroviral-mediated gene marking. (A) Protocol for simian hepatocyte isolation, retroviral transduction and transplantation. Hepatocyte transduction with HIV-1-derived lentivirus vectors avoids the culture steps. They are transduced in suspension and transplanted. (B) Hepatocytes are transplanted via the infusion chamber. (C) Freshly isolated simian hepatocytes at confluence after 3 days of culture. (D) Transduced hepatocytes in culture expressing the β-galactosidase. (E) Thawed hepatocytes after 3 days of culture. (see discussion on p. 90)

Color Plate 4: Liver preconditioning using monocratoline (MCT) for improving cell engraftment in DPPIV– rats. Transplanted F344 rat hepatocytes are shown in the recipient liver 4 and 7 days after cell transplantation. Panel a shows 1–3 transplanted hepatocytes with histochemically visualized DPPIV activity (red color, arrows) in periportal areas (Pa). By contrast, in MCT-treated rats (b) several-fold more transplanted cells are present. Original magnification, ×200; hematoxylin counterstain. Modified from Joseph B, et al. (20). (see discussion on p. 111)

Color Plate 5: Analysis of the kinetics of liver repopulation in DPPIV– rats preconditioned with retrorsine and partial hepatectomy. Foci of transplanted cells with DPPIV activity (red color) are seen 2 (a), 3 (b), and 4 weeks (c) after cell transplantation. Morphometric analysis of liver repopulation in panel d indicates linear increase in liver repopulation during this period. Original magnification, (a–c), ×40; hematoxylin counterstain. Modified from Wu Y-M et al. 18. (see discussion on p. 112)

Color Plate 6: Effect of immunosuppressive drugs, Rapamycin (Rapa) and Tacrolimus (Tacro), on liver repopulation in DPPIV– rats preconditioned with retrorsine and partial hepatectomy. Animals were treated with drugs subsequent to the completion of cell engraftment. Rapa- but not Tacro-suppressed transplanted cell proliferation as shown by DPPIV histochemistry and morphometric analysis of either the extent of liver repopulation (e) or individual transplanted cell foci (f). Original magnification (a–d), ×100; hematoxylin counterstain. Modified from Wu Y-M et al. (18). (see discussion on p. 114)

Color Plate 7: Transfection by Amaxa Nucleofection: Expression of GFP in primary mouse hepatocytes (isolated from C57BL/6 mice) nucleofected using an Amaxa mouse hepatocyte Nucleofector kit with a plasmid encoding maxGFP. Twenty-four hours after nucleofection, cells were analyzed by bright field (A) and fluorescence microscopy (B). The merged image is shown in panel (C). (see discussion on p. 124)
Color Plate 8: **Transfection using liposomes containing F protein of the Sendai virus:** Expression of LacZ in cells transfected with DNA-loaded F-virosomes as described in the text. After incubation for 24 h, cells were fixed with ethanol, stained for β-galactosidase and photographed. (magnification, ×20, Nikon, Japan). Hepa1 cells (A), HEK293 cells (B). Note, only asialoglycoprotein-expressed cells are transduced by this method. Structure of histidine lipid used to enhance F-virosome-mediated gene transfer (C). (see discussion on p. 127)

Color Plate 9: **Transduction of primary rat hepatocytes using a Lentiviral vector:** Isolated Gunn rat hepatocytes were transduced with Lentivirus pAlb-UGT1A1 at an MOI of 10 and immunostained with WP1, monoclonal primary antibody against UGT1A1, followed by anti mouse Alkaline Phosphatase substrate kit III as described in the text and control hepatocytes (A) and experimental hepatocytes (B) were photographed. (see discussion on p. 132)

Color Plate 10: **Lentiviral vector-mediated transduction of primary mouse hepatocytes, enhanced by Magnetofection®:** Isolated mouse primary hepatocytes were transduced with Lentivirus pAlb-LacZ at an MOI of 5 with or without Magnetofection® as described in the text, and were stained 48 h later for bacterial β-galactosidase activity (blue reaction products). (A) Untransfected control; (B) Lentiviral transduction without Magnetofection®; (C) Lentiviral transduction enhanced by Magnetofection®. (see discussion on p. 133)

Color Plate 11: Revealing that bone marrow cells (BMCs) have differentiated into non-haematopoietic cells can be achieved by transplanting lethally irradiated animals with new BMCs that can be tracked whatever their subsequent fate. This would include male BMCs to a female recipient, or GFP- or LacZ-positive BMCs to wild-type recipients. The male chromosome can be detected by in situ hybridisation, GFP by immunohistochemistry and β-galactosidase by X-gal histochemistry. (see discussion on p. 141)

Color Plate 12: Fluorescent and confocal microscopy. (A) Male cells (arrows) in male bone marrow-transplanted female mouse liver (green FITC dot). These cells are CK18 immunoreactive (red cytoplasm), suggestive of hepatocyte differentiation. (B) Human cell (green FITC, spotty nucleus, arrowed) in mouse liver (pink CY3 spots) after injection of human CD133+ cells into a NOD-SCID mouse. (C) BCR/ABL probe on human liver in a case of CML showing normal ploidy, with two copies of chromosome 9 (red signals) and two copies of chromosome 22 (green signals) in some cells (asterisks), but multiple copies (polyploidy) in another cell (arrow). (D) BCR/ABL fusion signal (green and red overlap producing orange, arrowed) seen in cell tentatively identified as a hepatocyte in a case of CML. There is one native chromosome 9 (red), one native chromosome 22 (green) and one small red signal (ASS gene). (E) Confocal images demonstrating liver polyploidy in a female mouse transplanted with male bone marrow, with multiple X chromosomes (green signals) showing that a Y chromosome (red signal, black arrow) is outside the nuclear membrane (view E), while a smaller nucleus (white arrow) has both X and Y chromosomes contained within it. (see discussion on p. 142)

Color Plate 13: Liver fibrosis in a mouse as viewed by bright field microscopy. (A) Demonstration of Y chromosome-positive cells (brown nuclear dots) in a female mouse liver after a male bone marrow transplant. (B) Demonstration of mRNA for pro(α1)I (black autoradiographic grains) in the same liver using a 3H-labelled antisense riboprobe. (C) Demonstration of Y chromosome detection (brown dot, arrow) and IHC for α-SMA expression (red staining) – a marker of myofibroblast differentiation. (D) Demonstration of the expression of mRNA for pro(α1)I, the Y chromosome and α-SMA in the same liver. One Y chromosome-positive cell is expressing neither α-SMA nor mRNA for pro(α1)I, but another cell (asterisk) is expressing all three markers. Note the reduced grain density when techniques are combined in comparison to when ISH for the mRNA is performed alone. (E and F) Examples of ISH for pro(α1)I mRNA.
expression and immunoreactivity for \( \alpha \)-SMA in the same section. (see discussion on p. 147)

Color Plate 14: The appearance of a Percoll gradient following centrifugation at \( 800 \times g \) for 30 min is shown. Layers 2 and 3 contain biliary epithelial cells (approximately 10\%) and are harvested for further purification of immature and mBEC populations by immunomagnetic separation. The supernatant and fractions 1 and 4–6 are discarded. (see discussion on p. 197)

Color Plate 15: Visualisation of the MRI contrast agent. (A) Adult human hepatocytes being labelled with the bimodal Iron Oxide Green Oregon (IOGO) contrast agent (in green). Note that some cells (cell nuclei in blue) are not labelled. It is noteworthy that the contrast agent seems strongly associated with the cell nuclei and does not fill the cytoplasm. It is likely that mainly phagocytic Kupffer cells incorporated this agent, whereas unlabelled cells represent a small fraction of undifferentiated hepatocytes. (B) In contrast, the Gadolinium Rhodamine Dextran (GRID) bimodal agent (in red) clearly labels the cytoplasm of cells that have the appearance of immature hepatocytes and is incorporated into all types of cells. (see discussion on p. 212)