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# Stem Cell Therapy for Acute and Chronic Liver Failure

Meghnad G. Joshi, Apurva Gadgil, and Ramesh R. Bhonde

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## Introduction

Liver disease is an exceptionally common cause of morbidity and mortality worldwide. The acute and chronic liver diseases are still treated with supportive rather than curative approaches. Orthotopic liver transplantation has so far been the only available therapy for patients with end-stage liver diseases. Unfortunately, the availability of donor organs is limited, and many patients die each year waiting for liver transplants. Prevalence rates and the demand for liver transplantation are rising annually worldwide. In Europe, nearly 5,500 orthotopic liver transplantations (OLTs) are performed [1]. In the UK, active transplantation list in March 2012 was 7,636 patients at the end of March 2012 and 508 patient died waiting for suitable transplant [2].

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M.G. Joshi (✉)  
Department of Stem Cells and Regenerative Medicine,  
D.Y. Patil University, D. Y. Patil Vidyanagar,  
Kasba Bawda, Kolhapur 416006, Maharashtra, India  
e-mail: [cmd@stemplusbiotech.com](mailto:cmd@stemplusbiotech.com)

A. Gadgil  
Stem Plus Biotech Pvt. Ltd., Shiv Meridian,  
Block #11, MG Road, Opp. Collector Bunglow, Sangli  
416416, MS, India  
e-mail: [info@stemplusbiotech.com](mailto:info@stemplusbiotech.com)

R.R. Bhonde  
School of Regenerative Medicine, Manipal Institute of  
Regenerative Medicine, Manipal University, GKVK Post,  
Bellary Road, Allalasanandra, Near Hotel Royal Orchid,  
Yelahanka, Bangalore 560065, India  
e-mail: [rr.bhonde@manipal.edu](mailto:rr.bhonde@manipal.edu)

Similarly, in USA as of October 2013, 123,392 liver transplants had been reported and as of today 16,479 patients are on waiting list [3].

Cell-based therapies with stem cells and their progeny is a promising new approach to this largely unmet medical need. Recent advances in the liver repopulation field and the use of alternative cell sources are under investigation. The major drawbacks and the most important advantages of adult or fetal hepatocytes and hepatocytes generated from different stem cells in the wide range of experimental and clinical applications are published. Even after 40 years of extensive experimental research, none of the procedures represent a “gold standard” in the clinical practice. The achievement of positive outcomes in many experimental and clinical studies involving liver progenitor cells has been handicapped by the limited engraftment of cells that can be of therapeutic significance. If stem cells are to be used as an alternative or bridge to organ transplantation, it is very important to reduce the cell loss during the transplantation, repeated cell infusions, and large numbers of cells for transplantation.

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## Acute Liver Failure

Acute liver failure (ALF) or fulminant hepatic failure (FHP) is characterized by rapid deterioration of hepatocyte function that leads to hepatic encephalopathy, coagulopathy, cerebral edema, infection, and multiorgan dysfunction syndrome [4]. ALF still has high mortality rate, and

orthotropic liver transplantation (OLT) is the only available treatment that gives satisfactory results [4, 5]. However, clinicians face difficulties in making a decision about the transplant due to limited patient history and rapid deterioration of the patient. Thus, delay results in a patient becoming un-transplantable due to other contraindications like multiorgan failure. Urgent OLT has become a standard treatment for ALF patients in USA where survival rates have shown improvement and 1-year survival exceeding 80 % [6].

The common causes of ALF are viral hepatitis, idiosyncratic drug reactions, acetaminophen, and mushroom ingestion [7]. Viral hepatitis B is the most common cause of ALF worldwide, responsible for about 70 % of cases, and it produces significant morbidity and mortality [8, 9]. Another causative factor for ALF in Western world is acetaminophen (APAP) overdose which is more commonly encountered. APAP intake leads to excessive production of its active metabolite *N*-acetyl-*p*-benzoquinone imine in the liver, causing depletion of the glutathione stores followed by centrilobular necrosis [10].

However, OLTs have numerous limitations like shortage of donor organs, the high costs, and the lifelong immunosuppressive treatments [11]. Various alternatives to OLT have been evaluated, such as split liver, cross circulation, plasma exchange, hemofiltration, hemodialysis, and hemoperfusion without any significant improvement [12, 13]. The alternatives such as stem cell transplant or artificial liver support systems can be helpful, as bridge to transplant that will increase the availability of suitable donor for patients who would otherwise have died might survive until transplantation.

Safety and efficacy of hepatocyte transplantation procedure has been studied in several animal models of ALF. The galactosamine-induced liver failure is the most commonly used models that include mice, rats, rabbits, guinea pigs, and dogs [14–16]; thioacetamide-induced liver injury in rabbits and rats [17, 18]; other models include complete hepatic devascularization [9, 19] and total [20] or subtotal (95 %) hepatectomy [21]. These models showed replacement of about 1–5 % of total hepatocyte mass, which is the

limiting factor for treatment of ALF [22]. In experimental animal models, improved survival rate in ALF was documented by these studies.

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## Chronic Liver Failure

Chronic liver failure (CLF) death toll is about 1.4 million annually, and nearly 150,000 patients die due to CLF in India [23]. The predominant reasons for CLF were cirrhosis due to hepatitis C, B, and D viral infection, followed by HCC, and alcoholic cirrhosis with or without concomitant infection with HCV. Autoimmune causes include primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), and biliary atresia. Non-alcoholic steatohepatitis (NASH) is associated with diabetes, protein malnutrition, obesity, coronary artery disease, and corticosteroid treatment. Other inherited causes are alpha-1 antitrypsin deficiency, hemochromatosis, Wilson's disease, galactosemia, and glycogen storage diseases [24].

Stem cell therapy for CLF patients has more limitations than acute or metabolic liver diseases. During the diseases progression, there is a major loss of hepatocytes and abnormal hepatic architecture due to scar formation in the liver.

Study of CLF in animal models is difficult due to lack of suitable animal model that can mimic the human situation. There are toxin-induced animal models such as carbon tetrachloride (CCl<sub>4</sub>) cirrhosis, phenobarbital, retrorsine, and end-to-side portacaval shunt [25–29]. In the animal experiments, liver toxins were injected to normal liver, and 4 weeks after the discontinuation of liver toxins, animals were subjected to cell therapy. During the experimental studies, different cell types applied intrasplenic, namely, fetal hepatocytes and mesenchymal, stem cells [29], rat or porcine hepatocytes [26], syngeneic rat hepatocytes [27], or immortalized rat hepatocytes [25]. These cell therapy experimental models clearly improved liver function and prolonged survival.

It is believed that at least 20 % of the normal hepatocyte mass is required to carry out normal physiological parameters [30]. Hepatocyte recovery from an average liver is about  $2.8 \times 10^{11}$  hepatocytes, occupying almost 80 %

of the total liver volume. Considering the safety of the cell transplantation, only  $2.4 \times 10^6$  hepatocytes per gram of liver can be transplanted, suggesting replacement of approximately 10 % of functional liver mass. The therapeutic mass of hepatocytes actually required to restore adequate liver function for CLF patient is extremely high and is not possible to transplant into the scar liver or spleen. In this situation, transplantation hepatocytes into other sites will accommodate the large number of therapeutic hepatocyte mass and carryout metabolic function for temporary support.

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## Types of Stem Cells Used for Liver Failures

### Embryonic Stem Cells

Embryonic stem cells (ES) are capable of generating unlimited hepatocytes, which can be used for transplantation. The study of ES is of great interest to clinicians, but it is also surrounded by controversies about its use. First critical step in generating ES from the definitive endoderm (DE) tissue can be achieved by treating the cultures with TGF $\beta$  family ligand activin [31]. Many investigators used DE as starting material to generate hepatocyte-like cells [32–34]. The hepatic maturation can be achieved by the combination of HGF, OSM, FGF, and dexamethasone to expand the hepatoblast population and to promote hepatic maturation. The mouse and human ES cells can be differentiated in to “hepatocyte-like” cells. These cells showed hepatocyte morphology, glycogen storage, express hepatic enzymes, and drug metabolism and secrete albumin [34–39].

Yamamoto et al. and Heo et al. demonstrated mouse ES have potential to differentiate in to hepatocyte-like cells. These cells, when transplanted in to mouse model of liver injury, engrafted and showed improved survival without malignancy [40, 41]. Bin et al. showed the therapeutic effect of embryonic hepatocytes in Wistar rats with CCl $_4$ -induced cirrhosis [42]. Transplantation of EC in different liver injury models showed evidence of repopulation of

recipient liver; however, undifferentiated ES showed teratogenic property in immunodeficient mice [40, 43].

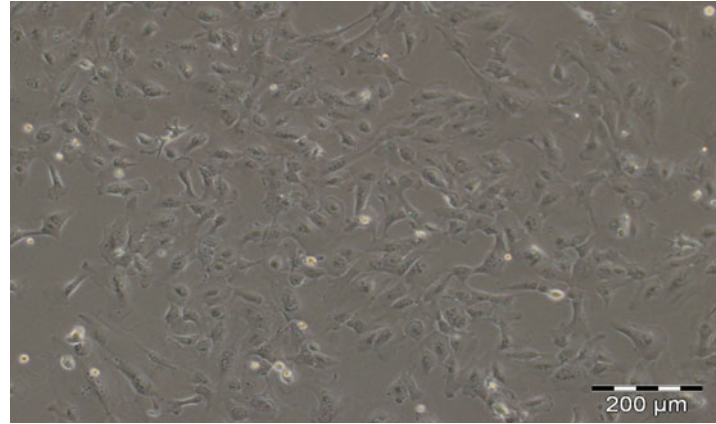
The major obstacle in the use of ES in the clinic is safety issue and ethical concerns. ECs are capable of unlimited proliferation and able to produce hepatocyte-like cells. They show reasonable functional capacity, although there is no consensus about which functional parameters are necessary for clinical use. ES-derived hepatocytes into animals with various liver injury models showed rescue of liver function, although engraftment of the cells was low.

### Fetal Hepatocytes

Human fetal liver (FL) has alternative source of liver progenitor that can be procured from donated tissues after indicated abortions. The FL, between gestation weeks 5–18, contains a large number of actively dividing hepatic progenitor cells that are termed as hepatoblasts. FL cells are committed progenitors, and the development is directed to differentiate to liver cells. FL can be an ideal candidate for cell therapy without concern of teratoma or cancer formation after transplantation.

It has been hypothesized that FL arise from bipotential mesendoderm precursor from mesoderm and endoderm [44]. Cells from the ventral midline of the endoderm lip, probably originating from the mesendodermal prechordal plate, give rise to part of the liver bud [45]. Many studies have shown that mesodermal multipotent adult progenitor cells in both mice and humans can differentiate into hepatocytes [46, 47]. FL tissue obtained during the first trimester mainly exhibits hematopoietic and endothelial markers [48]. During the second trimester development, the hematopoietic cells (HSC) shift towards the hematopoietic cells. Using markers originally expressed on HSC, including c-Kit (CD117) and CD34, can be useful for the identification and isolation of FH. Isolated FH were found to co-express hepatocytic or biliary phenotypic markers implying lineage progression [48]. We have previously isolated and characterized FH from

**Fig. 1** Primary fetal hepatocytes culture (Passage 3)



human FL expressing the markers CD34 and CD117 [29, 49, 50]. Flow cytometric and IHC analysis FH in early passages were positive for the hepatic stem cell markers EPCAM, CD133, and CD90, but not CD34 or CD45, indicating the non-hematopoietic origin of these cells. We have confirmed with IHC staining of CK19, CK18, and albumin the committed progenitors for biliary and hepatocytic lineages [29, 49, 50]. The isolation of fetal hepatocytes (FH) consists of mechanical disruptions into small fragment and then incubation with collagenase for digestion of connective tissue. Mechanical disruptions may yield about  $10^4$ – $10^6$  cells per donation (unpublished data). Our own experiments have yielded an average of  $5 \times 10^4$  cells (data not published) from 12 human fetal tissue isolations (gestation week 5–11) by mechanical disruption (Fig. 1).

There has been fair success in using FH in different animal models. These experiments show successful repopulation of immunodeficient mouse and rat liver with rodent fetal hepatocytes following liver injury [51–53]. In our previous study, we used retrorsine-induced liver injury mouse model, followed by 30 % partial hepatectomy. We infused  $2 \times 10^6$  intrasplenic injection of hFH and/or MSC. Overall animal survival rates were 100 % during the experiments. We confirmed the presence of engraftment and repopulation in the experimental model by immunohistochemistry and PCR. Immunohistochemical analysis showed cell colonies positive for human albumin, alpha-feto protein, c-met, human hepatocyte antigen, and

the human cytokeratins CK 8, 18, and 19. We also detected higher levels of human serum albumin and mouse serum. RT-PCR analysis showed intense expression of the important hepatic factors HNF 1 $\alpha$ ,  $\beta$ , HNF 4 $\alpha$  which provides useful information of persistence hepatocyte function and differentiation [29].

Isolated FH under GMP (good manufacturing practices) compliance can be useful for clinical transplant. In several clinical studies, FH was assessed for infectious diseases such as HCV, HBV, HIV, EBV, HEV, HDV, toxoplasmosis, rubella, cytomegalovirus, parvovirus, herpes simplex type 1 and 2, TPHA, sterility, and endotoxin testing. Therapeutic advantage of FH is useful for a variety of genetic/metabolic liver diseases and acute and chronic liver failure. In recent studies, a significant improvement of all liver serum biochemistry has been achieved after transplantation of FH in a patient with Crigler–Najjar syndrome and biliary atresia [54, 55]. FH isolated from second trimester (EpCAM + cells) and labeled with Tcd, 1-hexamethyl-propylene-amine oxime (Tc-HMPAO) were infused into the hepatic artery of 25 patients with end-stage liver cirrhosis. All patients showed marked improvement of clinical and biochemical parameters during 6 months follow-up [23]. In another study, FH was transplanted in a patient with end-stage chronic liver failure. The patient received 2 intrasplenic infusions ( $5 \times 10^8$ ) on day 0 and 80. The patient's Model for End-Stage Liver Disease (MELD) score improved from 15 to 10 within the first 18 months of observation [56].

Presently available clinical case reports on use of FH for liver disease give very limited information. This may be due to ethical concerns in getting donors. Larger clinical studies are essential to prove the clinical efficacy of FH.

## iPSC

Induced pluripotent stem cells (iPSCs) are adult cells that are genetically reprogrammed to an embryonic-like stem cell. This is achieved by introducing genes and factors important for maintaining the properties of ES. The reprogramming of adult cells into ES cells enables the generation of patient-specific stem cells and thus has enormous potential for the treatment of degenerative diseases.

In 2006, Kazutoshi Takahashi and Shinya Yamanaka first introduced factors for reprogramming of mouse fibroblasts through retroviral transduction with 24 transcription factors highly expressed in ES [57]. Similarly, Park et al. [58] generated iPSCs from human skin through ectopic expression of four genes (Oct3/4, Sox2, c-Myc, and Klf4). Human iPSCs and ES show similar morphologies, proliferation rates, and expression of a number of stem cell markers. However, the specific difference between ESCs and iPSCs is the origin of cell type. iPSCs are derived from adult tissue. In addition, genomic analyses of two types of cells show that hundreds of genes are differentially expressed [59]. Importantly, considering the adult origin, iPSCs can contain an epigenetic “memory” of the donor tissue, which restrict their differentiation capacity and therefore utility [60]. Jang and colleagues generated human iPSCs from a variety of adult human cells, including the liver cells, fibroblasts, bone marrow stem cells, and skin cells [61]. They found that though the iPSCs overall were molecularly similar to each other and to embryonic stem cells, they retained a distinct molecular “signature” inherited from the cell of origin. Regardless of their origin, the different iPSCs showed the same ability to develop into liver cells. A comprehensive study by Miura et al. using various mouse iPSC, has demonstrated that the origin of

the iPSC has a profound influence on the tumor-forming propensities in a cell therapy animal model. Mouse tail-tip fibroblast iPSC cells have shown the highest tumorigenic propensity, whereas gastric epithelial cells and hepatocyte iPSC cells have shown lower propensities [62].

Human iPSCs can be directed to differentiate into hepatocyte-like cells using different differentiation methods [63–66]. Jozefczuk et al. [67] demonstrated the 80 % similarities in gene expression responsible for normal liver physiology between human ES and iPSC. Hepatocyte-like cells generated from iPSC have been shown to secrete human albumin, synthesize urea, and express human cytochrome P450 enzymes [65]. Espejel et al. [68] demonstrated the iPSC-derived hepatocytes have both the functional and proliferative capabilities needed for liver regeneration in mice with fumarylacetoacetate hydrolase deficiency. Asgari et al. performed a growth factor-mediated differentiation of iPSCs and evaluated their potential for recovery of CCl<sub>4</sub>-injured mouse liver following transplantation [69]. In another study, transplantation iPSCs are engrafted, integrated, and proliferated in livers of an immune-deficient mouse model. iPSCs secreted human albumin and cells function was similar to primary human hepatocytes, including metabolic function [70].

The recent success in generating iPSC without viral vectors has brought iPSC one step closer to therapeutic application [71]. However, the suitability of individual iPSC for generating cell for therapy needs to be demonstrated. In spite of these, limitations iPSC-derived hepatocytes are a very promising population for cell therapies.

## Adult Hepatocytes

Adult hepatocyte (AH) transplantation has led its scientific foundations over 40 years. Transplantation of AH in animals has shown effectiveness in defective hepatic enzymes in metabolic models, improving survival rate in acute hepatic and chronic liver failure [72–79]. Animal models have given insight of mechanism of AH engraftment. However, limitations of animal models

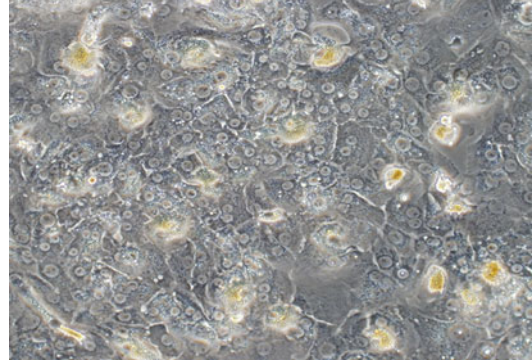


**Fig. 2** Cannulation of resected liver lobe for hepatocyte isolation

reflected on the clinical AH transplant. Animal studies showed that the AH get engrafted in the recipient liver and function normally, even when the engraftment accounted for only 1–5 % of the total hepatocyte mass [22]. This low percentage of engraftment is unlikely to support acute or chronic damage unless a significant liver repopulation is achieved.

The source of AH for the experimental and clinic use is livers discarded for liver transplantation, liver biopsies, or livers after tumor resection [80–82]. Isolation of AH from liver biopsies is quite difficult due to size variations and the paucity of visible vessels available for catheterization. Nationwide study by Bacarani et al. [82] found that organs rejected have steatosis more than 30 %, resulting in reduced hepatocyte yield, decreased viability, and reduced hepatocyte engraftment. Overall cell yield  $9.3 \times 10^9 \pm 8 \times 10^9$  hepatocytes, achieving  $7.2 \times 10^6 \pm 7 \times 10^6$  hepatocytes/g of liver tissue digested with an average viability of  $73 \% \pm 14 \%$  (Figs. 2 and 3).

Over the years, many researchers have attempted to optimize a hepatocyte isolation protocol. Unfortunately, there is no consensus on a single isolation protocol for use in all laboratories, so it is difficult to compare among the outcomes of different studies [83]. AH isolation process starts from surgical removal to transportation to the laboratory and the isolation process. Hepatocytes are exposed to a number of variables that affect their functional ability and viability.



**Fig. 3** Primary culture of adult hepatocytes

The surgical technique of resection, for example, necessitates restriction of blood supply to the organ, leading to warm ischemia and hypoxia of the affected portion of the liver and potentially reducing the viability of hepatocytes. Thus, a protocol that preserves the liver, minimizing the damage to hepatocytes, is essential. Berry and Friend in 1969 demonstrated a protocol involving a two-step collagenase perfusion method. This protocol has become the basis of all current hepatocyte isolation protocol [84]. To standardize the protocol of human AH isolation and functionality testing, European Centre for Validation of Alternative Methods (ECVAM) has recommended the protocol. One of the most debited recommendations by ECVAM is the source of the liver, which will be never accepted by the clinicians.

Furthermore, culture of primary AH is difficult to maintain and cannot be efficiently expanded. Primary AH during the culture loses the metabolic and biotransformation capacity [85, 86]. Cryopreservation of hepatocytes allows the banking of hepatocytes that could be used upon need. Various cryopreservation protocols successfully used in animal experiments when applied human hepatocytes results in loss of 60–65 % hepatocyte viability and 5–90 % attachment rate [30, 87, 88].

Mito et al. in 1993 attempted the first human AH transplantation, till date more than 60 clinical cases have been treated with AH transplantation either to bridge patients to OLT or to improve hepatic metabolic deficiencies [30]. Strom et al. reported the results of AH transplantation into 25

patients diagnosed with acute liver failure from a number of clinical trials in USA. Complete recovery without organ transplantation occurred in 2 patients, 6 were bridged to OLT (within 1–10 days), and the remaining 10 died between 18 h and 52 days after the first hepatocyte transplantation [89, 90]. Fisher et al. reported a 37-year-old woman with FHF who was infused with  $8.8 \times 10^8$  allogeneic AH into the liver through a catheter placed into the portal vein. This patient fully recovered, with a rapid fall in serum ammonia levels and was discharged from the hospital after 2 weeks [91]. Pareja et al. reported two patients received AH transplant as a bridge to whole-organ retransplantation. Patient already received a liver transplant (LT) in the past, with an end-stage liver disease due to recurrent hepatitis C virus cirrhosis while on the waiting list for an OLT. Both patients showed low blood ammonia levels and clinically improved the degree of hepatic encephalopathy, thus serving as a bridge to liver retransplantation in 1 patient [92].

The clinical use of hepatocyte transplantation is currently limited to support for the in-born errors of metabolism mainly because none of the “hepatocyte-like cells” were able to give metabolic support as AH. Importantly, treatment of acute and chronic liver failures required larger number of hepatocyte dose, which can be useful as temporary bridge to OLT. Successful cryopreservation protocol that could maintain the metabolic capacity after thawing is absolute clinical necessity.

## Bone Marrow

Petersen et al. first described the contribution of bone marrow-derived stem cells (BMSC) to liver regeneration [93]. Cell populations in BMSC that contribute to regeneration are hematopoietic stem cells, mesenchymal stem cells, multipotent adult progenitor cells, and very small embryonic-like cells.

Lagasse et al. reported that intravenous injection of adult bone marrow cells in the FAH<sup>-/-</sup> mouse (animal model of tyrosinemia type I) rescued the mouse and restored the biochemical

function of its liver. Moreover, hematopoietic stem cells gave rise to donor-derived hematopoietic and hepatic regeneration [94]. There is increasing evidence in the literature, suggesting BMS transplantation can be useful in liver rescue after acute or chronic injury [93–100]. Jang et al. reported that transplantation of BMS via the portal vein promoted functional improvement in mice with CCl<sub>4</sub>-induced acute liver injury. Liver function was restored 2–7 days after transplantation and fibrosis reduction was also reported [101]. Shizhu et al. transplanted BMS cells via tail veins of mice. BMSCs were found to populate the damaged liver around the portal and centrilobular regions, and they appeared to differentiate into albumin-producing hepatocyte-like cells. Animals showed toward improved liver enzymes as well as enhanced survival rates [102]. Many investigators showed successful BMSC engraftment in recipient liver, but repopulation was low.

Several clinical trials found that BMSCs were beneficial in the treatment of the patients with end-stage liver failure. Autologous BMSCs transplantation resulted in improvement of liver function tests [103–108]. Gasbarrini et al. reported the use of autologous unsorted BMSCs as rescue treatment for hepatic failure in a 67-year-old man who was ineligible for liver transplantation. Patient showed rapid improvement in hepatic synthetic function after the portal venous infusion of the cells. A liver biopsy performed 20 days after cell transplant was reported to show increased hepatocyte replication around necrotic foci [109]. Salama et al. conducted a study of 90 patients with end-stage liver disease. The patients received G-CSF for 5 days followed by autologous CD34<sup>+</sup> and CD133<sup>+</sup> stem cell infusion in the portal vein. Study reported that 54 % patient showed near-normal levels of liver enzymes. Couto et al. investigated BMSC therapy in patients with severe liver disease. BMSCs were isolated from autologous bone marrow and 10 % of the cells were labeled with <sup>99m</sup>Tc-SnCl<sub>2</sub>. Eight patients received  $2.0\text{--}15.0 \times 10^8$  cells. A patient developed a cutaneous immunomediated disorder, and another patient developed hepatocellular carcinoma (HCC) 12 months after infusion. A reduction in bilirubin was shown at 1 week,

while serum albumin increased above baseline up to 1 month after infusion. Couto et al. pointed out that the early improvement of liver function should be interpreted with caution, and controlled studies are needed to determine whether BMMCs infusion affects HCC development in cirrhosis [105].

Spahr et al. reported the feasibility of autologous bone marrow mononuclear cells (BMMNCs) for the treatment of patients with decompensated alcoholic liver disease (ALD). Administration of G-CSF and followed by the CD34<sup>+</sup> cells from BMSC showed significant improvement in liver function in many clinical trials [103, 110, 111]. In the study, 58 patients (mean age 54 years, mean MELD score 19, all with cirrhosis, 81 % with alcoholic steatohepatitis at baseline liver biopsy) were randomized. The procedure includes the combination of G-CSF injections and autologous BMMNCs into the hepatic artery. Adverse events were observed in BMNCs and standard medical treatment (SMT) groups. After 3 months, 2 and 4 patients died, respectively, in the BMNCs and standard medical treatment groups, respectively. The MELD score improved in parallel in both groups during follow-up with 18 patients (64 %) from the BMMCT group and 18 patients (53 %) from the SMT group [112].

Vast amount of experimental and clinical data denotes that transplantation of BMSC brings functional outcome into liver parenchyma, either by fusion or transdifferentiation of MSCs, though the amount of fusion or transdifferentiation is extremely low. One of the mechanisms behind transient hepatoprotective effect is different soluble growth factors produced by MSCs. Considering the outcome of many studies, the knowledge of biological properties and plasticity of BMSC is incomplete. In clinical settings, controlled studies are needed to determine the effectiveness of BMMNCs. Presently, 108 clinical trials are going on for the evaluation of therapeutic efficacy of BMSCs for end-stage liver diseases [113]. Overall, accessibility and availability of HSCs for transplant is an attractive tool for the liver regenerative therapies.

## Umbilical Cord Blood

Umbilical cord blood (UCB) cells are rich source of hematopoietic stem cells without any ethical concerns. Recent studies on UCB-derived stem cells (UCBSc) showed that these cells are capable of differentiating into adipocytes, osteocytes, chondrocytes, cardiomyocytes, neurons, and hepatocytes in vitro [114].

Newsome et al. demonstrated that human UCBSc could differentiate into hepatocytes after transplantation into immunodeficient mice without evidence of cell fusion. The percentage of human hepatocytes reached an average of 0.011 % after 16 weeks compared with mouse [115]. Kögler et al. reported that UCBSc differentiate into hepatocytes after transplantation into a pre-immune fetal sheep model. In vitro UCBScs lack HLA class II and costimulatory molecule expression [116]. Many experimental animal models of liver injury by carbon tetrachloride (CCl<sub>4</sub>), 2-acetylaminofluorene (AAF), and the Fas ligand showed that the repopulation of recipient liver by UCBSc is extremely low [117–123]. Two studies do report that UCBSc transplantation significantly reduces the mortality caused by induced liver injury [117, 118]. Burra et al. evaluated the therapeutic potential of UCB-derived mesenchymal stem cells (UCMSCs) in a murine model of acute liver injury using CCl<sub>4</sub>. UCMSCs-transplanted mice showed a more rapid damage resolution, lower inflammation level, and an increased catalase activity compared to CCl<sub>4</sub>-treated mice alone [124]. Chen Li demonstrated the therapeutic potential of human umbilical cord matrix stem cells (hUCMSCs) into nonobese diabetic-severe combined immunodeficient (NOD-SCID) mice with CCl<sub>4</sub>-induced ALF. hUCMSCs were engrafted in to recipient liver and showed the survival benefit and prevented the release of liver injury biomarkers. These data suggest that direct transplantation of native hUCMSCs can rescue ALF and repopulate livers of mice through paracrine effects to stimulate endogenous liver regeneration rather than hepatic differentiation for compensated liver function [125].



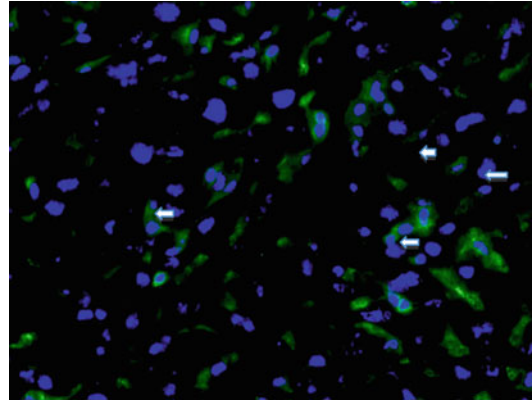
Zhang et al. evaluated the therapeutic use of hUCMSCs in 45 chronic hepatitis B patients with decompensated LC. During the study, 30 patients who received hUCMSCs showed significant reduction in the volume of ascites and improved liver function indicated by the increase of serum albumin levels, decrease in total serum bilirubin levels, and decrease in the sodium model for end-stage liver disease scores [126]. Presently, there are 4 ongoing clinical trials (phase I and II) using UCBSc, investigators thus studying the safety and efficacy of UCBSc on patients with liver cirrhosis.

Thus, there will be much excitement about the use of the UCBSc for the treatment of acute and chronic liver diseases. Collation of UCBSc is done by noninvasive methods, and there is no problem in the availability of donor. Importantly, UCBSc can be cryopreserved without major loss during cryopreservation. Furthermore, UCBSc are nonimmunogenic, which make them more suitable for allogeneic transplant.

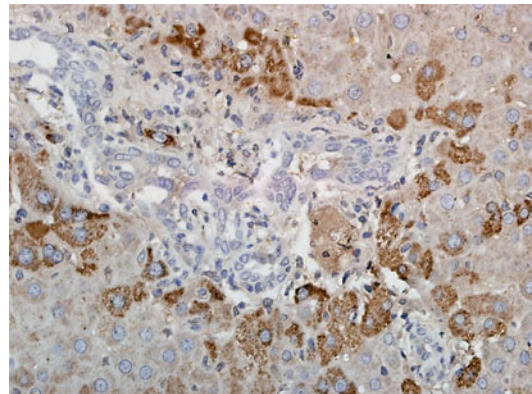
### Route of Administration

There are different approaches used for hepatocyte transplant in animal models. The most accepted approach for hepatocyte transplantation is intrasplenic transplantation. Hepatocytes injected in splenic pulp migrate through hepatic artery in liver parenchyma. The flow of the injection helps hepatocyte to invade the sinusoidal boundaries and entrap in vascular spaces [127]. Entrapped hepatocytes can integrate and proliferate into hepatic lobule. During intrasplenic transplantation, about 40 % of hepatocytes are entrapped in splenic pulp, and this serves as extra hepatic site for hepatocytes. The hepatocytes showed synthetic, metabolic, and biliary transport function [128] (Figs. 4 and 5).

Major limiting factors in using intrasplenic or intraportal route are portal vein thrombosis, portal hypertension, and pulmonary embolism due to transplanted cells [129]. Importantly, there are limitation of cellular dose that can be transplanted single time; thus, repetitive infusion of cells is not



**Fig. 4** D-galactose intoxicated C57BL/6 nude mice had 40 % partial hepatectomy followed by transplantation of FH ( $2 \times 10^6$ /mice). Fresh-frozen section of mice liver showing albumin (green staining), nuclei were counterstained with DAPI (blue)



**Fig. 5** D-galactose intoxicated C57BL/6 nude mice had 40 % partial hepatectomy followed by transplantation of FH ( $2 \times 10^6$ /mice). Paraffin section of mice liver showing human hepatocyte antigen (brown staining)

possible. However, portal hypertension usually resolves within hours after transplantation, and other measures taken to reduce these complications are by hepatic artery ligation to decrease the sinusoidal blood flow prior to hepatocytes infusion or the slow infusion of hepatocytes over a longer period [119, 130]. In our studies, we have used 70 % partial hepatectomy to mouse model of liver injury followed by  $2 \times 10^6$  fetal hepatocytes. During the experiments, we have seen portal hypertension and lung embolism as a

result of cell infusion. We could achieve survival rate of 50 % (unpublished data). Therefore, to overcome the limitation, we followed 30–40 % partial hepatectomy followed by cell infusion. From this modification, we could get 100 % survival. We have observed transient hypertension due to lung embolism, but it was transient [29].

Presently, different alternative sites to encourage hepatocyte attachment, proliferation, and survival are under investigation. In several animal studies, cells were directly injected into the liver parenchyma to avoid cell loss during the transplantation. But transplanted cells were observed in central veins indicating an increased risk of lung embolism [131–133]. Therefore, in the clinical setting, direct injection of cells into the liver may not be feasible. Ectopic hepatocyte transplantation is defined as a transplantation site for hepatocytes other than the liver or spleen. Cell therapy directed towards the liver may not be feasible in cirrhotic and fibrotic liver during end-stage disease. Thus, transplantation of cells in ectopic sites may give temporary relief for the metabolic and synthetic stress on hepatocytes. Researchers have evaluated different ectopic sites for hepatocyte transplant, including the intraperitoneal cavity, pancreas, mesenteric leaves, intrapulmonary artery, lung parenchyma, kidney capsule, interscapular fat pads, and lymph nodes [134–141]. Unfortunately, transplanted cells survive for a short time. The specific reason for short-term survival of cells is unknown; it is likely due to lack of liver specific growth factors or lack of neovascularization. To overcome this limitation, the use of growth factors, like SDF-1, HGF, EGF, or VEGFR, can play a crucial role for initial support for hepatocyte survival and proliferation. Yokoyama et al. injected fibroblast growth factor (bFGF) in subcutaneous space followed by transplantation of rat and mouse hepatocytes by providing a polyethylene terephthalate matrix. Transplanted hepatocytes survived from 4–8 months and retained their albumin synthetic and drug metabolizing capacity. Furthermore, the authors were able to transplant ten times the usual number of hepatocytes into the subcutaneous cavity [136].

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## Future Strategies

Stem cell therapy holds promises for acute and chronic liver diseases, but extensive research for the last 2 decades could not improve the outcome. Major limitation of the therapy is the availability of good quality hepatocytes for transplantation. Typically, hepatocytes are complex cells able to do multiple metabolic and secretory functions. The term “hepatocyte-like cells” fails to show major metabolic and secretory characteristics of adult hepatocyte [30]. There are ongoing efforts to make “hepatocyte-like cells” more functional like adult hepatocytes. One of the important problems clinicians face during the fibrotic or chronic liver failure is the availability of space to accommodate functional hepatocyte. To overcome this issue, study of ectopic sites where large number of functional hepatocytes can be transplanted without any other complication is ideal need to cell therapy. Considering the variables in the pathogenesis of acute and chronic liver diseases, it is highly important to tailor made the therapies to specific patient using different stem cells (ES, iPSCs, FH, BMMNCs, CBSCs).

Development in the strategies of generating hepatocytes, banking of hepatocytes, hepatocyte engraftment, therapeutic cell number, and functionality of hepatocytes will change the present scenario of liver-related cell transplant.

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## Conclusion

Available data on clinical hepatocyte transplantation indicated the limitations of cell therapy. Realistically, cell therapy can be advantageous to acute liver failure or metabolic support where therapeutic cell dose is less as compared to chronic liver failure. Considering the limitations on availability and storage of adult hepatocytes, alternative sources of hepatocytes generated from stem cell can be available for treatment. There are numerous advantages of stem cells generated hepatocyte transplant: (1) unlimited supply of hepatocytes; (2) cryopreservation and banking of hepatocytes; (3) complete metabolic

and secretory profile; (4) noninvasive treatment, without much hospitalization, and economical; (5) no need for posttransplant medication or immunosuppressant.

Many of the stem cell sources such as iPSCs, FH, BMMSCs can be alternative sources of hepatocytes and should be evaluated in larger clinical study. The present experimental and clinical studies demonstrate the advantages and limitations of each cell types used in therapy. Considering the information, revised clinical trials can be conducted in larger study groups; and this will take hepatocyte transplant in to clinical reality.

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