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# EXPERIMENTAL HEPATOCYTE XENOTRANSPLANTATION – A COMPREHENSIVE REVIEW OF THE LITERATURE

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## Abstract

**Background—**Hepatocyte transplantation is a potential therapy for certain diseases of the liver, including hepatic failure. However, there is a limited supply of human livers as a source of cells and, after isolation, human hepatocytes can be difficult to expand in culture, limiting the number available for transplantation. Hepatocytes from other species, e.g., the pig, have therefore emerged as a potential alternative source. We searched the literature through the end of 2014 to assess the current status of experimental research into hepatocyte xenotransplantation.

**Literature search and results—**The literature search identified 51 reports of *in vivo* cross-species transplantation of hepatocytes in a variety of experimental models. Most studies investigated the transplantation of human (n=23) or pig (n=19) hepatocytes. No studies explored hepatocytes from genetically-engineered pigs. The spleen was the most common site of transplantation (n=23), followed by the liver (through the portal vein [n=6]) and peritoneal cavity (n=19). In 47 studies (92%), there was evidence of hepatocyte engraftment and function across a species barrier.

**Conclusions—**The data provided by this literature search strengthen the hypothesis that xenotransplantation of hepatocytes is feasible and potentially successful as a clinical therapy for certain liver diseases, including hepatic failure. By excluding vascular structures, hepatocytes isolated from genetically-engineered pig livers may address some of the immunological problems of xenotransplantation.

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**Disclosure of conflict of interest**

None of the authors reports a conflict of interest.

**Keywords**

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## Introduction

Orthotopic liver allotransplantation is currently the treatment of choice for patients with end-stage liver disease. However, it is limited by a shortage of deceased human donors, which may result in a suitable allograft not being available when needed for a patient .with acute liver failure (ALF) or acute decompensation of chronic liver disease. Hepatocyte transplantation (Tx) is a potential alternative to whole liver Tx in the treatment of ALF or some liver-based metabolic disorders (1–23). Scaled-up isolation methods are available to isolate almost the entire hepatocyte population from human and pig livers (24–26). However, most healthy livers from deceased donors are prioritized for liver Tx, and so human hepatocytes that are healthy and functional are even more difficult to obtain than whole livers (11). Therefore, other species as sources of hepatocytes are being investigated, as discussed previously by others (27,28).

Pig hepatocyte xenoTx has several potential advantages (27,28), though evidence for some of these is limited :- (i) There could be an unlimited cell supply. (ii) Pigs have some metabolic similarities to humans (29–31). (iii) As the vascular endothelium of the liver is not transplanted, this may possibly reduce the risks of acute vascular rejection (32), though this is by no means certain. Our own preliminary studies indicate that there is less Gal expression and less human antibody binding to pig hepatocytes than to vascular endothelium (Ezzelarab M, et al, unpublished). There is also some evidence that hepatocytes show resistance to complement-mediated injury (33). (iv) Genetically-modified pigs should provide hepatocytes that to some extent are protected from the human humoral and cellular immune responses (34–36). (v) Conventional immunosuppressive therapy may possibly be sufficient to control rejection (37–39). (vi) Pig hepatocytes may be resistant to human viruses, such as the hepatitis and human immunodeficiency (HIV) viruses (40,41).

Before clinical trials of hepatocyte xenoTx are undertaken, evidence needs to be provided from animal studies that hepatocyte Tx across species barriers is likely to be successful. How long do hepatocytes from one species survive and function in another? Can adverse effects be anticipated?

We have reviewed the available literature on experimental hepatocyte xenoTx. We were unable to identify any report on clinical hepatocyte xenoTx. We did not review the literature on hepatocyte alloTx, which has been reviewed by others (5,6,42).

## Literature Search

In the 35 years from January 1980 to December 2014, we identified 51 reports of *in vivo* hepatocyte Tx across species barriers (Table 1). We were unable to identify any studies before 1980. There was peak experimental activity in the period 2007–2014 (Figure 1).

The majority of studies involved human (n=23) or pig (n=19) hepatocytes transplanted into a variety of other species (Figure 2). Rat (n=4), rabbit (n=4), and monkey (n=1) hepatocytes have also been transplanted. The most common recipient species were the mouse (n=24) and rat (n=22) (Table 1). Most commonly, the spleen (n=23) was the site of Tx, with the liver (via the portal vein, n=6) and peritoneal cavity (n=19) also being used. Details of all 51 experimental studies are provided in Tables 2–6.

## Results and Discussion

Hepatocyte alloTx has been carried out in an effort to correct an inborn error of metabolism (3,5,6,8–13,18–20,43–50) or to provide support in patients with hepatic failure (2,4,5,11,14,51,52). In view of the persistent shortage of hepatocytes from deceased human donors, if hepatocyte Tx is going to play a significant therapeutic role, an alternative source of hepatocytes will be required.

The pig could fulfill this need, but there are few data on whether pig hepatocytes will survive in humans and, if so, whether they will be able to carry out the functions of human hepatocytes. The latter question will be particularly important if pig hepatocytes are transplanted in an effort to correct a metabolic disease in which replacement of a specific enzyme or hormone is required, e.g., glycogen storage disease, Crigler-Najjar syndrome type 1 (Table 7) (5,43,44,48,50), rather than when only detoxifying functions are required.

In the study by Nagata et al (37), between 1–2 billion wild-type (genetically-unmodified) pig hepatocytes (in a 1% alginate matrix) were injected directly into the parenchyma of the spleens of three cynomolgus monkeys (weighing 5–9kg), who received relatively intensive, but clinically-applicable, conventional immunosuppressive therapy. Our own very preliminary data suggest that conventional immunosuppressive therapy (based on calcineurin inhibition), unless very intensive, may be insufficient to prevent an adaptive immune response against even genetically-engineered pig hepatocytes in nonhuman primates (Iwase H, et al. unpublished), and therefore immunosuppressive regimens (based on T cell costimulation blockade) proven to be successful in pig vascularized solid organ Tx (53–56) may be required.

In Nagata's study, graft function was determined by the measurement of porcine albumin. A peak of porcine albumin was detected in the blood within the first month. Following a single injection, the pig hepatocytes functioned for between 25 days (limited by death of the monkey from a cytomegalovirus infection) and >80 days. Following reTx on two occasions in one of the monkeys, porcine albumin was detected for >253 days (died from complications associated with replacement of a central venous catheter). Of considerable interest and relevance to future clinical trials was the observation that, although hepatocyte Tx was associated with a slight increase in anti-galactose-α1,3-galactose (Gal) IgG (considered to be within the normal range), there was no detectable increase in anti-nonGal antibody levels, suggesting that the Tx of hepatocytes from pigs genetically modified to delete expression of Gal (α1,3-galactosyltransferase gene-knockout pigs) might induce a minimal humoral immune response.

The considerable experience of encapsulated pig islet xenoTx, which includes several small clinical trials (57), suggests that encapsulation is not yet fully successful in protecting islets from the primate immune response, and therefore is unlikely to be fully successful in protecting pig hepatocytes. However, several groups have demonstrated some protection by hepatocyte encapsulation (58–64).

Currently, there appears to be no experience of the Tx of hepatocytes from genetically-engineered pigs into other species, though data from other models of xenoTx strongly suggest that hepatocytes from these pigs will provide a greater likelihood of success compared with those from wild-type pigs [reviewed in (65,66). We would suggest that, for Tx into humans, hepatocytes from genetically-engineered pigs in which both Gal *and* N-glycolylneuraminic acid (NeuGc) expression is absent (67), and which express at least one human complement-regulatory protein, will be advantageous to graft survival. If pig-to-nonhuman primate hepatocyte Tx is observed to be identified with a thrombotic reaction, then possibly the additional expression of a human coagulation-regulatory protein might be valuable. If hepatocytes are found to phagocytose human red blood cells and/or platelets (which we believe is unlikely), then steps may need to be taken to genetically engineer the pig to prevent this (discussed in 68).

Whether pig hepatocytes will carry out all of the functions required to maintain homeostasis in humans remains uncertain (31, 69–72). In a review of physiologic compatibilities between human and pig organ systems, Ibrahim et al drew attention to the 65% structural similarity between human and pig albumin (69). Porcine clotting factors (II, V, VII, X, XII) have been studied (73,74) and have been shown to trigger the human coagulation system (75–77). Other metabolic aspects, including the elimination of drugs by porcine hepatocytes, were discussed by Ibrahim et al (69).

There is a little evidence from orthotopic pig liver Tx in nonhuman primates that pig hepatocyte function will at least provide some factors required by primates, but this evidence is very limited (30,65,78,79). Ekser and his colleagues demonstrated that in baboon recipients of livers from genetically-engineered pigs, although follow-up was for less than one week, many parameters of hepatic function, including coagulation, remained in the near-normal range. Western blot demonstrated that pig proteins (albumin, fibrinogen, haptoglobin, and plasminogen) were produced by the pig liver, and production of several coagulation factors was also confirmed. Apart from the experience of Nagata et al (37), there is no evidence in the pig-to-nonhuman model of hepatocyte Tx.

Extracorporeal pig liver perfusion with human blood has generally been for such short periods of time (hours) that few conclusions can be drawn. However, decreased levels of vitamin K-dependent clotting factors (VII and X) were documented to be produced by the pig liver (80–82). There is also some evidence that pig hepatocytes can remove bilirubin from human blood (81,83). Although unlikely, there is also a risk that pig hepatocytes will phagocytose human erythrocytes (84–88) and/or platelets (86,89–93); it is unlikely this will occur in the absence of vascular endothelial cells and Kupffer cells.

It is therefore difficult to come to any realistic conclusion on the efficacy of pig hepatocytes to provide the necessary metabolic functions that will be required after their Tx into humans.

A recent study by Komori et al (94), however, provides some encouragement. This group reported that adult pig hepatocytes yielded a 100-fold higher serum albumin level in immunodeficient mice than adult human hepatocytes (which in turn yielded a 1,000-fold higher level than fetal human hepatocytes). However, these findings differ from previous reports which showed either no significant difference in albumin production between human and pig (95) or lower levels of albumin in pigs than in humans (although total serum protein levels were equivalent). Function of pig hepatocytes transplanted into humans might also be affected by such factors as whether the recipient has hepatitis (96).

Clinical hepatocyte *allo*Tx has demonstrated modestly encouraging results in the treatment of various inherited metabolic diseases, e.g., glycogen storage disease (44,48,50), Crigler-Najjar syndrome (43,50), ornithine transcarbamylase deficiency (50), and tyrosinemia type 1 (50).

The small survey reported here illustrates that there are data indicating that the Tx of hepatocytes can result in successful engraftment in widely-disparate species. For example, the Tx of human hepatocytes into the spleens of SCID or ALF mice, or into the portal vein of pigs with ALF, can provide life-supporting hepatic function and/or improvement in recipient survival (Table 2). Pig hepatocytes have been demonstrated to proliferate after implantation into extrahepatic sites in SCID mice or ALF rats (Table 4), and have also functioned for >8 months in a monkey receiving only conventional immunosuppressive therapy (37) (Table 3). Pig hepatocytes demonstrate some metabolic similarities to human hepatocytes (29–31). Rabbit hepatocytes have functioned in rats with ALF, and rat hepatocytes have engrafted and survived in ALF mice, allowing transient or definitive improvement of liver failure (Table 6).

However, in view of differences in metabolic function and immune responses between the various species combinations that have formed the experimental models, it is hard to draw conclusions relating to clinical pig-to-human hepatocyte Tx from most of the studies. Indeed, there has been only one study in the clinically-relevant pig-to-nonhuman model (37).

The minimum number of hepatocytes required to provide meaningful improvement in hepatic function in another species remains uncertain. The fewest human hepatocytes required to improve ALF in a xenoTx model has to date been reported to be $5\times10^5$, and the fewest pig and rat hepatocytes has been $2\times10^6$ and $1–2\times10^5$, respectively. The study by Nagata et al in the pig-to-monkey model suggested that the injection of approximately 1.5–$2.5\times10^8$/kg hepatocytes might be sufficient to have a clinical impact (37).

In summary, experimental experience to date provides optimism that pig hepatocytes transplanted into rat, mouse, and monkey are likely to engraft and function without excessive immunosuppressive therapy being required. However, any conclusion about the success of clinical pig hepatocyte Tx is clearly premature with respect to both metabolic function and immune response. Besides excluding vascular structures from the transplant

product, which should reduce the possibility of antibody-mediated xenogeneic rejection, hepatocytes isolated from genetically-engineered pigs may address other immunological concerns. The number of hepatocytes that will be necessary for the outcome to be of clinical relevance, e.g., correction of hepatic failure or correction of an inborn error of metabolism, remains uncertain.

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## Abbreviations

| | |
|---|---|
| **Alb-uPA** | albumin linked-urokinase type plasminogen activator |
| **ALF** | acute liver failure |
| **Gal** | galactose-α1,3-galactose |
| **SCID** | severe combined immunodeficient |
| **Tx** | transplantation |

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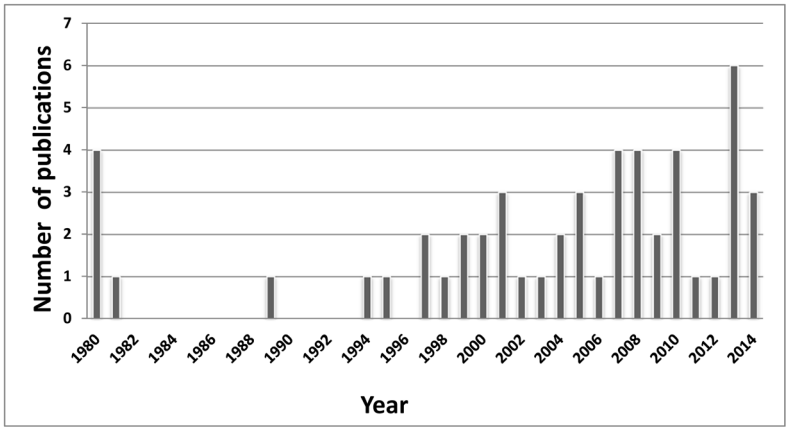


**Figure 1.**
Number of publications on hepatocyte xenotransplantation, 1980–2014.

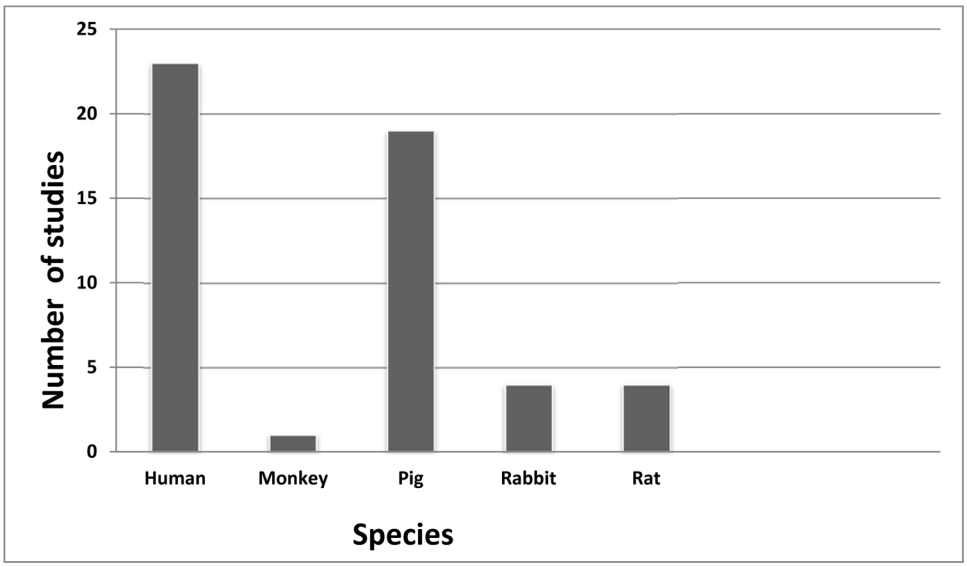


**Figure 2.**
Source species of hepatocytes in studies of xenotransplantation, 1980–2014

**Table 1**

Experimental hepatocyte xenotransplantation, 1994–2014: hepatocyte source species, recipient species, and number of published studies

| Source Species | Recipient Species | Number of Studies |
|---|---|---|
| Human | Mouse | 15 |
| | Rat | 5 |
| | Pig | 3 |
| Monkey | Mouse | 1 |
| Pig | Rat | 13 |
| | Mouse | 4 |
| | Monkey | 1 |
| | Rabbit | 1 |
| Rat | Mouse | 4 |
| Rabbit | Rat | 4 |
| **TOTAL** | | **51** |

## Table 2

Studies of human hepatocyte transplantation in other species

| Recipient species | Route of administration | Number of hepatocytes transplanted | Conclusions | Reference |
|---|---|---|---|---|
| Rat | Intraperitoneal | $1.5\times10^7$ | Cryopreserved hepatocytes remain liver function | Moscioni 1989 (97) |
| Rat | Intraperitoneal | $2\times10^7$ | Encapsulated hepatocytes survived and produced albumin | Wen 1998 (98) |
| Rat | Intraperitoneal | $2\times10^7$ | A 2-week course of cyclosporine resulted in graft function for 60 days | Wen 2000 (99) |
| Mouse (SCID/uPA) | Portal vein | $5\times10^5$ | Engrafted and partially repopulated liver | Dandri 2001(100) |
| Pig (pancreatec tomized) | Portal vein | $3\times10^7$ | Insulin-producing hepatocyte line controlled diabetes | Okitsu 2004 (101) |
| Mouse (SCID/uPA) | Spleen | 5.0 to $7.5\times10^6$ | Hepatocytes functioned | Tateno 2004 (102) |
| Mouse (SCID/uPA) | Spleen | $1\times10^6$ | Hepatocytes repopulated the liver and preserved normal function | Meuleman 2005 (103) |
| **Mouse ( ALF)** | **Intraperitoneal** | **$50\times10^6$** | **Hepatocytes functioned** | **Mai 2005** (61) |
| Pig (ALF) | Portal vein | $1\times10^6$ | Immortalized hepatocytes significantly prolonged the survival of ALF pigs | Totsugawa 2007 (104) |
| Mouse (SCID/uPA) | Spleen | 7.5 or $10\times10^5$ | Growth hormone enhanced proliferation of hepatocytes in SCID/uPA mice | Masumoto 2007 (105) |
| Mouse (SCID/ALF) | Spleen | $1\times10^6$ | Immortalized hepatocytes improved survival of ALF mouse | Tsuruga 2008 (106) |
| Rat (F344 nude) | Portal vein | $5\times10^6$ | Created immunodeficient rats that are suitable for hepatocyte Tx | Igarashi 2008 (107) |
| Mouse (SCID/uPA) | Spleen | $1\times10^6$ | Mouse NK cells played a critical role in the rejection of hepatocytes | Kawahara 2010 (108) |
| **Mouse (ALF)** | **Intraperitoneal** | **$40\times10^6$** | **Encapsulated immortalized hepatocytes improved survival** | **Sgroi 2011** (62) |
| **Mouse (ALF)** | **Intraperitoneal** | **$3\times10^6$–$6\times10^6$** | **Hepatocytes survived** | **Link 2012** (63) |
| Rat (F344 nude) | Portal vein | $1\times10^6$ | Hepatocytes engrafted | Tachibana 2013 (109) |
| Fetal pigs and piglets | In utero and percutaneous | $1\times10^7$ in utero (fetal pigs) and $5\times10^7$ percutaneous (piglets) | In utero Tx allowed for stable engraftment | Fisher 2013 (110) |
| Mouse (uPA/SCID) | Spleen | $2.5\times10^5$ | Hepatocytes functioned | Tateno 2013 (111) |
| Mouse (nude) | Subcutaneous | $2.5\times10^6$ | Overexpression of RhoC can enhance the tumorigenicity of HL7702 cells | Xie 2013 (112) |
| Mouse (uPA/NOG) | Intraperitoneal | $2\times10^6$ | Hepatocytes survived and expanded | Gutti 2013 (113) |
| **Mouse (uPA/SCID)** | **Spleen** | **$1\times10^6$** | **Hepatocytes functioned** | **Ohtsuki 2014** (114) |
| **Mouse (TK-NOG )** | **Intraperitoneal** | **$1\times10^6$** | **Human albumin produced** | **Kim 2014** (115) |
| **Mouse (nude/ALF)** | **Spleen** | **$1\times10^6$** | **Hepatocytes increased survival** | **Vidal 2014** (116) |

Abbreviations:

SCID/uPA mouse: The severe combined immunodeficiency/albumin linked-urokinase type plasminogen activator (SCID/Alb-uPA) human liver chimeric mouse model

F344 nude rat: F344 nude rats devoid of T cells were irradiated with X-rays and injected with bone marrow cells from SCID mice

ALF: Acute liver failure

uPA/NOG mice: Severe combined immunodeficiency/IL-2Rgc null (NOG) mice carry two copies of the mouse albumin promoter-driven urokinase-type plasminogen activator transgene.

RhoC: Ras homologous C (RhoC)

HL7702 cell: The HL7702 cell line was stably transfected with a RhoC expression vector and then subjected to cell proliferation, differentiation, colony formation, migration and invasion assays

TK-NOG mice: herpes simplex virus type 1 thymidine kinase [TK] transgene expressed within the liver of a highly immunodeficient mouse strain [NOG

## Table 3

Studies of monkey hepatocyte transplantation in other species

| Recipient species | Route of administration | Number of hepatocytes transplanted | Conclusions | Reference |
|---|---|---|---|---|
| Mouse (SCID) | Spleen | $1\times10^6$ | V5-treated hepatocytes improved survival | Tanaka 2006 (117) |

V5: anti-apoptotic pentapeptide, composed of Val-Pro-Met-Leu-Lys, has been demonstrated to suppress apoptosis in several types of human cells.

## Table 4

Studies of pig hepatocyte transplantation in other species

| Recipient species | Route of administration | Number of hepatocytes transplanted | Conclusions | Reference |
|---|---|---|---|---|
| Rat (ALF) | Intraperitoneal | $4\times10^7$ | Hepatocytes prolonged survival | Makowka 1980a (118) |
| Rat (ALF) | Intraperitoneal | $4\times10^7$ | Presensitization did not affect survival | Makowka 1980b (119) |
| Rat (ALF) | Intraperitoneal | $4\times10^7$ | Hepatocytes prolonged survival | Makowka 1981 (120) |
| Rat (ALF) | Spleen | $1\times10^8$ | Cryopreserved hepatocytes supported hepatic function | Papalois 1997 (121) |
| Rabbit (Watanabe) | Portal vein | $1–2\times10^8$ | Lowered serum cholesterol by 30–60% for >100 days | Gunsalus 1997 (122) |
| Rat | Subcutaneous | $1\times10^7$ | Hepatocytes survived | Elçin 1999 (123)78) |
| **Rat (cirrhotic)** | **Spleen** | **$1\times10^7$** | **Hepatocytes functioned** | **Stefan 1999** (124) |
| Rat (ALF) | Intraperitoneal | $6\times10^6$ | After 95% hepatectomy, cryopreserved encapsulated hepatocytes improved survival | Sarkis 2000 (125) |
| Rat | Intraperitoneal | $45\times10^6$/capsul e×3 capsules | Encapsulated hepatocytes remained functional for >15 days | Benoist 2001 (58) |
| **Rat (ALF)** | **Intraperitoneal** | **$6\times10^7$** | **Macroencapsulated hepatocytes prevented death** | **Sarkis 2001** (126) |
| Mouse (Balb/c) | Spleen | $2\times10^6$ | Hepatocytes synthesized albumin | Nishitai 2002 (127) |
| Rat (cirrhotic) | Spleen | $5\times10^6$ | Hepatocytes prolonged survival | Nagata 2003 (128) |
| Mouse (SCID) | Spleen | $1\times10^6$ | Freshly isolated hepatocytes had better viability than cultured, preserved or cryopreserved hepatocytes | Nishitai 2005 (129) |
| Cynomolgus monkey | Spleen | $2\times10^6$ | With conventional immunosuppressive therapy, hepatocytes functioned for 253 days | Nagata 2007 (37) |
| Rat | Intraperitoneal | $30–40\times10^6$ cells/mL ×1.8ml | Cryopreserved hepatocytes retained biological activity without immunosuppressive therapy | Baldini 2008 (59) |
| Mouse (ALF) | Intraperitoneal | $1\times10^7$cells/ml | Encapsulated hepatocytes sustained hepatic function | Mei 2009 (60) |
| **Rat (DPPIV- deficient)** | **Intraperitoneal** | **$1\times10^7$** | **Hepatic irradiation advantageous** | **Yamanouchi 2009** (130) |
| Mouse (SCID) | Spleen | $1\times10^6$ | Hepatocytes cultured in SAPNFs corrected ALF and prolonged survival | Yamamoto 2010 (131) |
| Rat (ALF) | Spleen | $3\times10^7$ | Immortalized hepatocytes improved hepatic function and prolonged survival | Pan 2010 (132) |

SAPNF: self-assembling peptide nanofiber (SAPNF) to provide a provisional three-dimensional (3-D) support to interact with cells to control their function in vivo.

DPPIV: dipeptidyl peptidase IV.

**Table 5**

Studies of rabbit hepatocyte transplantation in other species

| Recipient species | Route of administration | Number of hepatocytes transplanted | Conclusions | Reference |
|---|---|---|---|---|
| Rat (ALF) | Intraperitoneal | $4\times10^7$ | Hepatocytes prolonged survival | Makowka 1980a (118) |
| Rat (ALF) | Intraperitoneal | $4\times10^7$ | Presensitization did not affect survival | Makowka 1980b (119) |
| Rat (ALF) | Spleen | $1\times10^8$ | Hepatocyte graft survival was improved by daclizumab | Papagoras 2007 (133) |
| Rat (ALF) | Spleen | $1\times10^8$ | Rapamycin offered no survival advantage | Lytras 2010 (134) |

## Table 6

Studies of rat hepatocyte transplantation in other species

| Recipient species | Route of administration | Number of hepatocytes transplanted | Conclusions | Reference |
|---|---|---|---|---|
| Mouse | Spleen/ intravenous/subcutaneous | $1\times10^6$ | Intravenous or intrasplenic Tx was followed by a significantly lower DTH response than subcutaneous Tx | Tanabe 1994 (135) |
| Mouse (Alb- uPA) | Spleen | $1–2\times10^5$ | Livers were reconstituted with rat hepatocytes | Rhim 1995 (136) |
| Mouse (ALF) | Spleen | $0.5\times10^6$ | Hepatocyte survival was maintained by weekly Jo2 administration | Vidal 2008 (137) |
| Mouse (uPA/SCID) | Spleen | $5.0–7.5\times10^6$ | Hepatocytes maintained their viability | Hata 2013 (138) |

Alb-uPA mice: albumin-urokinase (Alb-uPA) transgenic mice

DTH: The delayed type hypersensitivity

Jo2: specific anti-mouse Fas monoclonal antibody

**Table 7**

Hepatic metabolic disorders that potentially could be treated by hepatocyte xenotransplantation

| |
|---|
| $\alpha_1$-antitrypsin deficiency |
| Arginino-succinate lyase deficiency |
| Bile acid synthesis disorders |
| Crigler-Najjar syndrome |
| Galactosemia |
| Glycogen storage disease type I (Van Gierke's disease) |
| Glycogen storage disease type IV (Debrancher enzyme deficiency) |
| Hemochromatosis |
| Hereditary fructose intolerance |
| Hereditary tyrosinemia type I |
| Inherited Factor VII deficiency |
| Ornithine transcarbamylase deficiency |
| Peroxisomal biogenesis disease |
| Progressive familial intrahepatic cholestasis types 1, 2, and 3 |
| Urea cycle disorders |
| Wilson's disease |