Transplantation of engineered chimeric liver with autologous hepatocytes and xenobiotic scaffold.

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Objective:
Generation of human livers in pigs might improve the serious shortage of grafts for human liver transplantation, as well as enable liver transplantation without the need for deceased or living donors. We developed a chimeric liver (CL) by repopulation of rat hepatocytes in a mouse and successfully transplanted it into a rat recipient with vessel reconstruction. This study was designed to investigate the feasibility of CL for supporting the recipient after auxiliary liver grafting.

Methods:
Hepatocytes from luciferase transgenic or luciferase/LacZ double-transgenic rats were transplanted into 20-30-day-old urokinase-type plasminogen activator/severe-combined immunodeficiency (uPA/SCID) mice (n=40) to create CLs with rat-origin hepatocytes. After replacement of mouse hepatocytes with those from rats, the CLs were transplanted into wild-type Lewis (n=30) and analbuminemia (n=10) rats, followed by immunosuppression using tacrolimus with/without cyclophosphamide or no immunosuppression. Organ viability was traced by in vivo bioimaging and Doppler ultrasonography in the recipient rats for 4-6 months. Rat albumin production was also evaluated in the analbuminemia rats for 4 months. In addition, histological analyses including Ki67 proliferation staining were performed in some recipients.

Results:
Both immunosuppressive protocols significantly improved graft survival and histological rejection of CLs as compared to the non-immunosuppressed group. While rat albumin production was maintained in the recipients for 4 months after transplantation, ultrasonography revealed patent circulation in the grafts for 6 months. Ki67 staining analysis also revealed the regenerative potential of CLs after a hepatectomy of
the host native liver, whereas immune reactions still remained in the mouse-origin structures.

**Conclusions:** This is the first report showing that engineered CLs have potential as alternative grafts to replace the use of grafts from human donors.


Xenotransplanted embryonic kidney provides a niche for endogenous mesenchymal stem cell differentiation into erythropoietin-producing tissue.

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**Abstract**

Recent findings have demonstrated that stem cells can differentiate into mature tissue when supplied with a niche containing factors identical to those in the normal developmental program. A niche for the development of an organ can be provided by xenotransplantation of a similar developing organ. However, this process has many technical, safety and ethical concerns. Here, we established xenotransplantation models that control endogenous mesenchymal stem cell (MSC) differentiation into mature erythropoietin (EPO)-producing tissue in a niche provided by a developing xenometanephros. Transplantation of rat metanephroi into mouse omentum, and similarly pig metanephroi into cat omentum, led to the
recruitment of host cells and EPO production. EPO-expressing cells were not differentiated from integrating vessels because they did not co-express endothelial markers (Tie-2 and VE-cadherin). Instead, EPO-expressing cells were shown to be derived from circulating host cells, as shown by EGFP expression in the grown transplants of chimeric mice bearing bone marrow from a transgenic mouse expressing EGFP under the control of the EPO promoter. These results suggest that donor cell recruitment and differentiation in a xenotransplanted developing organ may be consistent between species. The cells responsible for EPO expression were identified as MSCs by injecting human bone marrow-derived MSCs and endothelial progenitor cells into NOD/SCID mice. Furthermore, using metanephroi from transgenic ER/E2F1 suicide-inducible mice, the xeno-tissue component could be eliminated, leaving autologous EPO-producing tissue. Our findings may alleviate adverse effects due to long-lasting immunosuppression and help mitigate ethical concerns.


Impact of Normothermic Preservation with Extracellular Type Solution Containing Trehalose on Rat Kidney Grafting from a Cardiac Death Donor

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Background
The aim of this study was to investigate factors that may improve the condition of a marginal kidney preserved with a normothermic solution following cardiac death (CD) in a model of rat kidney transplantation (RTx).

Methods
Post-euthanasia, Lewis (LEW) donor rats were left for 1 h in a 23°C room. These critical kidney grafts were preserved in University of Wisconsin (UW), lactate Ringer's (LR), or extracellular-trehalose-Kyoto (ETK) solution, followed by intracellular-trehalose-Kyoto (ITK) solution at 4, 23, or 37°C for another 1 h, and finally transplanted into bilaterally nephrectomized LEW recipient rats (n = 4–6). Grafts of rats surviving to day 14 after RTx were evaluated by histopathological examination. The energy activity of these marginal rat kidneys was measured by high-performance liquid chromatography (HPLC; n = 4 per group) and fluorescence intensity assay (n = 6 per group) after preservation with UW or ETK solutions at each temperature. Finally, the transplanted kidney was assessed by an in vivo luciferase imaging system (n = 2).

Results
Using the 1-h normothermic preservation of post-CD kidneys, five out of six recipients in the ETK group survived until 14 days, in contrast to zero out of six in the UW group (p<0.01). Preservation with ITK rather than ETK at 23°C tended to have an inferior effect on recipient survival (p = 0.12). Energy activities of the fresh donor kidneys decreased in a temperature-dependent manner, while those of post-CD kidneys remained at the lower level. ETK was superior to UW in protecting against edema of the post-CD kidneys at the higher temperature. Luminescence intensity of successful grafts recovered within 1 h, while the intensity of grafts of deceased recipients did not change at 1 h post-reperfusion.

Conclusions
Normothermic storage with extracellular-type solution containing trehalose might prevent reperfusion injury due to temperature-dependent tissue edema.
Cardiac cell sheet transplantation improves damaged heart function via superior cell survival in comparison with dissociated cell injection.

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Abstract

Regenerative therapies have currently emerged as one of the most promising treatments for repair of the damaged heart. Recently, numerous researchers reported that isolated cell injection treatments can improve heart function in myocardial infarction models. However, significant cell loss due to primary hypoxia or cell wash-out and difficulty to control the location of the grafted cells remains a problem. As an attempt to overcome these limitations, we have proposed cell sheet-based tissue engineering, which involves stacking confluent cultured cells (two-dimensional), cell sheets, to construct three-dimensional cell-dense tissues. Cell sheet transplantation has been able to recover damaged heart function. However, no detailed analysis for transplanted cell survival has been previously performed. The present study compared the survival of cardiac cell sheet transplantation to direct cell injection in a rat myocardial infarction model. Luciferase-expressing neonatal rat cardiac cells were harvested as cell sheets from temperature-responsive culture dishes. The transplantation of cell sheets was compared to the direct injection of isolated cells dissociated with trypsin-ethylenediaminetetraacetic acid. These grafts were transplanted to infarcted rat hearts and cardiac function was assessed by echocardiography at 2 and 4
weeks after transplantation. In vivo bioluminescence and histological analyses were performed to examine cell survival. Cell sheet transplantation consistently yielded greater cell survival than cell injection. Immunohistochemistry revealed that cardiac cell sheets existed over the infarcted area as an intact layer. In contrast, the injected cells were scattered with relatively few cardiomyocytes in the infarcted areas. Four weeks after transplantation, cardiac function was also significantly improved in the cell sheet transplantation group compared with the cell injection. Twenty-four hours after cell grafting, significantly greater numbers of mature capillaries were also observed in the cardiac cell sheet transplantation. Additionally, the numbers of apoptotic cells with deterioration of integrin-mediated attachment were significantly lower after cardiac cell sheet transplantation. In accordance with increased cell survival, cardiac function was significantly improved after cardiac cell sheet transplantation in comparison to cell injection. Cell sheet transplantation can repair damaged hearts through improved cell survival and should become a promising therapy in cardiovascular regenerative medicine.

*Image of bioluminescence and histological analysis*


**Bone marrow-derived mesenchymal stem cells ameliorate hepatic ischemia reperfusion injury in a rat model.**

Abstract

BACKGROUND:
Ischemia-reperfusion (I/R) injury associated with living donor liver transplantation impairs liver graft regeneration. Mesenchymal stem cells (MSCs) are potential cell therapeutic targets for liver disease. In this study, we demonstrate the impact of MSCs against hepatic I/R injury and hepatectomy.

METHODOLOGY/PRINCIPAL FINDINGS:
We used a new rat model in which major hepatectomy with I/R injury was performed. Male Lewis rats were separated into two groups: an MSC group given MSCs after reperfusion as treatment, and a Control group given phosphate-buffered saline after reperfusion as placebo. The results of liver function tests, pathologic changes in the liver, and the remnant liver regeneration rate were assessed. The fate of transplanted MSCs in the luciferase-expressing rats was examined by in vivo luminescent imaging. The MSC group showed peak luciferase activity of transplanted MSCs in the remnant liver 24 h after reperfusion, after which luciferase activity gradually declined. The elevation of serum alanine transaminase levels was significantly reduced by MSC injection. Histopathological findings showed that vacuolar change was lower in the MSC group compared to the Control group. In addition, a significantly lower percentage of TUNEL-positive cells was observed in the MSC group compared with the controls. Remnant liver regeneration rate was accelerated in the MSC group.

CONCLUSIONS/SIGNIFICANCE:
These data suggest that MSC transplantation provides trophic support to the I/R-injured liver by inhibiting hepatocellular apoptosis and by stimulating regeneration.
Luminescence technology in preservation and transplantation for rat islet.


Abstract
The development of organ preservation solutions and associated technology has been a major effort in tissue transplantation recently. However, this research takes a great deal of time and resources. In this study, a novel method for the evaluation of preservation solutions was established by using islet cells. Primary islets were obtained by hand-picking method from the luciferase transgenic (Luc-Tg) rat pancreas. The viability rate and living condition of islets preserved with several solutions were evaluated by relative photon intensity. Preserved islets were transplanted to the renal capsule of streptozotocin (STZ)-induced type 1 diabetic NOD-scid mouse, and the intraperitoneal glucose tolerance test (IPGTT) and histology were analyzed. The Luc-Tg rat islet viability was increased in a relative photon intensity-dependent manner. In the recipients of ET-Kyoto (ET-K) or University of Wisconsin (UW) solution preserved Luc-Tg rat islet at 1 day, hyperglycemia induced by glucose injection declined to the normal range. In conclusion, this study demonstrates that the ET-K preservation method allowed tissue ATP synthesis and amelioration of cold ischemic tissues damage during extended 24 h isolated-islet preservation. This simple method will be adapted easily to the clinical setting and used to maximize the utilization of islet transplantation as well as for pancreas sharing with remote centers.
Luminescence imaging of regenerating free bone graft in rats.

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Abstract

BACKGROUND:
Bone transplantation is an important procedure often used for bone defect reconstruction after trauma and malignancies. However, the kinetics of free bone graft-derived cells remains unclarified. The authors examined the kinetics of graft-derived cells using transgenic rats systemically expressing firefly luciferase.

METHODS:
Free iliac bone grafts (5 × 5 × 2 mm, n = 10) derived from luciferase transgenic rats were transplanted into the subcutaneous space of the back of wild-type Lewis rats, and the kinetics of graft-derived cells were evaluated over time by determining the level of luminescence emission.

RESULTS:
Although the luminescence level emitted by luciferase decreased after transplantation, a substantial luminescence level (mean, 1.6 × 10(7) photons/second) was emitted from donor-derived cells even at 180 days after transplantation, suggesting a long-term survival of graft-derived cells. In a computed tomographic image analysis of bone grafts retrieved 180 days after transplantation, high-luminescence grafts with a sufficient number of viable graft-derived cells (mean, 2.6 × 10(7) photons/second; n = 4) showed significantly higher bone graft volume (3.1-fold) and polar moment of inertia of area (7.2-fold) than low-luminescence grafts (mean, 1.0 × 10(7) photons/second; n = 4), indicating that high-luminescence grafts maintain better conditions.

CONCLUSION:
These results suggest that bone graft-derived cells can survive for a long time and that the presence of a sufficient number of viable graft-derived cells is essential for graft engraftment and remodeling.