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Alternative Usage of Recellularized Liver Graft as Clinical Application

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Introduction: Although liver transplantation is the only curative and established treatment for end-stage liver disease, patients that could have benefited from transplantation are restricted by the severe donor organ shortage. The transplantable whole-liver engineering using decellularization technique is one of the approaches to resolve this problem, however, coagulation in the decellularized liver stopped the blood flow and there was no study to achieve clinical relevant recellularized liver grafts. Inappropriate cell distribution and coagulation with blood perfusion were tasks to be solved for practical use. The current study proposed a method substituted for transplantation as clinical application of recellularized liver graft, reproducing appropriate cell distribution.

Methods: The Lewis male rats were used for generating the whole-organ decellularized scaffolds and cell isolation. Isolated primary hepatocytes and liver sinusoidal endothelial cells (LSECs) were recellularized via bile duct and portal vein, respectively, and perfusion culture was performed. Histological analysis was evaluated for cell distribution and function in recellularized liver. After perfusion culture, we put our engineered liver graft into the extracorporeal perfusion system using an alive Lewis rat.

Results: Histological analysis revealed appropriate distribution of hepatocytes into the parenchymal space without intra-portal thrombosis, resulting in LSECs attachment along the portal vein, maintaining characteristic morphology and phenotype. Platelet deposition evaluated by measuring fluorescent intensity of integrin αIIb+ was significantly decreased in hepatocytes and LSECs co-seeded group than in hepatocyte alone seeded group (p < 0.001). Blood clotting did not stop the blood flow during 3 h extracorporeal perfusion.

Conclusion: Biliary duct seeding of parenchymal cells could allow endothelial cell attachment along the vasculature and did not obstruct blood flow into vessels. LSECs could maintain their morphology and phenotype, and possess anti-coagulation ability in engineered liver grafts. Extracorporeal perfusion might be one of the solutions for clinical use of recellularized liver graft. Further study is needed to investigate our fabricated liver graft could support liver function in the extracorporeal perfusion system.

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Hydrogen Rich Solution Attenuates Cold Ischemia-Reperfusion Injury in Rat Liver Transplantation

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Introduction: Liver transplantation (LT) is considered as the standard treatment for end stage liver disease. However, there is a problem of donor shortage, and the need of grafts from marginal donors has increased. Attenuation of ischemia and reperfusion injury (IRI) in such marginal donors is crucial for less possibility of primary non-function and the graft loss. There have been some reports that hydrogen (H2) shows the antioxidative and anti-inflammatory effects, and eventually prevents IRI in some non-hepatic transplant models. Therefore, we investigated whether the H2 attenuates IRI in LT model using rats.

Methods: We made and used the H2 rich water bath (HRWB), in which the H2 ion was dissolved in the UW solution. Isogenic LT model of Lewis rats was used. Without arterial reconstruction, orthotopic LT was performed according to Kamada’s cuff method. The animals were divided into four groups: sham operation (Sham), not preserved (NP), preserved 12 hours in UW solution (UW), preserved 12 hours in H2 rich UW solution (UW+H2). H2 ion solution in the graft liver was measured every hour after preservation in the preliminary study. Blood and tissue samples were collected 6 hours after the reperfusion. Hepatic enzymes in serum were measured. Pathological findings including the expressions of cytokines and heme oxygenase-1 (HO-1) in liver tissues were evaluated.

Result: H2 concentration of graft tissue increased depending on the storing time in the HRWB, and it became plateau after 1 hour. AST, ALT, and LDH levels of serum showed significantly lower in UW+H2 groups. In the UW group, liver histology showed focal hemorrhage, cell ballooning, and infiltration of neutrophils and macrophages, and those findings were much attenuated in the UW+H2 group. UW+H2 group also showed less oxidative damage and hepatocyte apoptosis. UW+H2 groups tended to have lower proinflammatory cytokines and higher HO-1 levels in mRNA expressions, and protein levels of HO-1 increased significantly.

Conclusion: By using the HRWB, sufficient H2 distribution in the liver graft was obtained. Storage of the liver grafts in H2 rich UW solution presented superior functional and morphologic protection for IRI. Up-regulation of HO-1 was suggested as one mechanism of this effect. Result of our present study demonstrated that H2 rich solution decrease oxidative stress and inflammatory changes by IRI in rat LT model.

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