Decellularlized Matrix in Organ Transplantation

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Liver transplantation is the only known treatment for patients with end-stage liver failure, but this therapy is limited by the shortage of donor organs. Hepatic tissue engineering combining biomaterial scaffolds and cells have been used as a promising strategy to create engineered liver graft for liver regeneration. Despite significant progress in this field, attempts to create clinically transplantable whole organs have not been as nearly successful. Recently, whole organ decellularization techniques have emerged as a new therapeutic strategy for organ replacement and provided feasibility for clinical translation. The perfusion decellularization method was applied to the whole organ for efficient removal of cellular components and generated organ scaffolds that can maintain the extracellular matrix (ECM) and vascular structure of the native organ. This review paper describes current progress in organ bioengineering for the development of transplantable liver grafts.

Key Words: Liver Transplantation; Hepatic Tissue Engineering; Organ Engineering; Decellularization; Recellularization

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INTRODUCTION

Liver disease is one of the leading causes of death in the world. Each year, approximately 25,000 patients die from end-stage liver disease in the United States alone. The orthotopic liver transplantation is the only known treatment for patients with end-stage liver failure but this therapy is limited by the shortage of donor organs. Many alternative treatments, including hepatocyte transplantation [1,2], transplanted tissue engineered liver [3-5], and extracorporeal bioartificial liver (BAL) device [6,7] have been attempted to treat acute liver failure, but have shown limited success.

LIVER TISSUE ENGINEERING AND CELLULAR INTERACTIONS

Hepatic tissue engineering has evolved as a novel therapeutic approach for drug testing and liver regeneration with engineered hepatic grafts [8-10]. The reconstruction of functional hepatic tissue is dependent on the ability to control factors that influence the cell environment, including cell-matrix interactions, soluble stimuli, and cell-cell interactions [11-14]. Three-dimensional scaffold-based tissue-engineered hepatic constructs have been commonly used for tissue engineering approaches [15-18]. A major challenge in the engineering of any tissue is the inability of providing a sufficient blood supply immediately post implantation. Engineering grafts for thick complex tissues such as the liver and heart require an adequate vasculature to sustain physiological requirements since the diffusion limit for oxygen is 100-200 μm [19]. This challenge is intensified when engineering a physiological demanding tissue such as the liver. The native liver tissue is supplied with a rich vasculature which is necessary to quench the immense demand for oxygen and nutrients required for its continuous, vigorous contractile activity. In addition to serving as conduits for the blood supply, endothelial cells are vital for promoting hepatocyte survival and function. Although some advances have been made in the area, none of the methods have provided an absolute solution for the problem. As in other tissue engineering problems, a liver regeneration system has many parameters that need to be optimized in order to enable a robust system design for restoring the function of the damaged liver.

Cell-cell interactions play a critical role in tissue morphogenesis, embryogenesis, and organ development. Primary adult hepatocytes co-cultured with other cell types have been shown to retain viability and function [20,21] through mechanisms dependent on heterotypic cell–cell contacts [22,23]. Hepatocyte morphology and function varies somewhat depending on co-culture cell type [24,25]. Optimal hepatic tissue for therapeutic applications should involve a mixture of hepatic cells including hepatocytes, fibroblasts, and endothelial cells in order to mimic hepatic structure and function *in vitro*. During vasculogenesis, endothelial cells recruit mural cells to the outside of the growing tube, where they adhere and differentiate to form the new vessel. Endothelial cells can also recruit hepatocytes in a similar fashion. When cultured in a three-dimensional scaffold, endothelial cells form typical vascular networks with capillary-like tubes. When endothelial cells and hepatocytes are cultured together, however, the endothelial cells form tube-like structures and the hepatocytes position themselves on the outside of these tubes [26]. These data suggest that an endothelial factor directs the assembly of hepatocytes on the capillary-like endothelial tube. Despite significant progress in this field, attempts to create a clinically transplantable whole organ have not been successful. One of the major challenges for hepatic tissue engineering is to produce large organs for clinical applications.

DECELLULARIZED MATRIX FOR WHOLE LIVER

Recently, whole organ decellularlization techniques have emerged as a new therapeutic strategy for organ replacement. The technique was first reported for a decellularized organ scaffold from cadaveric hearts [27]. The perfusion decellularization method was applied to the whole organ for efficient removal of cellular components and generated organ scaffolds that can maintain native extracellular matrix and vascular structure of the native organ. The scaffold was reseeded with cells and cultured in a perfusion-based system for the development of bioengineered whole organs. The technique was then applied for other organs, including the liver [28,29], lung [30-32], and kidney [33] and showed great promise in regenerative medicine.

Uygun et al. [29] first reported whole liver engineering using perfusion decellularized liver scaffolds and transplantation of recellularized liver grafts. In this study, ischemic rat livers were used for decellularization by portal vein perfusion. Liver was perfused with various concentrations of sodium dodecyl sulfate (SDS) for

72 hours, followed by Triton X-100 treatment for decellularization. A translucent acellular liver matrix was generated after the decellularization process. Histological examination confirmed no nuclei or cytoplasm in the matrix. Immunofluorescence staining of the liver matrix showed positive expression for collagen type I, collagen type IV, fibronectin, and laminin similar to native liver. The decellularized matrix retained 100% of the fibrillar collagen and -50% of the glycosaminoglycans in native liver. This was because many glycosaminoglycans are associated with cellular membranes that are solubilized in the decellularization process. The amount of DNA in the liver matrix was minimal ($< 3\%$). In order to assess the vascular network structure, the liver matrix was perfused with Allura red dye through the portal vein. The vascular tree and microvasculature structures were clearly apparent and remained intact. Scanning electron microscopy (SEM) images of the matrix confirmed the presence of the vascular network structure. The decellularized liver matrix was seeded with primary rat hepatocytes for the recellularization process via portal vein perfusion recirculation. The four-step seeding protocol with 10-minute intervals between each step showed better cell viability and distribution than single-step infusion for 40 minutes. Recellularization efficiency with the four-step protocol was approximately 95%. Further characterization of the *in vitro* perfusion culture of the recellularized liver graft demonstrated the maintenance of liver-specific functions; including albumin production, urea synthesis, and cytochrome p450 metabolism, similar to static collagen gel sandwich culture controls [34]. Histological analysis of recellularized graft cultured for 5 days exhibited cells distributed throughout the matrix and engrafted around the vessels. Nonparenchymal cell seeding using microvascular endothelial cells to the recellularized liver graft showed the capability to form vasculature. For heterotopic transplantation of the recellularized liver graft, nephrectomy of the recipient rat was carried out after clamping the left renal artery and vein. The graft was placed in the left renal space of the recipient and perfused after unclamping the artery. The recellularized liver graft was kept for 8 hours *in vivo* and harvested for further analysis. Results of this study demonstrated the maintenance of hepatic survival and function with minimal ischemic damage. Further transplantation experiments by ex vivo whole-blood perfusion for 24 hours as an orthotopic rat liver transplant model showed that hepatocytes remained viable and functional in the blood perfusion culture. This study indicates that decellularized liver matrix using organ tissue engineering technique can be used as a trans-

plantable liver graft for potential treatment of liver disease.

Other groups have demonstrated reproducibility and feasibility of these decellularization techniques in small and large animal models [28,35-39]. Baptista et al. [28] used whole organ decellularization for the generation of a vascularized liver organoid. Endothelialization of the vasculature lumen is critical to prevent thrombosis and to provide vascular function. In this study, perfusion cell infusion through the vascular channels of the decellularized liver matrix was employed to improve seeding efficiency and engraftment. Triton X-100 solution with ammonium hydroxide was used to decellularize the livers from mice, rats, ferrets, and pigs. The decellularization solution was perfused through the vascular network for 1 hour for mice, 2 hours for ferrets, 3 hours for rats, and 24 hours for pig livers. After treatment, the liver became transparent and preserved the clear vascular network structure. The decellularized matrix was extensively characterized by histological, immunohistological, western blot, spectrophotometric, and SEM analysis. Histological stainings showed the extracellular matrix composition of the matrix, including collagen, elastin, and glycosaminglycans with vascular channels. Western blot analysis revealed the presence of collagen type I, III, and IV, fibronectin, laminin, and decorin. These were mainly observed in the vascular structure and parenchymal areas of the matrix scaffold, similar to native liver. To evaluate the structure of the vascular network, fluorescein-labeled dextran was infused through the portal vein. The matrix scaffolds retained a vascular network with fine branching structures. To test re-endothelialization of the vascular network of the scaffold, GFP-labeled endothelial cells were seeded through the vena cava and the portal vein for comparison. The cells seeded via the vena cava were distributed throughout the larger blood vessels concentrating in the central veins and smaller branches. The cells seeded via the portal vein were distributed throughout the scaffold with higher cell concentration in the periportal areas of the liver lobule. Both seeding approaches showed an aligned vascular network. Using the methods developed for re-endothelialization of the scaffolds, the primary human cells were used to recellularize the matrix scaffolds for potential clinical applications of organ tissue engineering. The matrix scaffolds were co-seeded with freshly isolated human fetal liver cells and human umbilical vein endothelial cells through the portal vein of the scaffolds by perfusion with culture medium over 16 hours at 3 mL/min and constant perfusion at 0.5 mL/min. The seeded cells were able to repopulate areas throughout the scaffolds. Phenotypic and func-

tional analysis of the recellularized scaffolds demonstrated that the scaffolds can support the proliferation and differentiation of the fetal hepatocytes as well as the maintenance of liver-specific functions.

Barakat et al. [35] first reported human size whole liver engineering using decellularized the porcine liver scaffolds toward developing a functional liver graft for clinical application. Ischemic porcine livers were removed and decellularized by perfusing with 0.5% SDS via the portal vein. After the decellularization procedure, the matrix was further cross-linked with collagen and 10% formalin to maintain its strength and stability. Immunohistochemical analysis confirmed that the decellularization method successfully removed the cellular components while retaining most ECM proteins, native structure, and the vasculature. For recellularization of the matrix, human fetal hepatocytes were used because they have a higher proliferative activity than adult hepatocytes [40]. To maintain the viability and function of cultured hepatocytes, the hepatocytes were co-cultured with human stellate cells [41,42]. The decellularized liver matrix was co-seeded with human fetal hepatocytes and human stellate cells and perfused at 90 mL/hr for 3, 7, and 13 days. After 13 days of perfusion culture, the engrafted hepatocytes co-cultured with stellate cells maintained their viability (>70%) and proliferative capability in the recellularized porcine matrices. Differentiation of the fetal hepatocytes into mature hepatocytes was observed by immunohistochemical analysis. *In vivo* transplantation of decellularized liver matrix into a recipient porcine model was performed to evaluate its behavior and ability to withstand the shear stress under portal venous flow and pressure. The matrix was implanted in the infrahepatic space by using the recipient portal vein and infrahepatic inferior vena cava as an inflow and outflow, respectively. After reperfusion, the implanted matrix was filled with blood immediately. The matrix was uniformly perfused with blood in 5 minutes and maintained an intact structure. This study provided a foundation using whole organ engineering of a large animal model for transplantation in humans.

CONCLUSION

The liver is a large, complex organ involved with many metabolic functions. Using decellularization techniques of the whole organ, researchers were able to create organ scaffolds that can maintain the extracellular matrix and vascular structure of the native organ. Although organ reengineering shows promise, further im-

provements are needed to develop transplantable engineered liver grafts for clinical application. To engineer whole liver organs requires human parenchymal hepatocytes and nonparenchymal cells, including sinusoidal endothelial cells, Kupffer cells, stellate cells, and biliary epithelial cells. Re-endothelialization of the decellularized vascular structure is necessary for a sufficient supply of oxygen and nutrients to the recellularized liver graft. Organ preservation technique is also essential to extend the amount of time human organs can survive outside the body before transplantation. Current methods can store livers outside the body for approximately 24 hours. Recently, a new supercooling technique was developed to increase the preservation time of livers [43]. This technique was able to preserve rat livers for three days, advancing the practice of organ storage for clinical use. Together, the current advances in whole organ engineering shows great promise toward developing fully functional engineered liver grafts for clinical transplantation.

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