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Application of whole-organ tissue engineering in hepatology

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Abstract

Initially hailed as the ultimate solution to organ failure, engineering of vascularized tissues such as the liver has stalled because of the need for a well-structured circulatory system that can maintain the cells seeded inside the construct. A new approach has evolved to overcome this obstacle. Whole-organ decellularization is a method that retains most of the native vascular structures of the organ, providing microcirculatory support and structure, which can be anastomosed with the recipient circulation. The technique was first applied to the heart and then adapted for the liver. Several studies have shown that cells can be eliminated, the extracellular matrix and vasculature are reasonably preserved and, after repopulation with hepatocytes, these grafts can perform hepatic functions *in vitro* and *in vivo*. Progress is rapidly being made as researchers are addressing several key challenges to whole-organ tissue engineering, such as ensuring correct cell distribution, nonparenchymal cell seeding, blood compatibility, immunological concerns, and the source of cells and matrices.

Introduction

The only definitive treatment for severe hepatic failure is orthotopic transplantation. However, a critical shortage of organs is causing an increasing deficit of organs, currently around 4,000 livers per year.[1] Tissue engineering has the potential to cover the donor organ gap via the creation of artificial tissues. However, despite decades attempting to create tissues by fabricating scaffolds, beginning with microarchitecture at the cellular level and using synthetic biomaterials, tissue-engineered organs have not yet been successfully introduced into a clinical setting.[2,3] The commercial success of regenerative medicine has mainly been restricted to noncellular products; examples are acellular skin matrix components and cartilage replacements.[3]

This Perspectives article describes the current status of the field of liver-tissue engineering and outlines some of the challenges being encountered. Some of the novel solutions being developed to overcome those challenges are discussed; hopefully, these solutions will bring whole-liver engineering a stage closer to being used in the clinic to address organ shortages.

Hepatocyte transplantation

Hepatocyte transplantation is an alternative to whole-organ transplantation. Cells from a healthy donor are transplanted to a recipient with liver dysfunction.[4] The major problems with the cell transplantation approach are low rates of engraftment and poor survival of transplanted hepatocytes.[5] One approach to overcome this issue is to transplant hepatocytes

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that are contained in a carrier material, such as microcapsules (in which the cells are contained within a uniform covering of microspheres),[6] or within biodegradable scaffolds,[7] to provide a microenvironment suitable for cell attachment and to protect the cells from the recipient's immune system.[8] Another reason for poor *in vivo* survival of transplanted hepatocytes is inadequate access to blood circulation for nutrient delivery and waste removal. Hepatocytes are highly metabolically active cells and without an adequate blood supply they rapidly die from a lack of oxygen and nutrients or exposure to the toxic products of metabolism.[9]

Creating a liver-graft structure

Traditional tissue engineering approaches for the development of liver grafts have been hampered by a lack of oxygen and nutrient diffusion, which limits the thickness of the cell scaffolds to being sheet-like structures, usually engrafted in highly vascularized regions.[10,11] A hepatic mass that is sufficient to provide adequate metabolic function needs to contain a microvascular network connected to the circulatory system to prevent the internal mass of the cells being subject to ischaemic damage.[12] A number of approaches to overcoming this problem have been tested, such as attempting to mimic the native liver architecture using tissue engineering and micro-fabrication techniques. these techniques can be used to fabricate micrometer scale channel networks on a solid substrate that resembles the tissue microvascular structures, such as the sinusoids and bile canaliculi in a hepatic lobule. these networks can then be seeded with hepatocytes, endothelial cells and cholangiocytes to establish an artificial perfusable, functional hepatic unit. Using this tissue engineering technology, an implantable, hepatocyte-containing device could be created that can be connected to a recipient's circulatory system.[12,13] Other approaches include the transplantation of cells or constructs into vascular tissue[7,14] and use of scaffolds in tandem with angiogenic factors, such as vascular endothelial growth factor and basic fibroblast growth factor to stimulate rapid neovascularization of the implant.[15,16] Only one study has demonstrated any success in increasing survival rates in animal models of liver failure.[7] However, it has been nearly two decades since the first promising demonstration of engineered-liver implantation into rats and many problems have had to be overcome.[17] Past attempts at hepatic tissue engineering can be summarized as an iterative search for an ideal scaffold that has the necessary matrix cues,[8] such as adhesion factors and matrix-bound growth factors, and appropriate architecture[12] to stimulate liver cell engraftment, function and survival *in vivo*.

Whole-organ tissue engineering

Tissue decellularization

The first generation of tissue engineering materials used in scaffold fabrication, such as polylactic acid or polyglycolic acid, were of limited use as they lacked sufficient ability to modulate the repair and regeneration of the host tissue.[8] Consequently, interactions between materials and host tissues, such as control of specific cell-binding interactions and response to environmental cues (for example, signals provided by soluble and insoluble growth factors, cytokines and extracellular matrix [ECM] molecules),[18] became important considerations in the design of new materials. Whole-organ decellularization is an attractive scaffold-preparation technique for tissue engineering as the resulting structure can potentially retain the architecture of the original tissue and functional components of the native microvasculature,[19] as well as the native ECM composition, to a certain extent.[20] Potential applications of decellularized matrix in tissue engineering have already been demonstrated for a number of tissues, including bladder,[21] skin[22] and trachea.[23] The decellularized human skin Alloderm® (LifeCell Corporation, New Jersey, USA), and small

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intestinal submucosa are among the most commercially successful examples of engineered tissue.[3]

Decellularization of an entire heart through perfusion was first shown in 2008.[22] After the decellularization process the original ECM composition and its perfusable microvascular network were preserved.[24] Subsequent reports showed successful application of this technique to the liver[25–30] and lung;[31–33] preliminary results for the kidney and pancreas have also been published.[34]

Decellularized whole-liver scaffolds can be obtained from donor organs that are discarded because of damage and deemed unsuitable for transplantation. The approach for making whole-liver grafts is to repopulate the decellularized matrix scaffold with parenchymal and nonparenchymal cells, ideally derived from pluripotent stem cells, and conditioning the graft *in vitro* through perfusion with nutrients. The repopulated graft is heterotopically transplanted as an auxiliary support to a dysfunctional organ (Figure 1).

First recellularized rat livers

Our research group was the first to report the generation of a transplantable liver graft using a perfusion-decellularized liver matrix[25] and other reports followed.[26–30] The decellularization process preserved the structural and functional characteristics of the native microvascular network, which allowed subsequent recellularization with adult rat hepatocytes.[23] Recellularization efficiency with primary rat hepatocytes was high—over 90% of the hepatocytes infused into the portal vein remained in the scaffold.[23] This efficiency figure indicates that the cells were retained within the graft, but does not necessarily indicate attachment, engraftment or survival. *In vitro* perfusion culture of the recellularized liver graft showed that the graft supported liver-specific functions, including albumin secretion, urea synthesis and cytochrome P450 expression, at approximately 30% of the functional capacity of normal liver. Furthermore, after the recellularized liver grafts were transplanted in rats they supported hepatocyte survival and function with minimal ischaemic damage, albeit for a very short duration of 8 h. This report was followed by the creation of functional and viable transplantable rodent liver grafts using human foetal liver cells,[27,29,30] preparation of pig liver grafts using human foetal hepatocytes and stellate cells and very short-term (2 h) transplant time.[30] Heterotopic transplantation of some recellularized rat liver grafts survived up to 4 days.[26] However, the process of creating re-engineered liver grafts involves several complicated procedures and there are still many hurdles to overcome.

Role of the extracellular matrix in liver

Changes in the expression of a variety of ECM components in the developing liver revealed by immunohistochemical analysis provide strong evidence that ECM has a major role during hepatogenesis.[35] Maturation of sinusoidal vasculature, develop ment of hepatic architecture and hepatocyte growth and differentiation are some of the processes that are influenced by the ECM and several studies have successfully utilized natural ECM components to stabilize the adult hepatocyte phenotype[36,37] and induce embryonic stem cells to differentiate into hepatocyte-like cells.[38] Basement membrane proteins, such as laminin and fibronectin, as well as proteoglycans and their glycosaminoglycan side chains are directly involved in the integrinassociated cell-signalling networks that determine cell responses to extracellular cues.[39] The importance of the ECM components in modulating hepatocyte function and hepatic architecture highlights the advantage of using decellularized liver matrix as the scaffold material in successful liver engineering. However, few studies have directly evaluated the effect of the decellularized liver matrix on stem-cell fate[40,41] and this effect remains an important direction for research.

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Preserving the extracellular matrix

Liver ECM has a mixture of type I and type IV collagen, glycosaminoglycans, fibronectin, laminin and growth factors, as well as specialized spatial architecture of the sinusoids, forming the support for resident cells; all of these ECM factors have been shown to have a marked effect on cell behaviour, from attachment and differentiation to function.[20] The ideal decellularization technique would retain as much of this native composition and structure as possible, after removal of the cells. A comprehensive overview of the detergents and other methods that can be used for this purpose is beyond the scope of this article and has been reviewed elsewhere.[42] This article focuses on several insights gained from our research group's work on rat liver decellularization.

The liver is considered to have a limited ECM compared with connective tissue organs such as cartilage and bone,[43] but it has a surprisingly strong matrix that fully retains its original shape after decellularization—a decellularized liver does not feel very different from a nondecellularized liver when handled. The vessels are supple but robust and can be easily anastomosed with the recipient circulation. Although the vessels are able to withstand the pressure of blood flow, as a precaution, it is best to avoid exposing the organ to full arterial flow pressure by adjusting the catheter size used in anastomosis.

Rapid decellularization with highly concentrated detergents leads to rapid release of cellular debris and increase of internal pressure, which is evident by ballooning of the organ, leading to the rupture of Glisson's capsule. As such, it is advisable to carry out the procedure slowly using a multi-step procedure over about 4 days.[44] Correct positioning of the cannula for introducing the detergent is also critical to ensure the equal distribution of detergent over the entire organ, otherwise the peripheral tissue might not be adequately decellularized.

Detergents used to disrupt the cell membrane and dissolve cytosolic material also dissolve many ECM components, such as growth factors and glycosaminoglycans. Several studies have indicated retention of the structural proteins in ratios similar to their normal composition; however, two reports on growth factors indicate that there are reductions in the level of growth factors such as HGF and EGF, to ~20% of the normal liver levels in a pig model[45] and ~50% in a rat model.[28] As the concentration and relative ratios of these factors can have considerable effects on cell behaviour, it might be necessary to reintroduce some of them to the decellularized graft. Studies to elucidate postdecellularization ECM changes in the context of cell attachment, differentiation and maturation are urgently needed.

Rebuilding the liver

Choosing a cell source

Choosing the source of cells is a complex question with various competing approaches. There are several considerations when deciding whether to use autologous or allogeneic cells (Box 1). Possible hepatocyte sources for human liver tissue engineering include primary human cells, cell lines, xenogeneic hepatocytes, and bipotent or pluripotent stem cells (including foetal hepatoblasts, embryonic stem cells and induced pluripotent stem cells). Induced pluripotent stem cells are the optimum cell type for the treatment of chronic liver diseases as they would be autologous cells and would reduce or remove the need for immune suppression. However, methods used to derive these stem cells are prone to problems such as retaining epigenetic memory, which could alter their potential to differentiate into cells of lineages other than those they were derived from.[46–48] The similarity of induced pluripotent stem cells to human embryonic stem cells has not been fully elucidated, but practical and scalable methods to differentiate embryonic stem cells into mature hepatocytes that are equivalent to primary cells have not been established.[49]

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The cell source is not a major obstacle to tissue engineering but is an important consideration if preliminary clinical successes are to be translated to commercial production scales. A potential cell source for initial studies is primary human hepatocytes recovered from cadaveric livers, which can be used to generate hepatocytes with equivalent or better viability and function compared with fresh hepatocytes in a rat model.[50] Foetal hepatoblasts are another ideal initial solution since they can be propagated easily *in vitro*, and readily differentiate into hepatocytes and cholangiocytes. The ability of foetal hepatoblasts to differentiate into cholangiocytes could help to rebuild the biliary tree within the grafts.[51] Moreover, a promising study has already tested these cells for liver recellularization and showed the formation of cytokeratin-19-positive ductules in the recellularized graft.[27]

Recreating liver microarchitecture

It is important to recreate the correct spatial distribution of parenchymal and nonparenchymal cell types within the whole-organ scaffold. Infusion of hepatocytes through the vasculature has been shown to be an efficient seeding technique for the distribution of cells into the parenchymal spaces of the scaffold compared with direct injection into the parenchyma.[25,28] However, the infusion techniques used so far are the results of initial guesses and in our experience lead to highly uneven cell distribution within the tissue, as well as considerable variation between experiments. It is not difficult to direct hepatocytes into the parenchyma with continuous perfusion,[25,28] but it is not known whether this is simply because the vascular bed is very porous after cells are removed and because of the flow pressure during perfusion culture, or if there is a migration and homing factor involved; for practical purposes this is not an important question. However, lack of even distribution is a practical problem that creates a barrier to consistency in the manufacture of recellularized grafts.

Multi-step dynamic seeding of hepatocytes results in their uniform distribution.[25] If nonparenchymal cells are introduced into the scaffolds, endothelial cells and hepatocytes seem to attach to their respective niches more readily than if the nonparenchymal cells are not introduced.[25,27] Infusion through different circulatory systems, the portal and venous routes[27] or sequential seeding of different cell types (hepatocytes followed by endothelial cells)[25] can be used to restrict the distribution of the endothelial cells into the vascular spaces and hepatocytes into the parenchyma. Repopulation of the biliary tree with cholangiocytes has not been reported but it is thought that the cells would be physically contained in the biliary channels that are preserved in the whole-liver scaffolds[28] if they are infused into the bile ducts. In addition, it has been suggested that ECM matrix components have zonal variation in the decellularized scaffold;[40] therefore, it is likely that the biliary channel ECM would contain signals that favour the engraftment of cholangiocytes at the site.

When hepatocyte density is limited to ~20% of that in normal liver tissue, histological investigations indicate a mixture of zones.[25] The first zone consists of tissue that is similar to healthy liver. The second zone has high concentrations of dead cells (typically within the vascular bed), probably because of occlusion and consequent necrosis owing to inadequate perfusion. The third zone is very sparsely populated with cells that are mostly dead, most likely as a result of apoptosis caused by the absence of cell–cell contact. Cell density should be increased to create confluent hepatic mass within the parenchyma although, from experience with *in vitro* culture of primary hepatocytes and recellularized grafts, it is clear that there is an optimum range of seeding density. If there are too few cells, insufficient cell–cell attachment and interactions leads to formation of sparse colonies rather than a liver-like confluent hepatocyte mass. However, seeding too many cells leads to a large number of dead cells, adversely affecting the culture viability, probably owing to the cell debris causing immune or apoptotic signalling events in the otherwise healthy hepatocytes.

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Overseeding is a particular problem in decellularized whole-liver grafts as excessive numbers of cells can create occlusion in the vessels and massive cell death. Our own experiences indicate that ~80% of the cell density found in normal liver tissues could be effective, although optimization of the seeding process is needed. Multi-step seeding could prevent the increased density creating even more occlusion zones.[28] Studies are urgently needed to explore this technical aspect.

Generally, obtaining human endothelial cells is not problematic because most endothelial cell subtypes can be expanded *in vitro*. However, liver sinusoidal endothelial cells are difficult to obtain and maintain, and two reports on endothelialization of liver scaffolds have used other types of endothelial cells, such as rat cardiac microvascular endothelial cells[25] and human umbilical cord vascular endothelial cells.[27] In our opinion, this is a satisfactory shortterm solution. Hepatocytes and cholangiocytes, however, do not expand well *in vitro* and can be problematic when used for recellularization.

Preventing graft rejection

Liver origin and immune rejection—Sourcing livers for decellularization is, surprisingly, not an immediate problem. One option is pig livers, which are easy to obtain and ensure quality. However, use of xenogeneic tissues could lead to hyperacute transplant rejection because of recipient antibodies against galactose-α-1,3-galactose (the α-gal epitope), which is expressed by pig tissue.[52] However, after decellularization, xenogeneic tissue does not seem to generate an adverse immune response, but a constructive remodelling response was shown in an African Green monkey abdominal wall resection model.[53] Although there was an initial increase in serum α-gal antibodies with normal xenogeneic grafts when compared with xenogeneic grafts obtained from α-gal-deficient pigs, the serum antibody levels reached normal levels 90 days after transplantation.[53] Furthermore, an α-gal-knockout pig has been engineered and implantation of decellularized dermal matrix from knockout pigs in a nonhuman primate model resulted in a minimal or no response to α-gal.[54]

Human donor organs are often unused and are available for decellularization. Livers are rejected for transplant because of excessive steatosis, fibrosis and cirrhosis, or levels of ischaemia that are beyond acceptable limits. It is estimated that the number of mildly ischaemic livers is in the order of 6,000 per year[55] and these livers could potentially be recovered for transplantation by machine perfusion;[56] therefore, many more thousands of livers are accessible for human liver decellularization. Importantly, some of the pathologies that lead to rejection for transplant could have effects on the quality of ECM and it is not yet clear if this damage will impair recellularization. In our opinion, human livers will be the preferred source of organs for clinical trials of decellularization techniques; however, if this process is used for commercial scale production, pig livers could be preferable in order to reduce costs and for ease of standardization and quality assurance.

Preventing coagulation—Prevention of blood coagulation is difficult to achieve and failure to do so hinders long term *in vivo* testing of the recellularized liver grafts. Blood contact is normally restricted to the endothelium and the coagulation cascade is initiated if blood is exposed to tissue collagen underlying the damaged endothelium at an injury site, through direct and indirect interactions of collagen with platelet glycoprotein surface receptors.[57] Unless the graft has been fully recellularized to conceal collagen, coagulation will occur when the graft is exposed to circulating blood. Coagulation can be prevented *in vivo* by covering the vascular bed in the decellularized matrix scaffold with endothelial cells.[24,25,27,31] In practice, achieving near-perfect endothelial cell coverage of the vasculature in the scaffold is challenging. Administration of systemic anticoagulants has

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been used in experimental models for heterotopic transplantation of the recellularized liver grafts; however, although successful in preventing thrombosis, this led to complications such as haemorrhage and the recipients did not survive beyond 8 h. For similar reasons, most attempts at transplantation of the whole-liver scaffolds have been limited to only a few hours.[25,27,30,58] A promising solution is to deposit heparin layers on the decellularized matrix scaffold and render the whole-liver graft blood compatible.[26] Recipients of heparinized and recellularized grafts survived for up to 96 h compared with rats that had received recellularized grafts without heparin, which only survived for 16 h after 90% hepatectomy.[24] Although heparinization of the grafts is a major improvement, the efficacy and safety of this technique still needs to be tested.

Transplantation of recellularized grafts

Ideally, a recellularized graft would be equivalent to a native liver, featuring the hepatic artery, vena cava, portal vein and the bile duct, and could be orthotopically transplanted to the recipient to replace their dysfunctional liver. Heterotopic transplantation might be more practical, particularly for early *in vivo* investigations, as it would not be necessary for the recellularized graft to fully substitute for the liver. As such, heterotypic transplantation has been the preferred approach in the *in vivo* studies performed so far.

In a rat model study by our research group,[25] left nephrectomy was carried out to provide an arterial blood supply for the graft, which was connected to the portal vein and the renal vein to draw blood via suprahepatic vena cava. A heparinized catheter was used for anastomosing the vessels. This approach also enabled the pressure of the arterial blood being delivered into the graft to be reduced by using slightly narrower catheters, which was necessary as the pressure could cause leakage through the capsule of the graft. The leakage was probably because the graft was not fully recellularized. Although nephrectomy is feasible in animal models to create space as well as vascular connection points it is a clinically unlikely scenario.

An alternative approach is to transplant the graft into the intra-abdominal cavity in a serial configuration with the recipient liver without removal of any organs or tissues.[27] In the study that used this approach, the recipient superior mesenteric vein and vena cave were anastomosed to the graft portal vein and vena cava respectively; however, the graft survived for 60 min *in vivo* before clotting occurred.[27]

In a different approach, the native liver has been hepatectomized, the recellularized graft transplanted in series and the graft portal vein and the superior hepatic vena cava anastomosed to the recipient's inferior and superior portal vein using the cuff method.[59,60] In one study that took this approach the recipients lived up to 4 days.[26] Low blood flow and high resistance were observed within the graft, as well as small-for-size syndrome, which consists of portal hypertension, gastrointestinal congestion and ascites. However, the recipient had 90% hepatectomy and without the graft, survival was <2 days. Furthermore, the grafts were heparin coated to prevent or reduce blood coagulation, making comparisons difficult with other works. Ectopic, serial approaches are generally complicated surgical procedures, particularly in small animal models like the rat; however, they could be viable in humans, particularly when only a section of the liver (such as the smaller right lobe) is transplanted.

Another approach we have used combines nephrectomy and serial approaches. Right nephrectomy was performed to create space for the graft and the renal artery was tied-off. The graft was transplanted in series with the intrahepatic vena cava using the cuff method. Coagulation reduced post-transplant compared with our previous approach because of the cuff method, and decellularized graft recipients survived up to 4 weeks without

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heparinization. Although the periphery contained coagulated blood, the main flow sections appeared histologically normal, and would probably be able to support viable hepatocytes (K. Uygun, unpublished data). Extending recipient survival to a month means that factors such as cell survival and matrix remodelling can be studied in extensive detail, which was not possible previously.

Several technical and conceptual questions are yet to be resolved. The graft can be transplanted without removing any abdominal organs, particularly if a single lobe is used. High-pressure arterial blood flow risks causing damage to the graft, although it provides highly oxygenated, pulsatile flow. By contrast, the venous flow is less likely to cause pressure-related damage and is easier to manage surgically, but it contains less oxygen and laminar flow. In the liver, arterial flow primarily feeds the biliary tree and ~90% of the blood supply in the rat liver comes from the portal vein [**Au:OK?**].[61] Therefore, the absence of an arterial blood connection to the graft might not prove critical for hepatocyte survival. It is not known whether pulsatile flow characteristics, vessel pressure or graft location will have a major effect on cell survival within the graft, and further research into these factors is required.

Moving towards clinical trials

Each step of the whole-liver engineering process discussed above presents a set of challenges that need to be addressed; however, it is encouraging that for each step there is, at least, some preliminary success in small animal models. Therefore, the main challenges revolve around scaling-up to clinically applicable graft dimensions and achieving *in vivo* viability and function in order to progress to clinical trials.

Although decellularized whole-liver grafts are promising as a breakthrough for vascular tissues, major challenges need to be overcome. First and foremost is achieving adequate capillary structure and, therefore, long-term *in vivo* viability. Although perfusion-decellularized grafts retain an intact circulatory system at a clinically relevant scale, the decellularization process still destroys the capillary structures, which are composed of endothelial cells. The limitation of oxygen diffusion beyond ~200 μm means that a capillary-type structure needs to be reconstituted within the graft to keep the hepatocytes alive after transplantation. Therefore, the first goal is to create capillary structures with endothelial cells in the decellularized liver grafts.

The second major challenge to address is to ensure that there is a functioning biliary system, which might require the entire sinusoidal microarchitecture to be formed. It has been demonstrated that the biliary tree architecture in the liver is preserved after decellularization,[45] and retrograde infusion of cholangiocytes through the bile duct could be a way to seed these cells with proper spatial distribution. In order to create fully functional liver grafts, it is necessary to either induce the cells to reform the biliary tree, preferably *in vitro* but it could possibly be done *in vivo* during remodelling, as components of bile are toxic for hepatocytes. For temporary, auxiliary grafts used to provide short-term liver support, some degree of hyper bilirubinaemia might be tolerated without inducing major harm to the patient or the graft, as bilirubin toxicity is more of a chronic problem. Mildly elevated bilirubin is well-tolerated and in more severe cases such as Crigler-Najjar syndrome, maintenance therapies, for example phototherapy, can be used for short-term management while the patient is awaiting a donor liver for transplantation.[62,63]

Successfully treating acute liver failure with tissue-engineered liver grafts would justify the research into this technique, enabling treatment of 1,600 more patients with acute liver failure per year in the USA alone,[64,65] and some of the livers that are currently transplanted

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into patients with acute liver failure could be used to treat patients with chronic liver failure. The regenerative properties of the liver mean that it is the only organ with a large error margin for creating tissue-engineered replacements. Therefore, in our opinion, the liver (particularly in an acute failure setting) is an excellent stepping stone in the creation of complete laboratory-grown organ substitutes.

Conclusions

Whole-organ tissue engineering is complex and involves many technically challenging steps that need to be performed successfully in series. As such, it is possible that even if individual issues are addressed, the resulting protocol will be so prone to failure that it will only ever have a few limited successes. However, these difficulties are counterbalanced by the fact that whole-organ tissue engineering is a unique platform that allows integration of the fields of biomaterials, ECM and developmental biology, biomedical device engineering and transplant surgery, creating a truly multi-disciplinary field with an emphasis on translational success.

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References

1. Punch JD, Hayes DH, LaPorte FB, McBride v. Seely MS. Organ donation and utilization in the United States, 1996–2005. Am. J. Transplant. 2007; 7:1327–1338. [PubMed: 17428283]
2. Burg T, Cass CAP, Groff R, Pepper M, Burg KJL. Building off-the-shelf tissue-engineered composites. Philos. Transact. A: Math. Phys. Eng. Sci. 2010; 368:1839–1862.
3. Lysaght MJ, Jaklenec A, Deweerd E. Great expectations: private sector activity in tissue engineering, regenerative medicine, and stem cell therapeutics. Tissue Eng. Part A. 2008; 14:305–315. [PubMed: 18333783]
4. Fox IJ, et al. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. N. Engl. J. Med. 1998; 338:1422–1426. [PubMed: 9580649]
5. Smets F, Najimi M, Sokal EM. Cell transplantation in the treatment of liver diseases. Pediatr. Transplant. 2008; 12:6–13. [PubMed: 18186884]
6. Mei J, et al. Improved survival of fulminant liver failure by transplantation of microencapsulated cryopreserved porcine hepatocytes in mice. Cell Transplant. 2009; 18:101–110. [PubMed: 19476213]
7. Navarro-Alvarez N, et al. Intramuscular transplantation of engineered hepatic tissue constructs corrects acute and chronic liver failure in mice. J. Hepatol. 2010; 52:211–219. [PubMed: 20022655]
8. Mooney DJ, Vandenburgh H. Cell delivery mechanisms for tissue repair. Cell Stem Cell. 2008; 2:205–213. [PubMed: 18371446]
9. Chung S, King MW. Design concepts and strategies for tissue engineering scaffolds. Biotechnol. Appl. Biochem. 2011; 58:423–438. [PubMed: 22172105]
10. Ohashi K, et al. Engineering functional two- and three-dimensional liver systems *in vivo* using hepatic tissue sheets. Nat. Med. 2007; 13:880–885. [PubMed: 17572687]
11. Soto-Gutierrez A, et al. Construction and transplantation of an engineered hepatic tissue using a polyaminourethane-coated nonwoven polytetrafluoroethylene fabric. Transplantation. 2007; 83:129–137. [PubMed: 17264808]
12. Kulig KM, Vacanti JP. Hepatic tissue engineering. Transpl. Immunol. 2004; 12:303–310. [PubMed: 15157923]
13. Carraro A, et al. *In vitro* analysis of a hepatic device with intrinsic microvascular-based channels. Biomed. Microdevices. 2008; 10:795–805. [PubMed: 18604585]

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14. Yokoyama T, et al. *In vivo* engineering of metabolically active hepatic tissues in a neovascularized subcutaneous cavity. Am. J. Transplant. 2006; 6:50–59. [PubMed: 16433756]
15. Kedem A, et al. Vascular endothelial growth factor-releasing scaffolds enhance vascularization and engraftment of hepatocytes transplanted on liver lobes. Tissue Eng. 2005; 11:715–722. [PubMed: 15998213]
16. Hou YT, Ijima H, Takei T, Kawakami K. Growth factor/heparin-immobilized collagen gel system enhances viability of transplanted hepatocytes and induces angiogenesis. J. Biosci. Bioeng. 2011; 112:265–272. [PubMed: 21640648]
17. Uyama S, Kaufmann PM, Takeda T, Vacanti JP. Delivery of whole liver-equivalent hepatocyte mass using polymer devices and hepatotrophic stimulation. Transplantation. 1993; 55:932–935. [PubMed: 7682739]
18. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat. Biotechnol. 2005; 23:47–55. [PubMed: 15637621]
19. Badylak SF. The extracellular matrix as a biologic scaffold material. Biomaterials. 2007; 28:3587–3593. [PubMed: 17524477]
20. Badylak SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. Annu. Rev. Biomed. Eng. 2011; 13:27–53. [PubMed: 21417722]
21. Yoo JJ, Meng J, Oberpenning F, Atala A. Bladder augmentation using allogenic bladder submucosa seeded with cells. Urology. 1998; 51:221–225. [PubMed: 9495701]
22. Schechner JS, et al. Engraftment of a vascularized human skin equivalent. FASEB J. 2003; 17:2250–2256. [PubMed: 14656987]
23. Macchiarini P, et al. Clinical transplantation of a tissue-engineered airway. Lancet. 2008; 372:2023–2030. [PubMed: 19022496]
24. Ott HC, et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat. Med. 2008; 14:213–221. [PubMed: 18193059]
25. Uygun BE, et al. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat. Med. 2010; 16:814–820. [PubMed: 20543851]
26. Bao J, et al. Construction of a portal implantable functional tissue-engineered liver using perfusion-decellularized matrix and hepatocytes in rats. Cell Transplant. 2011; 20:753–766. [PubMed: 21054928]
27. Baptista PM, et al. The use of whole organ decellularization for the generation of a vascularized liver organoid. Hepatology. 2011; 53:604–617. [PubMed: 21274881]
28. Soto-Gutierrez A, et al. A whole-organ regenerative medicine approach for liver replacement. Tissue Eng. Part C Methods. 2011; 17:677–686. [PubMed: 21375407]
29. Zhou P, et al. Decellularized liver matrix as a carrier for the transplantation of human fetal and primary hepatocytes in mice. Liver Transpl. 2011; 17:418–427. [PubMed: 21445925]
30. Barakat O, et al. Use of decellularized porcine liver for engineering humanized liver organ. J. Surg. Res. 2012; 173:e11–e25. [PubMed: 22099595]
31. Ott HC, et al. Regeneration and orthotopic transplantation of a bioartificial lung. Nat. Med. 2010; 16:927–933. [PubMed: 20628374]
32. Petersen TH, et al. Tissue-engineered lungs for *in vivo* implantation. Science. 2010; 329:538–541. [PubMed: 20576850]
33. Price AP, England KA, Matson AM, Blazar BR, Panoskaltsis-Mortari A. Development of a decellularized lung bioreactor system for bioengineering the lung: the matrix reloaded. Tissue Eng. Part A. 2010; 16:2581–2591. [PubMed: 20297903]
34. Song JJ, Ott HC. Organ engineering based on decellularized matrix scaffolds. Trends Mol. Med. 2011; 17:424–432. [PubMed: 21514224]
35. Amenta PS, Harrison D. Expression and potential role of the extracellular matrix in hepatic ontogenesis: a review. Microsc. Res. Tech. 1997; 39:372–386. [PubMed: 9407547]
36. Dunn JC, Tompkins RG, Yarmush ML. Hepatocytes in collagen sandwich: evidence for transcriptional and translational regulation. J. Cell Biol. 1992; 116:1043–1053. [PubMed: 1734019]

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37. Sellaro TL, et al. Maintenance of human hepatocyte function *in vitro* by liver-derived extracellular matrix gels. Tissue Eng. Part A. 2010; 16:1075–1082. [PubMed: 19845461]
38. Ishii T, et al. Effects of extracellular matrixes and growth factors on the hepatic differentiation of human embryonic stem cells. Am. J. Physiol. Gastrointest. Liver Physiol. 2008; 295:G313–G321. [PubMed: 18535293]
39. Kim SH, Turnbull J, Guimond S. Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. J. Endocrinol. 2011; 209:139–151. [PubMed: 21307119]
40. Wang Y, et al. Lineage restriction of human hepatic stem cells to mature fates is made efficient by tissue-specific biomatrix scaffolds. Hepatology. 2011; 53:293–305. [PubMed: 21254177]
41. Marongiu F, et al. Hepatic differentiation of amniotic epithelial cells. Hepatology. 2011; 53:1719–1729. [PubMed: 21374689]
42. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole-organ decellularization processes. Biomaterials. 2011; 32:3233–3243. [PubMed: 21296410]
43. Horbett, TA.; Ratner, BD.; Schakenraad, JM.; Schoen, FJ. Biomaterials Science: an Introduction to Materials in Medicine. Ratner, BD.; Hoffman, AS.; Schoen, FJ.; Lemons, JE., editors. Academic Press; San Diego, CA: 1996. p. 147-164.
44. Uygun BE, et al. Decellularization and recellularization of whole livers. J. Vis. Exp. 2011:e2394.
45. Yagi H, et al. Human-scale whole-organ. bioengineering for liver transplantation: a regenerative medicine approach. Cell Transplant. 2012 in press. Au: Has this been accepted?
46. Ohi Y, et al. Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. Nat. Cell Biol. 2011; 13:541–549. [PubMed: 21499256]
47. Kim K, et al. Epigenetic memory in induced pluripotent stem cells. Nature. 2010; 467:285–290. [PubMed: 20644535]
48. Polo JM, et al. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nat. Biotechnol. 2010; 28:848–855. [PubMed: 20644536]
49. Kakinuma S, Nakauchi H, Watanabe M. Hepatic stem/progenitor cells and stem-cell transplantation for the treatment of liver disease. J. Gastroenterol. 2009; 44:167–172. [PubMed: 19214659]
50. Izamis ML, et al. Better than fresh: simple *ex vivo* perfusion optimizes ischemic and fresh donor livers for transplantation and significantly enhanced hepatocyte yields. Hepatology. 2011; 54:688A–688A.
51. Oertel M, et al. Purification of fetal liver stem/progenitor cells containing all the repopulation potential for normal adult rat liver. Gastroenterology. 2008; 134:823–832. [PubMed: 18262526]
52. Galili U. Induced anti-non gal antibodies in human xenograft recipients. Transplantation. 2012; 93:11–16. [PubMed: 22146315]
53. Daly KA, et al. Effect of the alphaGal epitope on the response to small intestinal submucosa extracellular matrix in a nonhuman primate model. Tissue Eng. Part A. 2009; 15:3877–3888. [PubMed: 19563260]
54. Xu H, et al. A porcine-derived acellular dermal scaffold that supports soft tissue regeneration: removal of terminal galactose-alpha-(1,3)-galactose and retention of matrix structure. Tissue Eng. Part A. 2009; 15:1807–1819. [PubMed: 19196142]
55. Abt PL, Fisher CA, Singhal AK. Donation after cardiac death in the US: history and use. J. Am. Coll. Surg. 2006; 203:208–225. [PubMed: 16864034]
56. Tolboom H, et al. Recovery of warm ischemic rat liver grafts by normothermic extracorporeal perfusion. Transplantation. 2009; 87:170–177. [PubMed: 19155970]
57. Coller BS, Beer JH, Scudder LE, Steinberg MH. Collagen-platelet interactions: evidence for a direct interaction of collagen with platelet GPIa/IIa and an indirect interaction with platelet GPIIb/IIIa mediated by adhesive proteins. Blood. 1989; 74:182–192. [PubMed: 2546619]
58. Bao J, et al. Construction of a portal implantable functional tissue-engineered liver using perfusion-decellularized matrix and hepatocytes in rats. Cell Transplant. 2011; 20:753–766. [PubMed: 21054928]

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59. Delrivière L, et al. Detailed modified technique for safer harvesting and preparation of liver graft in the rat. Microsurgery. 1996; 17:690–696. [PubMed: 9588714]
60. Delrivière L, et al. Technical details for safer venous and biliary anastomoses for liver transplantation in the rat. Microsurgery. 1998; 18:12–18. [PubMed: 9635788]
61. Nahmias Y, Berthiaume F, Yarmush ML. Integration of technologies for hepatic tissue engineering. Adv. Biochem. Eng. Biotechnol. 2007; 103:309–329. [PubMed: 17195468]
62. Strauss KA, et al. Management of hyperbilirubinemia and prevention of kernicterus in 20 patients with Crigler-Najjar disease. Eur. J. Pediatr. 2006; 165:306–319. [PubMed: 16435131]
63. van der Veere CN, et al. Current therapy for Crigler-Najjar syndrome type 1: report of a world registry. Hepatology. 1996; 24:311–315. [PubMed: 8690398]
64. Bernal W, Auzinger G, Dhawan A, Wendon J. Acute liver failure. Lancet. 2010; 376:190–201. [PubMed: 20638564]
65. Bower WA, Johns M, Margolis HS, Williams IT, Bell BP. Population-based surveillance for acute liver failure. Am. J. Gastroenterol. 2007; 102:2459–2463. [PubMed: 17608778]

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Box 1 | Autologous and allogeneic cells

Autologous

- Derived from self
- Low risk for exposure to transmissible agents
- Low risk of rejection, reducing the need for immunosuppressants
- Difficult to harvest—numbers are often insufficient for graft creation

Allogeneic

- Obtained from another individual of the same species
- Increased risk of transmissable agent exposure
- Likely to induce immune reaction and immunosuppression will probably be required
- Can be harvested in large numbers from healthy individuals
- Can be preprepared, possibly as an `off-the-shelf' treatment

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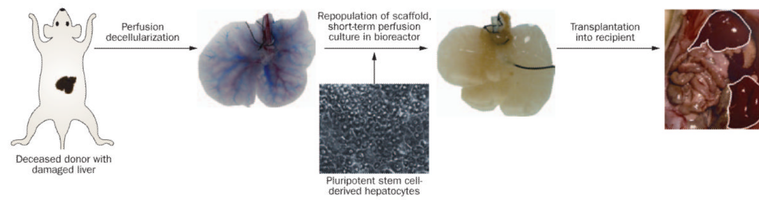

Figure 1.

Principles of whole-liver engineering. A liver is removed from a deceased donor but damage could render the organ unsuitable for transplantation. The organ can be decellularized with a detergent and the process retains the extracellular matrix, which is a scaffold that can be repopulated with hepatocytes, ideally derived from induced pluripotent stem cells. The repopulated graft can then be transplanted into a recipient. Permission obtained from Nature Publishing Group Ltd © Uygun, B. E. *et al. Nat. Med.* 16, 814–820 (2010).