World Journal of
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EDITORS FOR THIS ISSUE

Bioengineered functional humanized livers: An emerging supportive modality to bridge the gap of organ transplantation for management of end-stage liver diseases

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Abstract

End stage liver diseases (ESLD) represent a major, neglected global public health crisis which requires an urgent action towards finding a proper cure. Orthotropic liver transplantation has been the only definitive treatment modality for ESLD. However, shortage of donor organs, timely unavailability, post-surgery related complications and financial burden on the patients limits the number of patients receiving the transplants. Since last two decades cell-based therapies have revolutionized the field of organ/tissue regeneration. However providing an alternative organ source to address the donor liver shortage still poses potential challenges. The developments made in this direction provide useful futuristic approaches, which could be translated into pre-clinical and clinical settings targeting appropriate app-

lications in specific disease conditions. Earlier studies have demonstrated the applicability of this particular approach to generate functional organ in rodent system by connecting them with portal and hepatic circulatory networks. However, such strategy requires very high level of surgical expertise and also poses the technical and financial questions towards its future applicability. Hence, alternative sites for generating secondary organs are being tested in several types of disease conditions. Among different sites, omentum has been proved to be more appropriate site for implanting several kinds of functional tissue constructs without eliciting much immunological response. Hence, omentum may be considered as better site for transplanting humanized bioengineered *ex vivo* generated livers, thereby creating a secondary organ at intra-omental site. However, the expertise for generating such bioengineered organs are limited and only very few centres are involved for investigating the potential use of such implants in clinical practice due to gap between the clinical transplant surgeons and basic scientists working on the concept evolution. Herein we discuss the recent advances and challenges to create functional secondary organs through intra-omental transplantation of *ex vivo* generated bioengineered humanized livers and their further application in the management of ESLD as a supportive bridge for organ transplantation.

Key words: Bioengineered liver; Omentum; Secondary organ; Transplantation; End stage liver diseases

Core tip: The concept of bioengineering functional humanized neo-organs relies on finding more appropriate immunologically tolerable transplantation site. We have experienced omentum as more appropriate ectopic site with excellent properties of angiogenesis, regeneration, fibrotic reconstruction, and immunological compatibility which together endorse vascularisation, promote tissue healing, and minimize rejection of foreign body. However, regeneration of liver tissue in omentum is still unknown. Despite the amazing breakthroughs in the bioengineered organs, there is much work left to do. The approach described herein harbours enormous potential to overcome the limitations of organ transplantation and may support failing liver through ectopic transplantation as secondary organ.

INTRODUCTION

End stage liver diseases (ESLD) have become the major reason for the increasing deaths worldwide. According to the World Health Organisation, the total deaths caused by cirrhosis and liver cancer have increased by 50 million/year since 1990[1]. Liver transplantation is the only standard treatment available so far. However, more than 20% patients die on the waiting list due to a shortage of organ donors[2]. In order to expand the supply of livers available for transplantation, transplant surgeons and physicians have explored several new approaches including split liver transplants, living-related partial donor procedures[3] and the increasing use of "marginal" organs such as older donors, steatotic livers, non-heart-beating donors, donors with viral hepatitis, and donors with non-metastatic malignancy[4]. Despite these medical and surgical developments, it is unlikely that the availability of good liver grafts will ever be sufficient to meet the increasing demand of patients with end stage liver disease.

In order to overcome these limitations, various other treatment options are being explored among which hepatocytes transplantation has been described as the first supportive modality in regenerative medicine. But major challenges with such treatment is its limited availability of therapeutic dose from surgical samples, liver grafts or biopsies and their maintenance *in vitro* which requires cell-to-cell and cell-to-matrix interactions for proper functioning of anchorage dependent hepatocytes[5]. Usage of hepatocytes from xenogenic sources such as rabbit, porcine or canine, pose the risk of immunogenicity and transmission of zoonosis. This limitation can be addressed to certain extent by the usage of cell lines which can be maintained for longer time with higher growth rates under *in vitro* culture conditions but modification of gene expression under culture conditions might lead to problems and has issues related to its clinical applicability[6].

The first land mark study to bring hepatocyte transplantation into clinics was by Mito *et al*[7] in cirrhotic patients. In line with this study, our centre has treated seven acute liver failure patients by intra-peritoneal transplantation of human primary hepatocytes extracted from human foetus's which showed clinical improvement and support to the failing liver[8]. Following this, various other studies have reported successful transplantation of primary hepatocytes in treating various metabolic diseases[9,10]. Although higher successful rate has been reported using hepatocyte transplantation, yet use of fetal hepatocytes poses major hurdle of ethical issues for its wider clinical applicability. Other potential treatment alternatives discovered in recent years included induced pluripotent stem cells, Mesenchymal stromal cells (MSC) which have the ability to differentiate into hepatocytes but still they couldn't completely mimic the fully functional hepatocytes pointing towards a need to identify better niche for functional utilization of these cells[11-14].

Other alternative of direct cellular transplantation includes the use of extracorporeal liver support devices which can support a failing liver for a short period of time before organ transplantation[15]. But all these above mentioned treatment strategies may not fulfil the requirements to treat ESLD and may not provide immediate support for a failing liver to maintain normal functions. Hence, there is a need to develop bioengineered transplantable liver grafts which can retain the natural three-dimensional extra cellular matrix (3D-ECM) components and intact vascular networks similar to the native liver with repopulated functional hepatocytes or human hepatic progenitor cells. Rapid progress in the area of stem cell research and organ bioengineering paved a way in generating alternatives to liver transplantation.

After addressing all these limitations next comes the question of choosing an exact transplantable site where in these bioengineered organs can be easily acceptable and can able to perform the function. Recently omentum has been discovered as a wonderful ectopic site for transplantation with excellent properties like remarkable angiogenic[16], stem cell[17,18], fibrotic[19], and immune activities[20], which together endorse vascularization, promote wound healing, and minimize infection. Several studies have already demonstrated the importance of intra-omental transplantation in diabetic animal models[21,22]. However, the regeneration of liver tissue in ectopic sites is still unknown. Few studies have shown the omentum as a reservoir for proliferating renal, pancreatic, splenic[23-25] cells and as a site for hepatocytes engraftment which can be used in tissue engineering[26]. Hence, opting omental transplantation of bioengineered liver may offer development of secondary liver for the treatment of ESLD. This particular approach should offer promising treatment strategy in future and may rule out above mentioned limitations to answer for shortage of organ donors for ESLD.

CURRENT STATE OF REGENERATIVE STRATEGIES IN ESLD

Since last two decades, significant developments have been made to overcome the limitations of liver transplantation in ESLD. Among these strategies cell transplantation, use of extra-corporeal devices and transplantable bioengineered organs have been explored extensively.

CELL TRANSPLANTATION

In cell transplantation strategies, hepatocytes transplantation has been the most preferred cell types for infusion into liver due to their ability to perform major liver specific functions. However, getting therapeutic dose of human hepatocytes represents major limitation towards its wider clinical application[27,28]. Although several studies have reported use of 10% liver tissues

to isolate enough number of hepatocytes post-*in vitro* expansion which can provide required clinical response in both animal models and human[5,29]. The *in vitro* enrichment of hepatocytes is challenging due to their contact-dependent growth, long-term survival and function and maintenance of normal phenotype without de-differentiation[5,30]. Therefore alternative strategies are highly desirable to overcome these limitations.

Recent studies have reported use of embryonic and adult pluripotent stem cells to generate desired number of functional hepatocytes for therapeutic applications. However, use of embryonic stem cells (ESCs) represents ethical hurdles and immune incompatibility for the transplant recipients[31-33]. Moreover, use of induced pluripotent stem cells (iPSCs) has been reported for effective differentiation into functional hepatocytes, however poses potential issues related to genetic instability and lack of functional transplantation studies[11,12]. Mesenchymal stromal cells (MSCs) represents another alternative type of pluripotent cells to generate functional hepatocytes and support liver regeneration[13,14]. However, multi-lineage differentiation of MSCs represents major challenge to control the effective trans-differentiation into desired number of functional hepatocytes while restricting other default lineage cells. Although, stem cell transplantation strategies have showed potential in liver regeneration through various mechanisms, still it has not been considered as durable solution to completely support the lost liver functions[15]. Hence, alternative strategies are highly desirable to generate therapeutic number of functional heaptocytes under controlled conditions.

MAJOR SOURCES OF REGENERATIVE CELLS FOR THE TREATMENT OF ESLD

Liver-derived stem sells

These are the stem cells that are derived from adult or fetal livers. Adult stem cells are known as oval cells which play an important role in liver regeneration when replication capacity of hepatocytes is impaired[34]. Fetal liver stem cells are known as bipotent hepatoblasts that has ability to differentiate into bile duct cells or hepatocytes[35-37]. Fetal liver stem cells have been used to repopulate liver in animal models[38,39] and cultured hepatoblasts transplanted into immunodeficient mice showed greater *in vivo* engraftment and differentiation[40]. But the limitation in use of liver derived stem cells is their low number around 0.3% to 0.7% of oval cells in adult liver[41], whereas fetal liver mass comprises only 0.1% of hepatoblasts[42] and has associated ethical issues. Thus isolation and expansion of these cells and usage for transplantation is challenging.

Bone marrow-derived stem cells

Stem cells derived from bone marrow comprise hematopoietic and MSCs[43]. Among these, mesenchymal stem cells consists greater potential in liver

regeneration[44] with immunosuppressive and immunomodulatory properties[45]. But they always pose problem with low rates of liver repopulation[46] and have low trans-differentiation ability to hepatoblasts which limits to restore normal liver function[47].

Annex group of stem cells

Stem cells derived from human umbilical cord, human placental tissue, amniotic fluid and human umbilical cord blood constitutes Annex group of stem cells. These are pluripotent stem cells with higher proliferation and differentiation rates than adult stem cells[46,48,49] and are not known to cause teratomas or teratocarcinomas formation in humans. Di Campli *et al*[50] study on diabetic severe combined immunodeficient mice after acute toxic liver injury when treated with intraperitoneal administration of human umbilical cord stem cells showed rapid liver engraftment, differentiation into hepatocytes, improved liver regeneration, and reduced mortality rates[50].

IPSCs

These are similar to ESCs and the limitation of ethical issue can be overruled by *in vitro* generation of iPSCs from somatic cells avoiding the usage of embryonic tissue or oocytes[51]. However, the use of these cells in clinical practice is limited due to major hurdles with the genomic instability of these cells.

EXTRA-CORPOREAL LIVER SUPPORT SYSTEMS

Extracorporeal liver support devices have been designed with a goal to carry out normal liver function in patients with end-stage chronic, acute-on-chronic and acute liver failure for a short period of time until donor organ gets available. Two types of liver support systems have been designed: (1) Non-biological; and (2) Bio-artificial liver support devices.

Non-biological liver support devices

Designed to filter and adsorb accumulated toxins that are not cleared by non-functional liver[52]. Three major types of such devices have been explored as follows:

Molecular adsorbent recirculating system: Molecular adsorbent recirculating system (MARS) has been well explored device which is a hollow fiber membrane hemodialyzer which removes soluble and protein-bound substances against albumin-rich dialysate. This device was approved by FDA in 2012 for the treatment of hepatic encephalopathy. However, the major limitation of such devices represents: (1) Short-term detoxification function; (2) Chance of getting sepsis; (3) Cost issues; (4) Can remove only albumin-bound toxins or drugs which are excreted in circulation; (5) Safety and efficacy of MARS has not been demonstrated in controlled, randomized trials; and (6) The effectiveness

of MARS in patients that are sedative could not be established in clinical studies and therefore can't be predicted in sedated patients.

Promethus fractionated plasma separator and adsorption system: Other type of devices includes, promethus fractionated plasma separator and adsorption system (FPSA) which is an artificial device which removes both albumin-bound and water soluble toxins from blood more effectively than MARS. However, its wide applicability has been limited due to following reasons: (1) Direct contact between fractionated plasma and the Prometh anion exchanger causes significant adsorption of procoagulant and anti-coagulant factors, associated with clinically relevant adverse events; (2) Broad disturbances of the coagulation system have been confirmed in FPSA treated liver failure patients; and (3) An *ex vivo* recirculation model demonstrated nonspecific adsorption of coagulation factors protein S and protein C on the anion exchange cartridge.

Single-pass albumin dialysis: Moreover, to overcome on the limitations of above mentioned extracorporeal liver assist device, single-pass albumin dialysis (SPAD) system was evolved which functions as one-pass dialysis against albumin solution to remove albumin-bound toxins and water-soluble substances. Detoxification system in SPAD is similar to or greater than MARS and is less expensive than MARS and FPSA. However, again the suitability and wide clinical applicability of SPAD is limited due to following limitations: (1) Only albumin bound or water soluble toxins can be removed; (2) Lipid soluble toxins can't be removed by SPAD; (3) Bleeding risk from acquired coagulopathy; (4) Albumin solution is discarded after a single passage of membrane without being recycled; and (5) Absence of clinical data.

Bioartificial liver support systems

These are the bioreactors containing viable hepatocytes in a 3D network of hollow fibers. These are designed to achieve plasma perfusion and enhance the activities of living liver cells. Conversely, the membranes separating cells from plasma are not capable of achieving enough *in vivo* perfusion rates, and lack sources of safe, reliable, strongly proliferating and functionally active human cells. Still following major challenges remain to resolve: (1) Bio-artificial livers should be able to provide at least 10% of liver functioning; (2) Very difficult acquiring this many hepatocyte cells; (3) Controversy over the use of porcine cells due to possible transmission of infections; (4) Hepatocytes and plasma have very different physio-chemical properties; (5) Hepatocytes do not perform well when in contact with plasma; (6) Have a very high oxygen uptake rate; (7) Hepatocytes undergo a lot of stress inside of bio-artificial liver; (8) Any stress above 5 dyn/cm^2 renders cells useless; (9) Limited volume of the bioreactor;

(10) Maximum blood/plasma that can be safely drawn out of liver failure patient is one liter; (11) Difficult to achieve 10% of liver functioning within one liter; and (12) Makes Bio-artificial liver designing very difficult.

TRANSPLANTABLE BIOENGINEERED ORGANS

Owing to the hurdles in above mentioned devices, there is need to develop transplantable biological systems to provide: (1) Suitable three-dimensional organ architecture; (2) Organ specific intact vasculature for homogeneous supply of oxygen and nutrients; (3) Long-term cell survival and function within the natural organ specific niche; and (4) Metabolic, synthetic and detoxification functions similar to native liver.

MAJOR COMPONENTS OF HUMAN LIVER FOR BIOENGINEERING

Major components of human liver for bioengineering includes (1) Organ specific 3D-bioscaffolds; (2) Organ capsule; (3) Organ vasculature; (4) Cellular distribution in spatial anatomical organization of liver; (5) Biomolecules and growth factors for enhanced survival and function to transplanted cells; (6) Types of cells required for long-term support; and (7) Long-term functional response.

To provide these crucial components recently two major technological advancements have been made: (1) Organ bio-printing; and (2) Humanized neo-organ development.

Organ bio-printing

With the advancements in tissue engineering it is possible to construct complex parenchymal organ structures along with intact vascular network by 3D bio-printing[53]. 3D bio-printing is one of the prevalent examples of bioengineered organs in the science world today, and it is growing and advancing quickly. This jaw dropping technology is one of the hot topics in bioengineering. It still fascinates that we have the potential to build organs from the push of a button. 3D bio-printing is a form of tissue engineering which utilizes inkjet printers and builds the scaffolding of a particular organ, layer by layer[54]. These inkjet printers allow the use of multiple cell types for printing. Robbins *et al*[55] developed a metabolically active 3D hepatic tissue where they identified increased liver specific function lasting for up to 135 h, and compartment-specific organization, along with a primitive hepatocyte microanatomy of hepatic stellate cells and endothelial cells. Researchers have also build bone repair constructs by coating the 3D printed scaffold with stem cells, which can grow into tissues over time[54]. The mild conditions used for bio-printing and material sintering have allowed viable cells and active therapeutic proteins to be incorporated into the construct production process. Today, this particular technology has been emerged only for *ex vivo* and its application *in vivo* has not been experienced which needs to be validated further.

Humanized neo-organ development

The recent concept of bioengineering functional humanized neo-organs has given a hope towards finding permanent cure as an alternative support to the failing organ. This concept of artificial organs was first originated in the radiation field post-World War Ⅱ, and was executed in the first bone marrow transplant in the 1970s[56]. According the Llames S tissue engineering has three main constituents: The *ex vivo* expansion of cells, seeding of these expanded cells in three dimensional structures that mimic physiological conditions and grafting the prototype. The technology relies on the development of whole intact organ scaffolds through whole organ perfusion acellularization procedure which retains extra-cellular matrix and circulatory networks of the native organ post-acellularization[57]. This important phenomenon allows three-dimensional intact acellular organ specific scaffold for efficient repopulation of desired cell population further to generate functional neo-organ system.

With advancement in regenerative medicine it has been possible to create bioengineered functional tissues or organs that can be used clinically[58,59]. So far several successful studies have been published in generating various organs and tissues based on these acellularization and stem cell repopulation[59-61] that can be used for treating patients. Significant progress in generating several types of complex organ biological scaffolds has led to development of an efficient acellularization protocols for whole organs through perfusion based techniques[62-66] (Tables 1 and 2). These acellularized whole organs combined with an efficient recellularization process[67-70] have made it possible to use these bioengineered organs for *in vivo* preclinical studies in small animal models[71-73].

Our centre has well expertise in generating various types of acellularized whole organ bioscaffolds including xenogeneic liver through detergent-based perfusion. So far, we have successfully generated acellularized and repopulated humanized whole liver and demonstrated its applicability as better natural 3D-drug testing model system[74]. Apart from liver, we have also generated acellularized kidney[75], heart[76], spleen, meninges, and many more. Still various other studies are in pipeline in generating humanized bioengineered organs from our centre.

WHOLE LIVER BIOENGINEERING

Highly specialized thick and complex organs like liver can be subjected to acellularization technology to obtain intact 3D-ECM. Due to delayed co-morbidity beyond marginal criteria or because of delayed ischemic time,

Table 1 Method adopted for whole complex organ acellularization techniques for different organisms

Organ	Acellularization agent	Perfusion method	Animal model	Reference
Heart	SDS, PEG, Triton X-100, and enzyme-based protocols deoxycholic acid	Antegrade coronary perfusion	Rat	[98]
	Trypsin, EDTA, NaN3, Triton X-100, and deoxycholic acid	Retrograde aortic perfusion	Pig	[65]
Lung	0.1% and 0.5% SDS	Antegrade pulmonary arterial perfusion	Rat	[63]
	CHAPS	Pulmonary artery and tracheal perfusion	Rat	[66]
	Triton X-100 and sodium deoxycholate	Right ventricle and tracheal perfusion	Mouse	[99]
Liver	Triton X-100 plus 0.1% SDS	Portal vein perfusion	Rat	[100]
	SDS		Rat	[70]
	1% Triton X-100 and 0.1% ammonium hydroxide		Mouse, rat, ferret, rabbit and pig	[69]
	0.25% and 0.5% SDS		Pig	[101]
	Sodium citrate + SDS + Triton-X-100	Hepatic artery perfusion	Rat	[74]
Kidney	0.5, 3, 6, 10% Triton X-100, 5 mM	Renal artery perfusion	Rat	[71]
	calcium chloride, 5 mM magnesium sulfate, 1 M sodium chloride, DNase, and 4% sodium deoxycholate		Rat	[72]
	3% Triton X-100, DNase, and 4% SDS			
	1% SDS and 1% Triton X-100			
	1% Triton X-100 and 0.1% ammonium hydroxide		Pig	[68]
	Heparin and antibiotic-containing physiological saline, 0.1-1.0% SDS, 0.1% Triton-X-100 and 0.0025% deoxyribonuclease 1		Goat	[75]

Table 2 Study outcome and major limitations of different types of acellularization techniques adopted for different types of whole organ scaffold development

Organ	Acellularization Method	Study out come	Limitation	References
Rat liver	Perfusion with detergents (SDS, Triton X-100)	Perfusion with SDS removes most of cells, damages the ECM when treated with Triton X-100 and removes 97 % of DNA	SDS damages the ECM	[69,74]
Porcine liver	Mechanical perfusion (electroporation)	Most of the cells are removed, preserves the blood vessels	Disruption of microfilament and microtubule	[102]
Mouse heart	Enzymatic, detergents, Acids	Cells are removed	Damages the ECM proteins, poorly maintains the 3D architecture	[103]
Porcine trachea	Enzymatic (trypsin) non-enzymatic (EDTA), detergent (Triton X-100) and deionized Water	Cells are removed, clear the cell debris	Disruption of glycosaminoglycan, reduce the laminin and fibronectin	[104]
Rat kidney	Perfuse with SDS, deionized water, dTriton X-100 and PBS along with antibiotics	Twice filtration is observed	Loss of cell-mediated functions like transport of solutes	[105]
Rat heart	Perfused with detergents		Long-term cell survival, oxygen tension and continuous rhythmic beating	[63,98]
Goat kidney	Perfused with Trypsin-EDTA in PBS, perfuse antibiotics and then with SDS in PBS	Cells are removed, pore to pore interconnection in the scaffold		[75,106]

ECM: Extra cellular matrix.

in United Kingdom livers offered for transplantation are usually discarded[77]. This act offers a way to use this kind of livers for acellularization. The liver is the largest gland in the body and carries out numerous essential functions such as metabolism, maintaining homeostasis, and the synthesis of amino acids[57]. Therefore, acellularization is extremely beneficial to the liver because it not only maintains the microstructure but also its bio signals such as extracellular matrix proteins and adhesion peptides[57].

Since extracellular matrices are similar from species to species, whole organ scaffolds have become possible for livers. Several recent studies have been reported for efficient acellularization of livers obtained from various xenogeneic sources[78-81] and the resulting 3D-ECM structure has become an outstanding source for generating highly functionalized liver cells *in vitro*[82,83]. As these extracellular matrices are conserved between species, the process of recellularization with human cells into an animal scaffold is easier[57] and this kind of approach does not elicit any kind of immune rejection, cross contamination and zoonosis. In our recent study, we have demonstrated development of humanized whole liver using human hepatic progenitor cells repopulation through hepatic artery infusion into acellularized liver scaffolds[74]. These humanized livers perform detoxification and metabolic functions similar to the native liver. However, the complete recellularization of a fully function human liver has not yet been accomplished[57]. Recent advances in isolating and culturing both native cells and stem cells, as well as the development of acellularized organ scaffolds and biocompatible synthetic biomaterials, suggest that we are making rapid progress towards providing new alternatives to donor livers for transplantation[56].

CHALLENGES NEED TO BE ADDRESSED IN GENERATING COMPLETE BIOENGINEERED FUNCTIONAL LIVER

Despite the amazing breakthroughs in the bioengineered organs, there is much work left to do. Simply reconstructing the whole organ will not be sufficient to replace organ transplantation. The approaches described above are fairly new and are still in the developmental stages. There has been only handful of successful transplantation of bioengineered organs into actual humans. Scientists are still working on ways to engineer more complex organs such as the liver. There are also long-term issues to resolve, such as the preservation of the overall function of these bioengineered organs. However, little is known about the mechanisms by which these grafts may integrate and maintain function. When more complex organs are involved, the scenario is completely different, as investigations are still in very early stages and clinical translation is not foreseeable on the basis of current knowledge and available data[84]. The following major

critical issues are yet to be resolved to make these approaches a clinical reality: (1) Liver is a complex organ with various cell types, hence rebuilding liver micro architectures with these cells is yet to be addressed; (2) The optimal cell source that can meet the criteria for recellularization of acellularized liver scaffolds still remains unclear; (3) The first and foremost challenge is the need to address the reconstruction of complete and functional uniform endothelial cell layer throughout acellularized liver scaffolds; (4) It is necessary to reconstruct biliary system which is needed for bile acid excretion to develop a fully functional bioengineered liver; (5) Assessing the functionality of these bioengineered livers after *in vivo* transplantation for long term needs to be studied clearly; (6) For organ functionality, maintaining its vascular structure is much more important. As hepatocytes require higher amounts of oxygen for their functionality, it is necessary to maintain hierarchical vascular network structure in acellularized liver scaffolds[85]. Critical step in engineering a transplantable liver is the creation of a functional vasculature capable of long-term perfusion following anastomosis. Without an appropriate endothelial lining of the vessels, continuous blood perfusion of the graft in the absence of anticoagulation quickly results in thrombosis; and (7) Finding an appropriate site for providing enough support to the failing liver has been one of the most challenging issue to use the bioengineered organs as secondary liver.

OMENTUM AS BETTER ECTOPIC SITE FOR TRANSPLANTATION TO GENERATE SECONDARY ORGAN *IN VIVO*

The major question for applying these humanized bioengineered livers relies on finding an exact and more appropriate transplantable site where in these bioengineered organs can be easily acceptable and are able to perform the function. Recently omentum has been discovered as a potential ectopic site for transplantation with excellent properties like remarkable angiogenic[16], stem cell[17,18], fibrotic[19], and immune[20] activities, which together endorse vascularization, promote wound healing, and minimize infection (Table 3). Several studies have already demonstrated the importance of intra-omental transplantation in diabetic animal models[22,86].

The omentum is a visceral adipose tissue derived from mesothelial cells[87] connected to the spleen, stomach, pancreas, and colon[88,89]. Although well known as a visceral fat depot, the role of the omentum in peritoneal immunity was not recognized until the early 1900s, when a British surgeon referred to it as 'the police man of the abdomen' due to its ability to attenuate peritonitis and promote surgical wound healing[90]. In fact, omentum was noted to move about the peritoneal cavity and occlude sites of inflammation, such as ruptured ovaries, inflamed

Table 3 List of recent studies reporting use of omentum as transplantation site to support the lost organ function from ectopic transplantation of engineered tissues or grafts

Animal model	Site of transplantation	Mode of graft used	Results	Reference
Femoral bone of New Zealand rabbit was	Greater omentum on the left side	Free transplant of the greater omentum	Process of the callus formation and its mineralisation are much quicker and thicker on the defect that was covered with the free transplant of the greater omentum.	[107]
Pancreatectomized dogs	Spleen or Omentum	Islet auto-transplantation	Beta cell response to mild non-insulin induced hypoglycemia was normal, whereas the alpha cell response was not.	[108]
Murine carotid artery injury model	Omentum was applied to the injured vessel	Omentum + Omental progenitor cells	Omentum can directly contribute reparative progenitor cells to injured tissues upon treatment with Tβ4.	[109]
Nondiabetic nude rats	Omentum/kidney capsule	Perinatal porcine islet cell grafts	In both sites, the A-cell volume increased fourfold between weeks 1 and 10 reflecting a rise in A-cell number. In the omental implants, however, the cellular insulin reserves and the percent of proliferating cells were twofold higher than in kidney implants. In parallel, the blood vessel density in omental implants increased twofold, reaching a density comparable with islets in adult pig pancreas.	[110]
Diabetic rat and nonhuman primate (NHP) models	Intra-omental	*In situ*-generated adherent, resorbable plasma thrombin biologic scaffold	Improved metabolic function and preservation of islet cytoarchitecture, with reconstitution of rich intrainsular vascular networks in both species.	[21]
Adult male Spraguee Dawley rats	Omental transposition	Hepatic tissue sutured into the omentum mobilization of the omentum and transposition onto the left hepatic lobe	Omental transposition provided adequate microcirculation for proliferation of ectopic hepatic cells after liver resection.	[111]
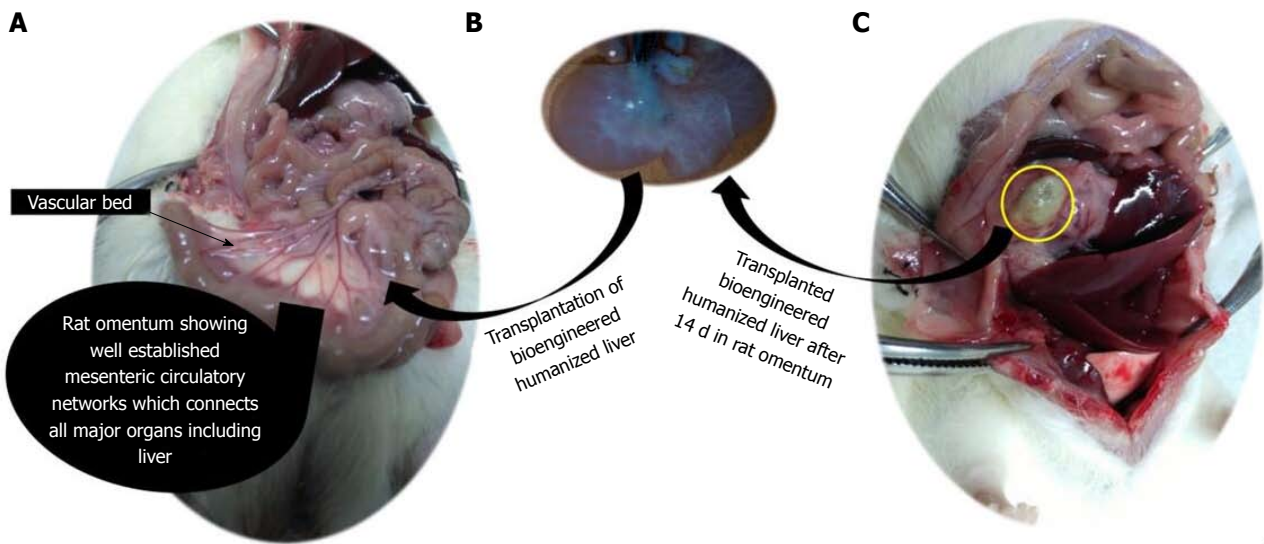
Figure 1 Intra-omental transplantation of bioengineered humanized livers showing development of secondary liver after 14 d. A: Anatomy of rat omentum showing well-established web of circulatory networks which connected with major organs; B: Developed bioengineered humanized liver in our lab *ex vivo*; C: Intra-omentally transplanted bioengineered humanized liver showing well engraftment with the surrounding tissue.

appendices, ulcerated intestines, or wounds due to trauma or surgery[90]. Consistent with this observation, the omentum has remarkable angiogenic[16], fibrotic[19], regenerative[17,18] and immune[20] activities, which together promote vascularization, accelerate wound healing, and limit infection. However, these same activities are also likely involved in pathological responses, such as the rapid growth of omental tumour metastases[91].

Once thought of as just a large amount of redundant fat overlying the intestines, surgeons' attitudes towards the omentum have changed. It is recognized as an organ in its own right, with many diverse functions ranging from its ability to attenuate the
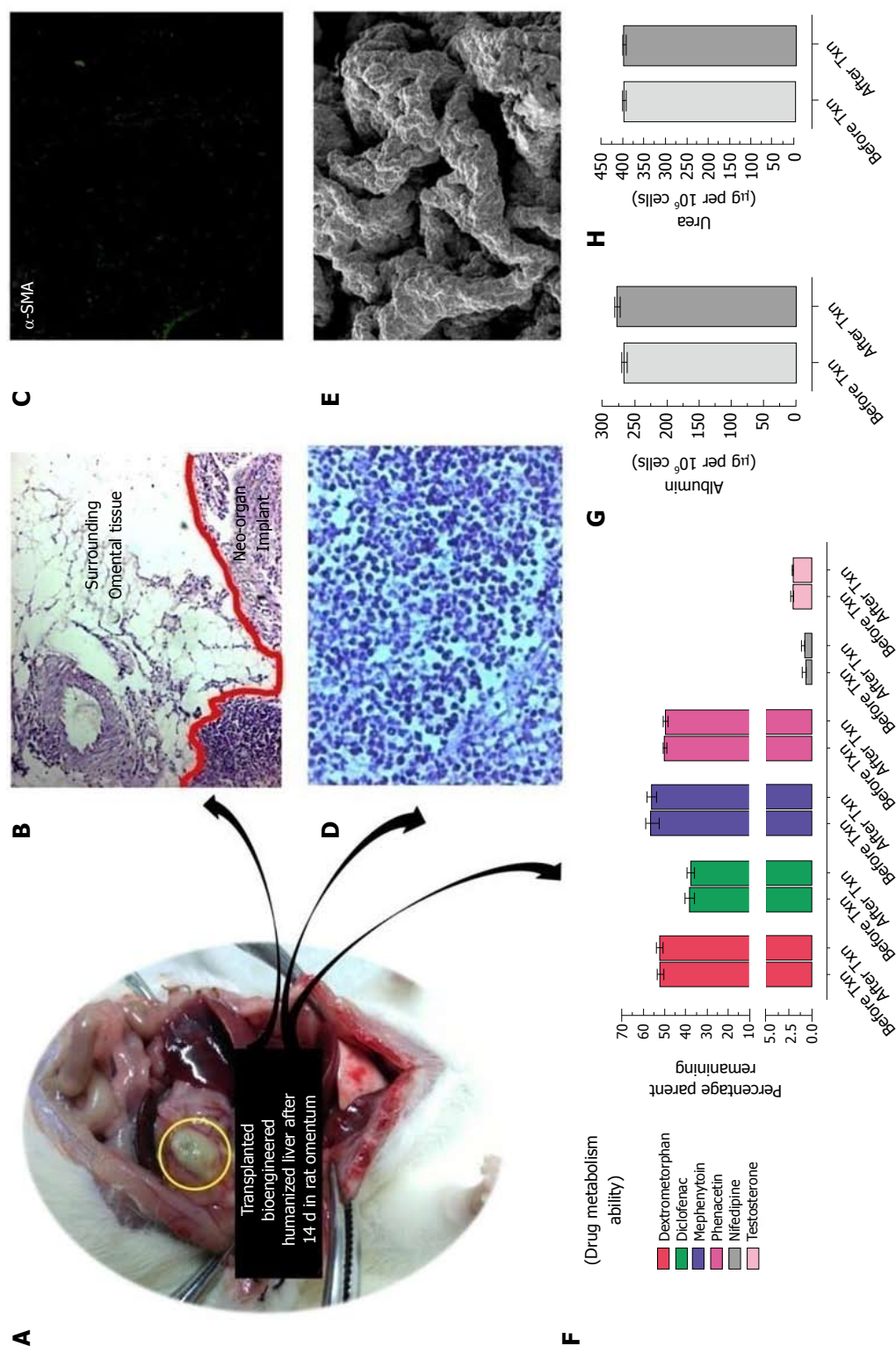
Figure 2 Intra-omental transplantation of bioengineered humanized livers showing no sign of fibrosis or immunological response at transplantation site. A: Optical image of transplanted implant at intra-omental site; B: Hematoxyeleine and eosin (HE) staining of the transplanted implant along with surrounding tissues showing no sign of immunological cells infiltration or tissue damage. Moreover, neo-vascularization was seen into nearby surrounding tissues which connects with the implant; C: Immunocytochemical staining using α-SMA showed no sign of fibrotic reactions to implant; D: HE staining showed well organized distribution and proliferation of hepatic cells into the implant post-transplantation; E: Scanning electron microscopy (SEM) image of retrieved graft at day 15 post-transplantation showing almost similar anatomy of bioengineered livers with natural liver; F: Retrieved livers at day 15 post intra-omental transplantation showed almost similar metabolic activity to before transplantation (*P* > 0.05). The other two important liver cell functions such as G: Albumin synthesis and H: Ammonia detoxification (*i.e.* urea production) is almost similar to the bioengineered humanized livers prior to transplantation (*P* > 0.05).
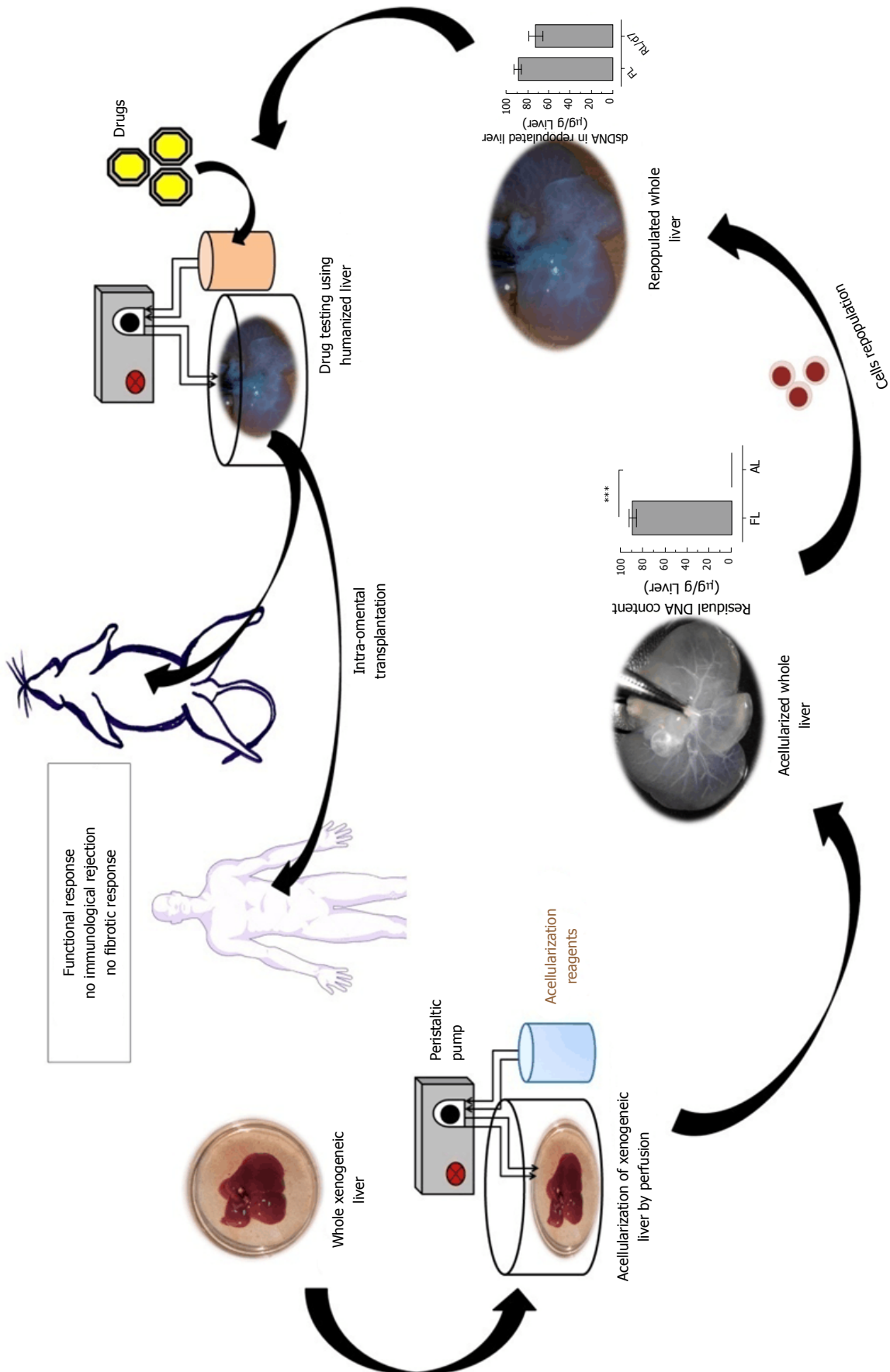
Figure 3 Brief overview of strategy for the development of immune-competent bioengineered humanized liver using acellularization and repopulation technology for future biomedical applications.

spread of sepsis in peritonitis to acting as a source of angiogenic and hemostatic factors involved in tissue healing and repair. The omentum has been identified as a source of adult stem cells which may have future prospects in the fields of tissue engineering and the synthesis of vascular grafts. Its regenerative properties have been exploited in virtually every field of surgery from the reconstruction of complex wounds to the protection of gastrointestinal anastomosis.

The regenerative properties of the omentum have been exploited by surgeons for over a century, ranging from the protection of anastomosis in gastrointestinal surgery, revascularization of arterial ulcers, to the reconstruction of head and neck deformities[92]. The advantage of the omentum is that it is an accessible and versatile source of growth factors, angiogenic factors, and leukocytes. It can be lengthened considerably by careful dissection to produce a mobile organ[93].

The regeneration of liver tissue in ectopic sites is still unknown. It has been discovered that the omnetum is a reservoir for proliferating renal, pancreatic, splenic tissues[23-25] and as a site for hepatocytes engraftment which can be used in tissue engineering[26]. Hepatocyte transplantation has been done in various tissues like spleen, pancreas and omentum[26,72,94-96]. With advancements in tissue engineering hepatocytes seeded onto polymer scaffolds and have been transplanted into omentum wherein engraftment of hepatocytes occurred due to elevated rates of angiogenesis into cell-polymer constructs within the omentum[96].

Thus intra-omental transplantation of bioengineered livers may provide adequate microcirculation for proliferation of ectopic hepatic cells repopulated within the bio-artificial liver. It has been observed that portal vein ligation does not affect the ectopic liver regeneration[97]. In our preliminary experiences, we have observed that intra-omental transplantation of bioengineered liver lobes gets easily accommodated into the site without eliciting immunological responses while maintain their biological functions and communicates blood borne growth factors for survival and function of the graft (Figure 1). We also observed that these bioengineered liver grafts survive at omental site in long-term and functions as secondary liver (Figure 2). These findings are well supported by earlier studies wherein other types of grafts have been transplanted into the omentum[21]. Future efforts at understanding mechanisms to regulate ectopic liver regeneration may assist the pursuit for liver tissue/organ bioengineering to support the failing liver functions in long-term.

CONCLUSION

Engineers and researches have been making monumental breakthroughs in the area of bioengineered organs. These bio-artificial organs may redefine transplants for human applications in future with more critical advancements. The introduction of cells into the human body is designed to stimulate regeneration, promote vascularization and/or supplement the production of hormones and growth factors[56]. Consequently, bioengineered biological substitutes present a new way to restore damaged tissue and maintain their functions. Not only does this provide a new source of organs, but probably even more reliable organs at that. Not only would people not need an organ donation, but their body will more readily accept a bioengineered organ through intra-omental transplantation, most likely reducing recovery time as well (Figure 3). In near future these potential strategies can overcome the limitation of organ donors and these bioengineered organs can even serve as a best natural 3D-drug testing models[74] and investigating precise molecular mechanisms in biomimetic natural organ system[112] and could support failing liver through ectopic transplantation as secondary organ in ESLD.

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