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Cell Delivery: From Cell Transplantation to Organ Engineering

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Abstract

Cell populations derived from adult tissue and stem cells possess a great expectation for the treatment of several diseases. Great efforts have been made to generate cells with therapeutic impact from stem cells. However, it is clear that the development of systems to deliver such cells to induce efficient engraftment, growth and function is a real necessity. Biologic and artificial scaffolds have received significant attention for their potential therapeutic application when use to form tissues in vitro and facilitate engraftment in vivo. Ultimately more sophisticated methods for decellularization of organs have been successfully used in tissue engineering and regenerative medicine applications. These decellularized tissues and organs appear to provide bioactive molecules, and bioinductive properties to induce homing, differentiation and proliferation of cells. The combination of decellularized organs and stem cells may dramatically improve the survival, engraftment, and fate control of transplanted stem cells and their ultimate clinical utility opening the doors to a new era of organ engineering.

Keywords

Cell transplant; Organ Engineering; Decellularized matrices; natural scaffolds

INTRODUCTION

Since the development of techniques for the isolation of individual cells, cell therapies promise to provide cures to multiple diseases and disorders, normally via tissue repopulation, which is the focus of this review. Potential cell therapies have been most classically introduced to the body via an injection of cells, suspended in an appropriate medium, either into the systemic circulation or directly into the tissue of interest. Transplantation of isolated hepatocytes has been considered a potential therapy for the treatment of liver disorders. Over the last several decades, laboratories have progressively shown that primary hepatocytes can engraft in the liver, spleen, peritoneal cavity and other extra hepatic sites, and can function following transplantation to correct liver-based errors of metabolism and prolong the survival of animals with liver failure (7,10,17,49,62).

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Liver cell transplantation presents distinct advantages over orthotopic liver transplantation (OLT) for organ replacement therapy: cell transplantation is technically simpler than OLT, requiring only injection/infusion of a cell suspension (31); liver cells can be cryopreserved for future use (22); and cells obtained from one donor can be used for multiple patients (33). Yet, major obstacles to the broad clinical use of cell transplantation include the competition with OLT for the few suitable donor livers, long-term cell engraftment and function, effective and adequate immunosuppressant protocols and the fact that primary hepatocytes cannot be readily expanded in vitro (1,7,49). These studies have confirmed the ability of transplanted liver cells to participate in the repopulation of damaged or diseased livers and demonstrated proof of principle for this new therapeutic approach. The results of many studies, however, are also making clear that at least some of the transplanted cell populations regulate liver regeneration in certain advantageous circumstances via the loss of reproductive integrity in endogenous hepatocytes giving preferential proliferation of transplanted hepatocytes.

A wide variety of cell populations have been transplanted in this manner, including different populations of fetal liver cells including Dlk-1(+) cells or Thy-1(-) cells (42,43) purified from unfractionated embryonic day (ED) 14 fetal liver stem/progenitor cells (43), xenogeneic hepatocytes (35), embryonic stem cell-derived hepatocytes, and even conditionally immortalized cell lines (24). However, studies have uniformly demonstrated large-scale death of the transplanted cells, extremely poor engraftment (typically <10% cells engraft) (62), and loss of control over the fate of the transplanted cells after their introduction into the body. Together, these issues are likely responsible for the limited clinical success of this approach to date and the repeated finding that success is greatest in small tissue volumes (e.g., rodent models).

It may be possible to improve the survival and function of transplanted stem cell populations by borrowing concepts from the tissue-engineering field originally developed for the transplantation of differentiated cells (12,40,46,55,56,59,64). In particular, the tissue engineering field routinely makes use of material carriers, functioning as synthetic analogs of the extracellular matrix, to provide a substrate for transplanted cell adhesion and differentiation to control the localization of the cells in vivo, and to serve as a template for the formation of new tissue masses from the combination of transplanted cells and interfacing host cells (Figure 1). These technologies may prevent anoikis in the transplanted cells and also regulate their gene expression. Moreover, the material's ability to induce or contain vascularization may dramatically improve cell survival and function in the host environment. Nevertheless, although clinical success to date in tissue engineering approaches to cell transplantation has been limited to skin, tendons and lately in trachea engineering (3,30), there is enough proof of principle for the engineering of many other tissue/organ types including the liver (12) (Figure 1).

This review will provide an overview of two complementary approaches, cell transplantation and tissue engineering; both under active investigation, to enhance the success of organ repopulation strategies and to create new grafts for transplantation by manipulating the biology of cells in advance of their transplantation and the development of biological scaffolds that carriers newly cells to manipulate transplanted cells in vivo and arrange the host reaction (Figure 1).

CELL TRANSPLANTATION

Two general approaches have been taken for cell therapies. The first has been rapid cell isolation and reapplication of the cells with minimal manipulations (10,49). The second, more common approach has been cell isolation, transport to the lab, and extensive in vitro manipulation before reimplantation (17,18). Advantages of the former approach include

minimal cost and minimal oversight by regulatory agencies, whereas the latter approach is costly and requires manipulation of the cells under current Good Manufacturing Protocols (cGMP) in clean rooms with extensive documentation and close regulatory supervision (9).

Liver cell transplantation (LCT) involves the transfer of normal hepatocytes into diseased liver, by injecting isolated hepatocytes into the spleen or splenic artery or directly into the portal vein. A couple of decades ago studies demonstrated that administration of 10^4 liver cells could replace more than 80% of the liver, indicating that donor hepatocytes were capable of at least 12 rounds of cell division (50).

So far, human LCT has been attempted in patients with acute liver failure (57), in chronic liver disease with cirrhosis (32,58), and in children with liver-based metabolic disease (10,49). To date, data concerning the efficacy of LCT for hepatic failure in humans has been difficult to interpret, and although clinical experiences lack the impressive stimulant to regeneration found in animal experiments, LCT remains as an alternative experimental treatment to bridge patients to orthotopic transplantation or auxiliary partial orthotopic liver transplantation (APOLT) with the purpose to reduce the risk related to liver transplantation in patients with clinical complications (49). The benefits of LCT have been extensively documented in both animal models and human trials. All this laboratory data that has been accumulated suggests that hepatocytes can function in ectopic sites with some clinical effectiveness (34,45,46,56).

The liver however appears to be the most accommodating site for engrafted hepatocytes, probably because of the availability of portal nutrients, contact with other hepatocytes and non-parenchymal cells, proximity to paracrine factors and the ability to secrete into the biliary system. Hepatocytes can be seeded into the liver by intrasplenic injection, where the spleen serves as a conduit for the distribution of cells into the liver parenchyma or by intraportal injection (26,44,62). The migration pattern and cell engraftment has been evaluated by confirmation of specific molecules of the transplanted hepatocytes in the serum of animals or employing transduced cells to express the β -galactosidase gene allowing localization by enzyme histochemistry and using transgenic rodents whose hepatocytes are dipeptidylpeptidase IV (DPP-IV) deficient has allowed localization using enzyme histochemistry (20).

Following infusion into the portal circulation, or implantation into the spleen, the vast majority of donor hepatocytes translocates to the liver and accumulates in the hepatic sinusoids, causing transient portal hypertension that resolves in hours. Trapping of transplanted cells in hepatic sinusoids is most likely mediated by passive occlusion of the sinusoids by hepatocytes as well as receptor-mediated interactions between the transplanted hepatocytes and hepatic endothelial cells and components of the hepatic matrix (62). Passage of hepatocytes into the space of Disse requires retraction of the sinusoidal endothelial cells. Shortly after entering the Space of Disse there is a transient disruption of the gap junction and tight junction between adjacent hepatocytes in the vicinity of the transplanted hepatocytes. The transplanted cells then insert themselves between host hepatocytes, with subsequent regeneration of the bile canaliculi and gap junctions in approximately 72 hours. Pharmacologic disruption of endothelial integrity and sinusoidal dilatation appear to improve engraftment (20).

The liver cell mass required to cure the various forms of liver disease is not known yet and animal studies are not definitive to answer this question. The number of liver cells and transplantations required to replace function will depend on whether the indication for transplantation is liver failure or metabolic deficiency. To date LCT has not been shown to completely correct any metabolic abnormality for long-term. This failure to completely correct enzyme deficiencies could be explained by the relatively small number of liver cells that engrafted in most transplant recipients due to cell quality and quantity and perhaps ineffective

immunosuppression protocols. Since the number of cells that can be transplanted is limited by concerns for the development of portal hypertension, repeated intraportal infusion of hepatocytes could be effective. Moreover, in most liver-based metabolic disorders, donor hepatocytes do not have a selective growth advantage over native hepatocytes. In laboratory animals with normal hepatic architecture, hepatic irradiation and the drug retrorsine can block the proliferation of host hepatocytes, while partial hepatectomy or infusion of hepatocyte growth factors, such as thyroid hormone or hepatic growth factor could be used to stimulate the expansion of donor hepatocytes. However to date transplantation of a number of hepatocytes corresponding to 1–5% of total liver mass can be expected to have a positive impact (19,27).

Cell transplantation is an elegant approach to treat or prevent liver failure that could save tens of thousands of lives each year. However, two key obstacles prevent it reaching widespread clinical utilization: the lack of transplantable cells, and poor transplanted cell engraftment leading to poor long-term functionality & viability of the transplanted cells. The evidence reviewed here strongly supports the conclusion that the realization of cell transplantation methods would be much enhanced with the engineering of an ideal transplantable scaffold that has all the necessary microstructure and extracellular cues for cell attachment, differentiation, functioning, vascularization, and which can be repopulated with liver cells derived from stem cells.

CELL SOURCE

Considering the limited availability of human donor livers for hepatocyte isolation, alternatives to primary hepatocytes have been explored. Stem cell populations, in particular, are appealing therapeutic agents due to their typically rapid and extensive proliferation and their potential to be personalized for patients. An unlimited supply of stem cell-derived liver cells could dramatically affect the development of cell-based therapies for the treatment of liver disease and could eventually lead to therapies that could improve the lives of other patients with less severe, but debilitating liver-based metabolic disorders. The availability of reliable source of high-quality liver cells would also facilitate the study of liver diseases and revolutionize the early stages of the drug discovery process.

There has been tremendous progress over the past decade in the identification of a variety of cell populations isolated from adult and embryonic tissues that are capable of contributing to the rebuilding of multiple tissues and organs (6,36,61), the development of techniques to direct the differentiation of embryonic stem cells to desirable cell populations (e.g., pancreatic beta cells) (25), and even recently the ability to reprogram human adult cells into pluripotent stem cells (iPS cells) (48). The major challenge facing this field is to transition rapidly from the identification of candidate cell populations to the development of effective delivery approaches (Figure 1).

Besides human liver cells, xenogeneic cells could be a potential cell source that could address many of the challenges of treating hepatic failure. The xenotransplantation of hepatocytes is not limited by the availability of donors, could be performed repeatedly if needed, and may be effective for the treatment of viral hepatitis, since xenogeneic hepatocytes do not seem to be vulnerable to infection by human hepatitis viruses (63). The most thoughtful obstacle to xenotransplantation is the immunological rejection of the organ graft; however major efforts are undergoing to elucidate the exact mechanism involve in rejection of the cells. A recent study showed survival of porcine hepatocytes for up to 3 months after injection into the spleen of immunosuppressed monkeys, thus suggesting that hepatocyte xenotransplantation from pigs could potentially become an option for patients with severe liver failure in the future (35) (Figure 1).

Fetal liver cell are an attractive candidate as cell source for liver cell therapies. Fetal liver progenitor cells have shown enormous replication and differentiation potential, including the capacity to generate mature liver parenchymal and non-parenchymal liver cells after transplantation in normal animals. The transplanted fetal liver cells have been reported to be capable of repopulating the liver by inducing apoptosis in neighboring host hepatocytes that proliferate more slowly than the transplanted cells in a phenomenon known as cell competition (43,44). The supply of fetal human tissues, however, is not unlimited and the possibility of oncogenic perturbations needs further study. It will also be important to determine whether cells derived from fetal livers before 20 weeks of gestation – when most elective abortions are performed – would express differentiated hepatocellular function after transplantation. Further studies are needed to corroborate the clinical significance of this technique (Figure 1).

The distinctive pluripotency and plasticity abilities of embryonic and induced pluripotent stem cells, turn them into an attractive target for the development of liver based therapies. The in vitro differentiation state of stem cells that leads to the best survival in vivo is unknown in most instances. However it is believe that these early-stage progenitor cells may be more efficient than fully differentiated liver cells for the growth and repopulation of diseased livers after transplantation because they can give rise not only to hepatocytes but also to other cell types (non-parenchymal liver cells) including bile duct ephitelial cells required for the formation of the sophisticated hepatic anatomy.

Hepatic differentiation usually involves in vitro culture of ES cells on extracellular matrices followed by the addition of growth factors and cytokines. For this, several combinations of growth factors have been successfully used. Among those, Activin A followed by the addition of FGF1 or FGF2, BMP, and HGF, to induce expression of hepatic fate genes and Oncostatin M (OSM) and dexamethasone (Dex) for maturation, or Activin A followed by sodium butyrate and HGF, have led to significant differentiation of a large number of cells with hepatic characteristics (4,6,14,55). The potential of those cells in the development of liver cell-base therapies has been relatively proved.

Care must be taken when evaluating studies describing the extent to which mature liver cells have been successfully derived from stem cells. Stem cell-derived “hepatocytes” have been generated using many strategies, and have been shown to secrete albumin and urea, and express cytochrome P450 (CYP) enzyme activity. However, a more detailed analysis of gene expression, metabolic activity, growth potential, secretory function and capacity to engraft and repopulate livers will be required to determine whether such cells can fully function as primary hepatocytes (4,54).

BIOENGINEERING SYSTEMS FOR CELL DELIVERY

Using tissue-engineering techniques, researchers have studied the microstructure of the liver in order to improve in vitro culture techniques that allow maintenance of signals similar to the intact hepatocyte microenvironment. These techniques have been incorporated to produce better cell delivery techniques and cell carriers. Ideally, these bioengineered systems should provide; in vitro conditions that reflects the in vivo environment and they should be useful for the growth, differentiation, and “conditioning” of stem cells for subsequent in vivo transplantation. Nutrient and oxygen transport and availability should be better regulated in these systems than in monolayer cultures.

Scaling up the engineered tissue approach that could be use for clinical applications will require significant improvements in the capacity to generate large-scale three-dimensional tissue in vitro. Otherwise, it could be also possible to promote the growth of small-engineered tissues subsequent to their introduction in vivo. Materials for cell transplantation have been traditionally designed from biomaterials science perspective, where the material should

provide appropriate biomechanical and chemical properties (3). More recently, however, the design of these materials has been expanded dramatically to include biological criteria that include: a) signals cells receive via adhesion to the carrier b) soluble signals available in the cellular microenvironment c) bio-inductive properties d) mechanical and material properties e) host response to these biomaterials f) degradation properties once in vivo of the materials. These various cues are intended to orchestrate the host cell response, in addition to regulating the transplanted cell population(s). Quantitative biological design criteria will be critical for materials used for stem cell culture, differentiation and transplantation, as these cues provided by the material will likely play a crucial role in signaling cell fate and differentiation. A possible approach to generating quantitative design criteria is to first develop a quantitative understanding of the relation between the cue and the target cell population response.

Bioengineers are then designing a material, most often a polymer, that provides this cue to transplanted and host cells in the desired quantity over time and space. For example, a 3D synthetic self-assembling peptide hydrogel has been used to delineate the role of concentrations and gradients of various morphogens on angiogenesis in vitro and design material systems to promote angiogenesis in vivo (39,40,46,60,66).

Other approaches have involved the use of natural extracellular matrix (ECM), the ECM is custom designed and manufactured by resident cells of each tissue/organ and is in a state of dynamic equilibrium with its microenvironment (3,12). The ECM also provides a supportive medium or conduit for blood vessels and lymphatics and for the diffusion of nutrients from the blood to the surrounding cells. Thus, ECM has all the characteristics of the perfect tissue engineered scaffold/biomaterial for cell culture, differentiation and transplantation.

Extracellular Matrix as a Biologic Scaffold Material for Cell Delivery

Researchers have looked into the microstructure of the liver to provide inspiration for culture models that maintain the signals from the hepatocyte microenvironment in vivo and provide survival and function after transplantation. Typical approaches involve the manipulation of the extracellular matrix environment, media composition, or promotion of cell–cell interaction (3,37,39).

Adhesion to a substrate is required to prevent anoikis and allow transplanted cell survival over even short time frames, and manipulating the presentation of adhesive cues further allows one to regulate major cellular processes over longer time. A variety of naturally derived ECM molecules (e.g., type I collagen and fibrin) are currently being used as cell vehicles due to their intrinsic cell binding capabilities, as are synthetic polymers to which adhesion is regulated by adsorbed proteins (52).

Biological carries composed by natural ECM have shown to facilitate the constructive remodeling of many different tissues in both preclinical animal studies and in human clinical applications. The complex three-dimensional organization of the structural and functional molecules of which the ECM is composed has not been fully characterized; therefore, synthesis of this biomaterial in the laboratory is not possible. Individual components of the ECM such as collagen, laminin, fibronectin and hyaluronic acid can be isolated and used both in vitro and in vivo to facilitate cell growth and differentiation. Various forms of intact ECM have been used as biologic scaffolds to promote the constructive remodeling of tissues and organs (3). These ECM scaffolds have been harvested from the small intestine, skin, liver, pancreas, and urinary bladder among other tissues. Many of these ECM materials have been even commercialized for a variety of therapeutic applications (2). A variety of these scaffolds have been discussed and characterized elsewhere (3).

The full potential of ECM scaffolds to promote constructive remodeling is not well understood yet. The factors that appear important for the constructive remodeling of these natural ECM cell-carriers are their ability to be rapidly and completely degraded with the generation of downstream bioactive molecules, the bio-inductive properties of the functional molecules that compose the native ECM material and the ability to engineer its mechanical properties at the time of implantation. However its sophisticated and complicated microstructure needs to be further characterized. In addition, the effect of various processing steps to produce the biological ECM may impact the host immune response.

Artificial Matrix For Cell Delivery

Synthetic peptides mediating adhesion can also be presented to cells as self-assembling hydrogels, coupled as side chains to polymer backbones, or as components of synthetic proteins that provide desirable combinations of cell interactive domains and overall physical/chemical properties.

In the past three decades, several biopolymers, including PLLA, PLGA, PLLA-PLGA copolymers, and other biomaterials including alginate, agarose and collagen gels, have been developed to culture cells in 3-D (8,21,28). These culture systems have significantly advanced our understanding of cell-material interactions and cell delivery and facilitated the perception of a new field of tissue engineering and regenerative medicine.

Attempts have been made to culture cells in 3-D using synthetic polymers or copolymers. These synthetic polymers are often processed into microfibers, (5–20 μm). To produce a true 3-D environment, a scaffold's fibers and pores must be substantially smaller than the cells. In order to culture tissue cells in a truly 3-D microenvironment, the fibers must be significantly smaller than cells so that the cells are surrounded by the scaffold, similar to the extracellular environment and native extracellular matrices (66).

Animal-derived biomaterials (e.g., collagen gels, polyglycosaminoglycans, and Matrigel) have been used as an alternative to synthetic scaffolds (51). Unlike artificial matrices, animal-derived biomaterials do contain residual growth factors, undefined constituents, or nonquantified impurities. This not only makes it difficult to use them to grow tissues for human therapies. Animal-derived biomaterials, for example, collagen gels, laminin, polyglycosaminoglycans, and materials from basement membranes including Matrigel™, have been used as an alternative to synthetic scaffolds. Although researchers are well aware of their limitations, it is one of the few limited choices.

The advantages of using the designer peptide nanofiber scaffolds are as follows: 1) One can readily modify the designer peptides at the single amino acid level at will, inexpensively and quickly. 2) Unlike Matrigel, which contains unknown ingredients and quality that varies from batch to batch, artificial scaffolds may contain pure components and every ingredient is completely defined. 3) Artificial matrices can be used to study controlled gene expression or cell signaling process. Thus, these scaffolds proved to be promising tools to study cell signal pathways in a selective way not possible with any unknown substrates that could result in confusing cell signaling activation. 4) These scaffolds could provide the opportunity to incorporate a number of different functional motifs and their combinations to study cell behavior in a well-defined ECM-analog microenvironment, not only without any chemical cross-link reactions but also fully bio-re-absorbable scaffolds.

In addition, a new technology that involves the creation of an artificial liver tissue by layering thermoresponsive cultured cell sheets in vitro and then transplanting them directly into the subcutaneous space has been recently introduced, which was demonstrated to stably persist for over 200 days (46). We still do not know, however, if graft survival lasting months in rodents

will translate into a more prolonged survival in patients, or whether a sufficient functional cell mass can be transplanted.

Even all the advances produced in the last years, there has not yet been a definitive demonstration that the mechanical properties of the adhesion substrate regulate tissue formation by transplanted cells *in vivo*. This likely relates to the difficulty in separating mechanical and degradative properties of materials, and the material degradation rate clearly plays an important role in the ability of transplanted cells to deposit new matrix and remodel the tissue.

Whole Organ Scaffolds for Tissue Engineering

The first generation tissue engineering materials have found limited use as they had limited ability to modulate the repair and regeneration of the host tissue. Consequently, material and host tissue interactions became an important consideration in the design of new materials such that the new generation materials should harness elements that regulate tissue regeneration by different mechanisms, such as controlling specific cell binding interactions and responding to environmental cues (29). In addition, since material microstructure has been found to play an important role for tissue morphogenesis (11,23), three dimensional aspects such as pore structure, surface area to volume ratio, texture and surface topography that define material architecture have become other important design parameters (5). Considering these design constraints, decellularized extracellular matrix (ECM) represents an ideal material for tissue engineering applications as it retains relevant aspects of complex structure and chemical composition of the ECM (12). Moreover, using perfusion decellularization technique described recently for heart (47) the native three dimensional structure of the organ can also be preserved which will most likely provide the necessary architecture for optimal cell distribution and subsequent tissue morphogenesis. The intent of most decellularization processes is to minimize the disruption and thus retain native mechanical properties and biologic properties. The most commonly utilized methods for decellularization of tissues involve a combination of physical and chemical treatments. The physical treatments can include agitation or sonication, mechanical massage or pressure, or freezing and thawing. These methods disrupt the cell membrane, release cell contents, and facilitate subsequent rinsing and removal of the cell contents from the ECM. These physical treatments are generally insufficient to achieve complete decellularization and must be combined with a chemical treatment. Enzymatic treatments, such as trypsin, and chemical treatment, such as ionic solutions and detergents, disrupt cell membranes and the bonds responsible for intercellular and extracellular connections. Tissues are composed of both cellular material and ECM arranged in variable degrees of compactness depending on the source of the tissue.

In addition, the scaffold obtained via perfusion-decellularization will contain a perfusable vascular tree that facilitates *in vitro* perfusion and reconnection to the blood torrent and this can greatly enhance nutrient delivery and waste removal in the tissue-engineered construct.

CELL TRANSPLANTATION TO ENGINEER ORGANS

A significant challenge to the successful implementation of cell therapies to engineered organs is the integration of the new cells within the vascular, lymphatic, and nervous systems of the host matrix or decellularized organ. Engineering of sophisticated cell organizations (e.g. liver) is especially challenging. The liver is composed of several distinct cell types, including hepatocytes, sinusoid endothelial, Kupffer, stellate and biliary epithelial cells (Figure 2). These cells are orchestrated through tight junctions and connective tissue such as type I and IV collagen and laminins.

The need for vascularization is motivated by the observation that transplanted cells will rapidly die if they are placed a significant distance from the host vasculature (53). Researchers have attempted various approaches via angiogenesis, by 1) providing local and sustained presentation of angiogenic molecules, this techniques have been demonstrated to enhance vascularization and perfusion in vitro and in the implant site (55), 2) other strategies have included the co-seeding of endothelial cells and supportive fibroblast that spontaneously form capillary-like networks, and the engineering of branching channels to mimic the vascular tree (38). However, these efforts have not yet produced a scaffold that has a natural vascular tree with centralized inlet and outlet vessels and a pervasive nutrient exchange capacity comparable to the capillary network in the body. Thus a significant advance in the field of organ engineering has been the utilization of decellularized tissue/organs as skeleton for cell implantation in a three-dimensional environment (3,47).

Lately, a work was published reporting that a whole-heart scaffolds with intact three-dimensional geometry and vasculature that was attempted by decellularization of cadaveric hearts by coronary perfusion with detergents. This work demonstrated that the natural scaffold could be then repopulated with neonatal cardiac cells or rat aortic endothelial cells and cultured these recellularized constructs under simulated physiological conditions for organ maturation. Cellularization of these decellularized hearts was performed by infusion of cells through direct perfusion of the coronary vasculature or direct puncture into the decellularized scaffold. Even though, the actual contractile function was almost 2% compared to normal contractile function, this work represents an example of organ engineering using decellularized natural scaffolds (47). Most interestingly, a report showed the first human clinical tracheal transplantation using a cadaveric trachea with cartilage structure as matrix. This tissue-engineered graft was successfully implanted in the left main bronchus of a 30-year-old woman. The trachea was decellularized over a period of 6 weeks and seeded with a combination of epithelial cells and bone marrow stromal cells from the own patient for a period of time of 96h. This important and innovative report demonstrates the principle that a cellular therapy combined with tissue engineering can produce engineered organs with important implications for serious clinical disorders (30).

However, cell seeding of these multi-dimensional artificial and naturally derived scaffolds presents challenges for whole organ engineering. Particularly, the human liver, who contains a dense cell mass (estimated on 10×10^{10} cells). Naturally, the intact liver vascular tree is accessible through one central inlet (portal vein), which branches into a capillary-like network and then reunites into one central outlet. Classically, liver cells are transplanted via portal vein or intrasplenic. Following rapid infusion into the portal circulation, the vast majority of donor hepatocytes translocates to the liver and accumulates in the hepatic sinusoids, causing important portal hypertension. Trapping of transplanted cells in hepatic sinusoids is most likely mediated by passive occlusion of the sinusoids by hepatocytes as well as receptor-mediated interactions between the transplanted hepatocytes and hepatic endothelial cells and components of the hepatic matrix. Only a small fraction of hepatocytes ultimately engraft in the liver after intraportal injection (5–20%) (Figure 2). Thus, intrasplenic transplantation has been the preferred site for transplantation, where the spleen serves as a conduit for the distribution of cells over time. On the other hand, pharmacologic disruption of endothelial integrity and sinusoidal dilatation appear to improve engraftment. Even so, a major limiting factor to intraportal engraftment is the number of viable cells that can be safely injected at one time (9,10,57).

Based on this funded knowledge of liver cell transplantation, theoretically, cellularization of naturally decellularized liver scaffold might be done over extended period of times, using limited cell numbers at each time. Even if these scaffolds lack of cellular components, they still have vasculature network that is well conserved, thus one can hypothesize that there might

be a barrier that hepatocytes might have to cross to fully integrate in the liver parenchyma. Additionally, it might be necessary to provide cells through retrograde flow from the vena cava that would enter the liver lobule through the central vein and deposit cells in the peri-central area. In this way, we could have cells seeded in the peri-portal and peri-central area.

Alternatively, endothelial cells or progenitors may be added in concert with the cell type of interest and it may also contribute to the formation of a new vascular network that enhances cell survival and tissue formation. Endothelial coverage of the decellularized vascular structures lumen of the bioscaffold would be essential to prevent thrombosis and to provide proper vascular function. Thus the addition of non-parenchymal cells would be ideal to reconstitute the liver sinusoid (Figure 2). Perhaps, the addition of supportive cells (e.g. fibroblast or mesenchymal stromal cells) would enhance the secretion of native matrices for adequate cell repopulation and reorganization. Yet the problem of the engineering of the biliary tract and reconnection to the existing biliary conducts should be address. But the use of stem cells and bipotent cells (e.g. fetal liver cells) might represent a source for this issue.

The liver Extracellular Matrix (ECM) presents an ideal scaffold for stem cell differentiation into hepatocytes, as well as cell transplantation. It is known that local environmental factors induce hepatocyte homing, differentiation, and proliferation, and studies indicate that stem cells may differentiate toward mature hepatocytes following transfer into injured liver. It is reasonable to expect that a similar beneficial response will be observed for liver grafts as well. Therefore, the decellularized liver matrix presents great potential as the scaffold for hepatocyte maturation and transplantation. This process may be further manipulated by sequential delivery of factors involved in the initiation and maturation of stem cells to liver cells, allowing temporal and spatial control over differentiation.

Although there has been little effort to actively drive formation of a new lymphatic system (41), the systems and approaches developed to drive angiogenesis may prove useful for this application. In addition, there has been relatively little progress toward understanding and regulating innervation in engineered tissues. A number of approaches to nerve regeneration, often involving electrical, chemical, or topographic stimulation are being actively pursued (13,15), but only a few studies have noted structures suggestive of nerves in tissues forming from transplanted cells (16). A lack of innervation will likely prevent full function and probably lead to atrophy in many situations. The recent recognition of significant molecular crosstalk between the nervous and vascular systems during development (65) and regenerative processes may provide multiple targets to simultaneously enhance innervations and vascularization of engineered organs.

FUTURE PERSPECTIVES AND CONCLUSIONS

Cell transplantation is an elegant approach to treat or prevent liver failure that could save tens of thousands of lives each year. However, two key obstacles prevent it reaching widespread clinical utilization: the shortage of transplantable cells, and poor engraftment leading to poor long-term functionality & viability of the transplanted cells. The evidence reviewed here strongly supports the conclusion that the realization of cell transplantation methods would be much enhanced with the engineering of an ideal transplantable scaffold that has all the necessary microstructure and extracellular cues for cell attachment, differentiation, functioning, vascularization, and which can be repopulated with liver cells derived from stem cells. An exciting possibility is utilizing decellularized liver extracellular matrix as the scaffold for cell transplantation, which could ultimately allow the development of an engineered auxiliary liver grafts for transplantation and open the doors to a new era of organ engineering.

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Figure 1. Cell Sources and Delivery Technologies for Cell Transplantation

Different cell sources are currently available or under investigation including porcine cells, fetal cells, induced pluripotent stem cells (iPS cells) and embryonic stem cells (ES cells). Cells are engineered and multiplied in culture prior to transplantation or used with minimal manipulation after harvesting or isolation and then delivered directly or utilizing bioengineering strategies. The transplanted cells are expected to home to the site of interest (e.g. liver parenchyma) or stay at the site of injection, depending on the particular application. When bioengineering delivery systems are used then cells may be allowed to adhere to a material carrier, typically a biodegradable polymer, in vitro, and subsequently be implanted or injected on the material to localize the cells to a specific anatomic site. The material serves as a guide and frame for tissue formation and typically is designed to degrade and be replaced by deposition of new extracellular matrix and cell proliferation.

Figure 2. Schematic Representation of Organ Engineering for Transplantation of Liver Grafts using Cell Transplantation Principles

Whole organ scaffolds serve as template for cell delivery to re-engineer the sophisticated liver organotypic structures by using different types of liver cells including hepatocytes, endothelial cells, cholangiocytes, stellate cells, neural cells and kupfer cells. (a) decellularized rat liver, (b) Re-engineered liver, (c) transplantation of engineered auxiliary liver graft in the rat.