Seminars in Pediatric Surgery 23 (2014) 150–155

Contents lists available at ScienceDirect

Seminars in Pediatric Surgery

journal homepage: www.elsevier.com/locate/sempedsurg

Liver cell therapy and tissue engineering for transplantation

Joseph P. Vacanti, MD[a,b,c,d,*], Katherine M. Kulig[a,b,c]

[a] Center for Regenerative Medicine, Massachusetts General Hospital, Boston, Massachusetts
[b] Department of Surgery, Massachusetts General Hospital, 55 Fruit St, WRN 1151, Boston, Massachusetts 02114
[c] Department of Pediatric Surgery, MassGeneral Hospital for Children, Boston, Massachusetts
[d] Harvard Medical School, Boston, Massachusetts

ARTICLE INFO

Keywords:
Hepatocytes
Liver transplantation alternatives
Organ engineering
Stem cells
Tissue engineering
Xenotransplantation
Regenerative medicine

ABSTRACT

Liver transplantation remains the only definitive treatment for liver failure and is available to only a tiny fraction of patients with end-stage liver diseases. Major limitations for the procedure include donor organ shortage, high cost, high level of required expertise, and long-term consequences of immune suppression. Alternative cell-based liver therapies could potentially greatly expand the number of patients provided with effective treatment. Investigative research into augmenting or replacing liver function extends into three general strategies. Bioartificial livers (BALs) are extracorporeal devices that utilize cartridges of primary hepatocytes or cell lines to process patient plasma. Injection of liver cell suspensions aims to foster organ regeneration or provide a missing metabolic function arising from a genetic defect. Tissue engineering recreates the organ *in vitro* for subsequent implantation to augment or replace patient liver function. Translational models and clinical trials have highlighted both the immense challenges involved and some striking examples of success.

Donor organ shortage creates a need for engineered tissue

Treatment for end-stage liver disease remains orthotopic liver transplantation. Human liver transplantation began as an attempt to treat a 3-year-old patient suffering from biliary atresia by Thomas E. Starzl over 5 decades ago at the University of Colorado. The hurdles required to be successfully overcome were enormous, and the child did not survive the procedure. Post-operative problems also resulted in the deaths of the next four adult recipients. A 4-year hiatus was used to better develop the surgical technique and immunosuppressive therapy. Eight children were transplanted upon re-initiation of the program in 1967; of them, four made it past the 1-year mark, with one patient surviving over 3 decades. The primary cause of failure was sepsis, which was attributed to imperfect immunosuppression, a problem that would not be solved until the early 1980s with the introduction of cyclosporine A. The procedure has been highly refined, and most liver transplant centers now report a 1-year survival rate of over 86% and a 5-year rate of 72%.[1] A severe and chronic shortage of available organs for transplants continues to result in over 1500 deaths each year of the 17,000 patients on the waiting list in the US. For those not deemed suitable transplant recipients, liver disease has become the 3rd most prevalent cause of death in the US. No adequate alternative treatment for liver failure is available.

Transplantation in the pediatric patient population has special challenges. First among them is the scarcity of size-matched cadaveric organs. This has been partially addressed by the development of procedures that rely upon the segmental nature of the liver. This problem was originally addressed by *ex vivo* liver reduction through bisection of the organ while avoiding major arteries and bile ducts, whereby the left lateral segment or full left lobe was retained for transplant. This has largely been replaced by the split graft technique, which while more complicated, provides two segments for transplantation into two recipients. Typically, the larger right lobe is transplanted into an adult, while the left lateral segment or lobe is provided to a child. This procedure has greatly increased the number of grafts available for the pediatric population, however not all transplant centers are willing to or capable of performing the complicated procedure.

Living donor liver transplantation (LDLT) developed directly from the experience of performing split liver harvesting in heart-beating cadaveric donors. A portion of liver is surgically removed from a living donor and immediately transplanted into the patient. The left lateral segment is typically utilized. Because of the regenerative properties of the organ, the donor regains normal hepatic mass and function in 1–2 months, with similar, but delayed recovery for the recipient. This procedure was originally

* Corresponding author at: Joseph P. Vacanti, M.D., Department of Surgery, Massachusetts General Hospital, 55 Fruit St, WRN 1151, Boston, Massachusetts 02114.
E-mail address: jvacanti@partners.org (J.P. Vacanti).

http://dx.doi.org/10.1053/j.sempedsurg.2014.05.001
1055-8586/

developed primarily as a donor mechanism for parents with children with diseased livers. Dr. Christoph Broelsch performed the first successful LDLT at the University of Chicago Medical Center in 1989, with a 2-year-old girl receiving part of her mother's liver. By expanding the potential donor pool to close relatives, the odds of finding an immunologically matched organ are greatly increased. Although the success rate for patients undergoing LDLT matches or bests that of cadaveric transplants, the harvesting procedure is not without significant risk to the donor. Most of the common post-operative complications are readily addressed; however, the risk of donor death from pulmonary embolism or other causes is not insignificant and ranges from 1 in 300 to 1 in 1000 in various transplant centers. This creates a very serious ethical issue as the donor does not directly benefit from the procedure and yet is under significant psychological pressure because the potential recipient is his or her child.

Because transplantation remains the only treatment of end-stage liver disease, an increase in the number of transplant centers and efforts to expand the donor pool have not been able to keep up with need. Statistics regarding outcome of the pediatric patients on the UNOS transplant list are difficult to interpret owing to the widespread non-adherence to the pediatric end-stage liver disease (PELD) scoring system.[2] The lack of suitable-sized organs has led to utilizing higher risk and more complex alternatives. However, it is clear that children under 1 year of age on the transplant waiting list have the greatest risk of death.

The etiology of the pediatric patients facing end-stage liver failure is quite different from that of adults, with almost one-half having biliary atresia (cholestasis). Other less prevalent conditions include autoimmune and sclerosing hepatitis, organ failure due to drug toxicity, and metabolic disorders. Several single locus genetic causes exist, including Wilson disease, alpha 1-antitrypsin deficiency, familial cholestatic syndromes, and tyrosinemia. Large, non-resectable hepatoblastoma, and hepatocellular carcinomas may also warrant a transplant. While the overall number of liver transplants performed in the US rose exponentially since its inception until leveling off to about 6300 procedures per year since 2005, the number of pediatric transplantations has remained almost unchanged at about 550 since 1990.[1] The aims of liver tissue engineering are to develop mechanisms to support hepatic function during the wait for a suitable organ, develop cell-based therapies to permanently provide specific missing biochemical functions in existing organs, and develop methodology to provide tissue-engineered alternatives to donor organs.

Liver structure guides tissue engineering

The architecture of the liver is elegant in its simplicity. The organ is largely composed of a few cell types, of which hepatocytes, the principal parenchymal cells, contribute to 80% of its mass. The liver receives over 25% of the total resting cardiac output and accounts for 20% of the oxygen consumption. Blood is supplied to the liver by both the hepatic portal vein and hepatic arteries. Within the liver, flow is divided and blood processed simultaneously by millions of hepatocyte-containing acini, the basic functional hepatic unit. Blood flow then coalesces in the hepatic veins and exits the organ.

Within each ascinus, capillary-like vessels called sinusoids support a single layer of hepatocytes. Blood flow is quite slow in the sinusoid, taking a few seconds to travel the 1–2 mm distance. During this transit period, hepatocytes take up toxins for processing and secrete serum proteins. Hepatocytes have very high oxygen requirements and so are situated about 2 μm from the lumen of the sinusoid. The drop in oxygen levels during the passage of blood through the sinusoid due to hepatocyte consumption regulates hepatocyte phenotype. Resulting zones display differences in the ability to detoxify ammonia, metabolize xenobiotics, and utilize glucose. Hepatocytes are shielded from direct blood flow by a thin, porous extracellular matrix (space of Disse) and a specialized endothelium that lines the sinusoids. Endothelial cells are fenestrated with an array of many dozens of 100 nm wide trans-cellular pores that control the two-way flow of plasma between the vessel lumen and the perisinusoidal space for processing. A single layer of hepatocytes, bound by tight junctions to form an impenetrable barrier, surrounds each sinusoid. Epithelial polarization is essential for hepatocyte function, with cellular domains specified by the circumferential tight junction ring. The specialized hepatocyte surface facing the sinusoid, the basolateral membrane, selectively takes up plasma components for processing and secretes newly synthesized serum proteins. The opposite, apical membrane, in conjunction with those of neighboring hepatocytes, forms the narrow, tubular channels called bile canaliculi. These join to form a three-dimensional network that drains secreted, processed toxins and digestive molecules to the bile ductules. The detailed architecture of both the organ and the hepatocyte is essential for its efficient and effective function; as much as it is practical, efforts to generate liver by tissue engineering would do well to replicate their structure.

Cell source considerations

Several source issues must be adequately addressed for liver tissue engineering to be fully successful.[3] First, the mass of cells required is substantial. As the liver is approximately 2.8% of total body weight, the organ of a 70-kg individual is about 1.5 kg. The minimum liver mass required for survival has been variously asserted to be 10–30% of the total organ or 200–600 g. At 120 million human hepatocytes/gram of tissue, a minimum of 2.5–7.5 billion cells would be required for a clinical product.

Primary hepatocytes

A healthy adult liver readily regenerates and maintains both volume and function after undergoing up to 70% resection. However, liver regeneration does not recapitulate its ontogenesis. *In situ* cellular hyperplasia of the remaining liver is responsible rather than regeneration of the excised lobes. Unfortunately, although much is known about normal liver development, regeneration occurs by a different and poorly understood mechanism. The process itself has been well characterized histologically, but the molecules that regulate the process have only been partially characterized. A highly controlled proliferation, involving ECM-sequestered latent signaling molecules, inflammatory cells, and angiogenesis, rapidly replaces the missing tissue.[4] Because most of the regeneration derives from cell cycle re-entry by mature hepatocytes rather than activating a rare stem cell population, a few rounds of cell division are sufficient to replace as much as a kilogram of tissue. What is known about liver regeneration is still insufficient to enable induction of *in vitro* proliferation of mature, isolated hepatocytes. Primary adult hepatocytes demonstrate minimal proliferation *in vitro*, rarely completing even a single round of cell division *in vitro*. Significant research has not yet solved this conundrum. Human fetal liver cells do demonstrate *in vitro* proliferation ability, although hepatic function remains quite low.[5] Regardless, it is doubtful clinical products would be developed from fetal tissue.