

# Tissue Engineering and Organ Structure: A Vascularized Approach to Liver and Lung

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**ABSTRACT:** Over the past two decades, great strides have been made in the field of tissue engineering. Many of the initial attempts to develop an engineered tissue construct were based on the concept of seeding cells onto an avascular scaffold. Using advanced manufacturing technologies, the creation of a preformed vascular scaffold has become a reality. This article discusses some of the issues surrounding the development of such a vascular scaffold. We then examine of the challenges associated with applying this scaffold technology to two vital organ constructs: liver and lung. (*Pediatr Res* 63: 520–526, 2008)

In 1985, we hypothesized that a living vital organ could be rationally designed and built for human therapy from cells and degradable polymer scaffolding. This was based on only two pieces of previous work: 1) an acellular artificial dermis by Yannas and Burke (1); and 2) a vascular conduit of contracted collagen and vascular cells (2).

Our first reports described the formation of hepatic, intestinal, and pancreatic tissue (3,4). In the ensuing 22 y, the fields of Tissue Engineering and Regenerative Medicine have become established and several tissues are available for human trials or use. In 1998, to overcome the problem of sufficient functional mass for human therapy, we hypothesized that we could build a vasculature as part of the tissue-engineered organ which would provide immediate exchange of oxygen- and nutrient-rich blood to the full volume of engineered tissue, much as a transplanted organ functions today. Summarized below are the most recent developments toward an engineered vascularized tissue with emphasis on liver and lung.

## VASCULARIZED SCAFFOLD FOR SOLID ORGAN TISSUE ENGINEERING

The fundamental challenge of developing a tissue for therapeutic use is scaling up the growth of cells from a culture dish to a three-dimensional scaffold. Multilayer cellular constructs are achievable on flat sheets or thin porous scaffolds. However, the creation of tissues for solid organ transplantation must overcome the limited distance of oxygen diffusion.

One approach is centered on angiogenesis, or the self-assembly of a vascular network within a scaffold by endothelial and smooth muscle cells. Although on a small scale this is achievable, this approach develops too slowly to support the mass of tissue that is required for an organ transplant, such as a liver. The Tissue Engineering and Organ Fabrication Laboratory at Massachusetts General Hospital proposed the creation of a scaffold with an integrated vascular network (5,6). A capillary-like vascular network within a three-dimensional scaffold can deliver oxygenated blood to within several hundred micrometers of all cells in the scaffold. These vascular networks are designed to replicate features of a capillary network within the bounds of what can be manufactured. Initial work centered on the utilization of photolithography techniques to create molds of capillary-like networks (7–12). The vascular networks of these original molds were designed to have arterial pressure drop across the network with physiologic shear stress in the channels. These molds were used to create polymer based vascular networks, which could be seeded with endothelial cells (8). A second generation vascular network for a liver scaffold is shown in Fig. 1 (13).

The vascular network is adjacent to parenchymal cells of the specific organ (*i.e.*, hepatocytes). A thin porous membrane in the scaffold allows the supply of oxygen and nutrients from the vascular network to the parenchymal cells and also removes waste products. A cross-sectional scanning electron microscope image of this scaffold design without cells is shown in Fig. 2. The posts are structural supports to maintain the desired height of the parenchymal chamber. Within this concept, the strategy has been to develop a scaffold with an integrated capillary-like vascular network, which can support a small amount of tissue. The planar network and scaffold can then be expanded by stacking and in parallel arrangements to create the necessary size of tissue or organ.

Recent developments by other groups in this area have focused on improving the design of the vascular networks to better mimic natural vessels and capillary beds. Zhou *et al.* hypothesized a generalized model of the minimum energy principle of natural vascular networks originally proposed by Murray (14,15). This theoretical model defines scaling laws of a vascular network and has been validated by comparing it to morphologic data on several native human and animal vascular networks (16). This proposed scaling law defines relationships between the length,

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**Abbreviations:** HepG2, hepatocellular carcinoma cell line; MSC, mesenchymal stem cells

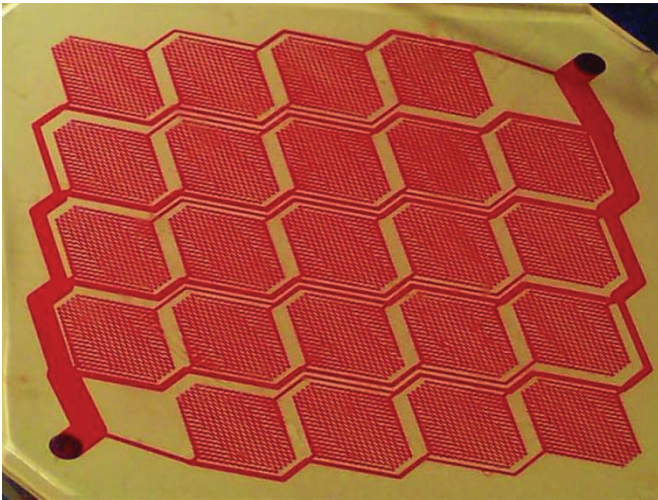


Figure 1. Second generation vascular network design.

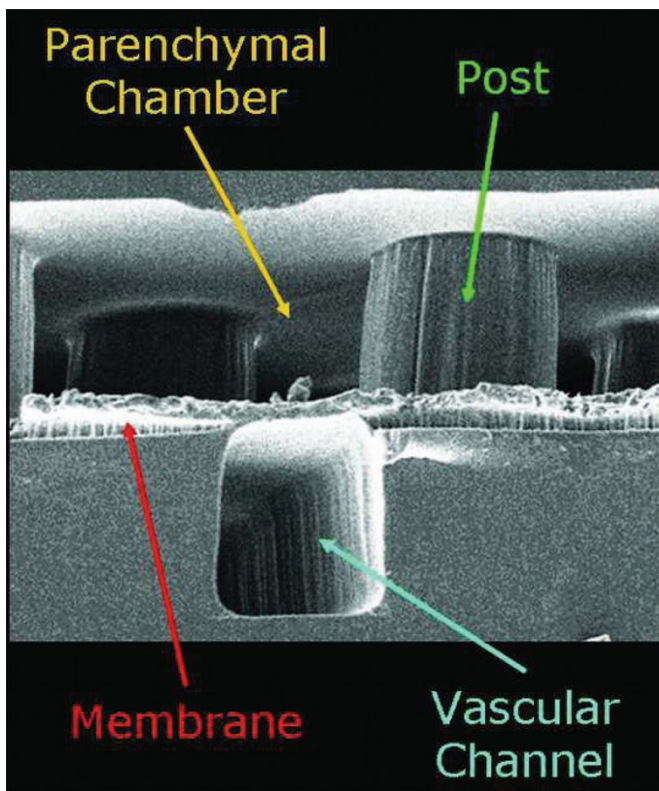


Figure 2. Cross-sectional image of vascular network channel and adjacent parenchymal chamber.

diameter, and flow rate of blood in each branch and in the vascular network as a whole. There is excellent correlation of this theory with distal coronary and pulmonary circulations. The correlation with arcade vascular structures such as the mesenteric arteries and the epicardial coronary arteries is not as strong. The vascular network designs proposed by this model exist in three dimensions similar to native vasculature. Although anatomically accurate, there will need to be a significant advance in tissue-engineered scaffold development and manufacturing before such a design could be used.

Janakiraman *et al.* developed a theoretical vascular network to support tissue-engineered skin (17). This network was

optimized for mass transport efficiency utilizing a conceptual porous material. The result is a symmetrical bifurcating design with up to six vascular generations. The conclusion is that rectangular ducts provide a more efficient transport of nutrients due to their increased surface to volume ratio over square shaped ducts. The work highlights an approach of utilizing computations to optimize the design of a vascular network.

Vozzi *et al.* and Bianchi *et al.* have developed a branching vascular network design based on Murray's Law and an allometric scaling scheme developed by West *et al.* (14,18–20). This planar design has been fabricated using an innovative method of precision polymer extrusion from a microsyringe. The result is a solid polymer fractal network made with a resorbable polymer, poly-lactide-*co*-glycolide. Endothelial cells and fibroblasts have been grown on the outside of the vascular network as a first step toward creating a microvascular scaffold. However, *in vitro* resorption of the polymer network to create a microvessel with a lumen has not been demonstrated.

Our vascular network, now in its third evolution, is based on biomimetic design parameters including asymmetric branching, physiologic branch lengths and bifurcation angles, and relative diameters of subsequent generations based on Murray's law. In addition to improvements in network design, the manufacturing of the network has advanced from a single depth vascular network created with photolithography to a three-dimensional planar network design utilizing an innovative manufacturing process. The result is a more physiologic vascular network that can be expanded both serially and in parallel and provides improved delivery of oxygen and nutrients to cells while minimizing the risk of thrombus formation in the network *in vivo*. The ongoing development of this vascularized scaffold continues to drive the ultimate focus of our lab, the development of tissue-engineered liver and lung constructs capable of replacing function at the organ level.

## LIVER

The field of hepatic tissue engineering has the potential to address far reaching clinical problems from in-born errors of metabolism to chronic liver failure. After addressing the critical issue of immediately supplying cells with oxygen and nutrients the field is principally limited by one factor: the lack of an adequate supply of functional hepatocytes. The liver is composed of several distinct cell types, including hepatocytes, sinusoid endothelial, Kupffer, stellate, biliary epithelial, and pit cells. These cells are joined together through tight junctions and connective tissue such as type I and IV collagen and laminins (21). Hepatocytes are responsible for most clinically measurable organ functions including metabolic homeostasis, protein synthesis, and detoxification. The remaining non-parenchymal cells support parenchymal cellular orientation and function (22–24). Using a standard protocol for dissociating cells from liver tissue, several cell types have been isolated and evaluated for their potential as a source of cells for hepatic tissue engineering including primary animal hepatocytes, hepatocellular carcinoma (HepG2) cell lines, and human primary hepatocytes from normal liver (25,26).

Hepatocytes derived from the livers of nonhuman mammalian species are termed xenogenic cells. The prime advantage these cells offer is a virtually unlimited supply of primary hepatocytes under strictly controlled production conditions (27). This is particularly true of porcine hepatocytes because they exhibit metabolic characteristics similar to human hepatocytes, can be procured in large quantities, and stored for many months with minimal loss of viability (28). However, the use of these cells in tissue-engineering applications is controversial due to the risk of zoonotic transmission of diseases such as porcine endogenous retrovirus (29). In the European community, the concern for porcine endogenous retrovirus transmission is so great that The Advisory Group on the Ethics of Xenotransplantation and the Nuffield Council on Bioethics have recommended a complete European moratorium on the clinical use of xenogenic hepatocytes (30,31). Despite the uncertain future of this cell source in the European community, The United States Public Health Service's *Draft Public Health Service Guideline on Infectious Disease Issues in Xenotransplantation* clearly supports xenogenic cell source research, provided that xenogenic tissues are prescreened for zoonotic disease before use and that patients in whom xenogenic cells have been implanted are followed long-term for signs of transmission (32). Based on the political climate surrounding these cells, it is unlikely that they will represent a near-term solution to the hepatocyte shortage and would likely be abandoned if a human source were identified (33). In addition, subtle differences in the metabolic response between animal and human hepatocytes are known to exist and these differences limit the performance of a tissue-engineered construct based on animal cells (34). Therefore, the ideal cell source for hepatic tissue engineering would clearly come from a human-derived source of hepatocytes.

The term "cell line" refers to human cells derived from metastatic tumors, which have become immortalized and demonstrate an unlimited capacity for growth *in vitro*. Within the field of hepatic tissue engineering, the most commonly used cell line is the C3A subclone of the HepG2 cell line (28,35). These cells are commonly used *in vitro* and have a very hardy nature. They tolerate repeated freezing and thawing cycles and will readily proliferate in culture, which makes them very easy to use in the laboratory setting. This cell line continues to demonstrate some normal hepatic metabolic function including albumin synthesis, p450 activity, and urea synthesis. Unfortunately, this cell line has an abnormal metabolic profile (36,37). One of the abnormal metabolic products of HepG2-C3A cells is alpha-fetoprotein, which is often used as a marker of metabolic activity in HepG2-based tissue-engineering constructs (35). Because HepG2 cells are derived from a known malignant tumor, they present a risk for the development of metastases in any clinical application, and most likely will be relegated to use exclusively in *in vitro* tissue-engineering applications (27,35,38).

Primary hepatocytes are fully mature adult hepatocytes that have been isolated from the extracellular matrix of human liver. These cells are the cells of greatest interest for hepatic tissue engineering and are broken down into two principal groups: allogenic primary hepatocytes and autologous primary

hepatocytes. Allogenic primary hepatocytes are cells that are derived from human sources other than the patient for whom they are intended. Further, a tissue-engineered construct containing these cells would present all the immune risks of transplantation and could complicate the patient's future rejection risk should a traditional transplantation be required at a later time (33). Moreover, these human-derived cells pose a greater risk for potential disease transmission to a patient than animal cells. However, allogenic primary hepatocytes may represent the best cell population for tissue-engineering applications, given their unimpaired metabolic function and relatively large quantities compared with autologous primary hepatocytes (28).

Autologous primary human hepatocytes are the preferred alternative to allogenic primary hepatocytes because they are derived directly from the patient for whom any tissue-engineered liver replacement therapy is intended (33). These cells already exhibit the same immunomarkers as the patient, eliminating the possibility of an immune rejection (28). However, although immunologically advantageous, the use of the patient's own cells has been considered an unsatisfactory solution to the cells source problem for two reasons. First, patients with liver dysfunction may not have the hepatic reserve to withstand the removal of hepatocytes in a separate procedure before the implantation of a tissue-engineered liver construct. Second, because these hepatocytes are already functioning poorly, once removed from the *in vivo* environment, they may lose function entirely (33).

An overriding limitation to the use of these cells is that primary human hepatocytes of either variety are very difficult to obtain in large quantities (39). In fact, primary human hepatocytes are hard to obtain at all. Only three potential sources exist: *a*) discarded liver removed before implantation of a liver transplant; *b*) surgically resected sections of a diseased liver (typically cancer); *c*) cells obtained from liver biopsy. In the first case, the organ dysfunction, which necessitated the transplant, may translate into a dysfunction on the cellular level and the scarring associated with the disease impairs the isolation of the cells for use *in vitro*. In addition, these grafts are only sporadically available and any organ considered unfit for use in transplantation may not contain many hepatocytes able to withstand processing for tissue-engineering applications (27). In a liver specimen resected for a focal disease such as cancer or metastases, normal cells are found surrounding the lesion. These cells typically exhibit normal cellular function, but the goal of a resection is to minimize the mass of liver removed and therefore these cells are not plentiful. Biopsies, although far less invasive than a liver resection, yield a population of cells orders of magnitude smaller than the cells derived from a liver resection. Although all of these sources of hepatocytes could provide allogenic primary hepatocytes, the only clinically viable opportunity to obtain autologous primary hepatocytes from a patient with liver disease is *via* a biopsy specimen. Given their scarcity, primary human hepatocyte populations of both varieties have been expanded and preserved through the use of immortalization techniques involving temperature-sensitive SV40Tag, suicide genes, and Cre-lox P-mediated oncogene excision (28,36,38). All of these techniques have shown promise *in vitro*

but have failed to generate adequate amounts of functional tissue to be clinically applied at this time. Even if primary human hepatocytes were available in large quantities, a larger problem exists: primary human hepatocytes are difficult to maintain in culture for extended periods of time (40). The reason for this poor *in vitro* performance may be due to the processing required to isolate primary hepatocytes in the first place.

A standard protocol for dissociating cells from liver tissue of multiple species is based on the enzymatic dissociation of all the liver cells from the supporting extracellular matrix. The parenchymal and nonparenchymal components are separated to further isolate the specific cell populations (25,26). When isolated in this manner, hepatocytes exhibit *in vitro* degradation in function and viability because hepatocytes require cell-to-cell contact to maintain their epidermal characteristics (29,41). A novel source of tissue for hepatic tissue engineering that may avoid these limitations is the use of precision-cut liver slices.

As early as the mid 1980s, it was recognized that coculturing primary hepatocytes with other cells significantly improved *in vitro* survival (42). This observation eventually led to the evaluation of intact liver slices as a model for hepatic metabolism, and several benefits were recognized. Specifically, primary hepatocytes cultured in isolation become disorganized and detached early in culture (42); however, when cocultured with nonparenchymal cells, primary hepatocytes survived for several weeks while retaining metabolic function (43). In addition, the liver has an enormous regenerative ability, which precision-cut liver slices may preserve (44). When primary hepatocytes regenerate *in vivo* they will proliferate several times until the mass and function of the organ is restored and then return to the G<sub>0</sub> state (45,46). By comparison, primary hepatocytes procured through enzymatic dissociation replicate once or twice *in vitro* but then demonstrate steady decline and dedifferentiation (47). This observed difference may be due to the disruption of critical cell-to-cell and cell-to-matrix interactions, and implies that nonparenchymal cells and extracellular matrix are necessary for hepatocytes to proliferate and regain function. Precision-cut liver slices preserve the cell-to-cell and cell-to matrix contact of the tissue while recreating the proper ratios of nonparenchymal cells for co-culture (43). Unfortunately, slices of human liver are also extremely difficult to obtain and to be clinically relevant these slices would have to be induced to regenerate *in vitro*. To date this has not been successfully reported in the literature.

One promising source of future hepatocytes that has only recently been investigated comes from the field of stem cells. The term "stem cell" is broadly used to refer to undifferentiated cells of any species that meet the following criteria: 1) they can convert from their undifferentiated form into one or several specialized cell types and; 2) they continue to generate undifferentiated genetically identical daughter cells capable of future differentiation (28,48). These cells may serve as the solution to the problems of inadequate quantity; poor *in vitro* longevity, and genetic incompatibility. Several sources of stem cells show promise for use in the field of tissue engineering including embryonic stem cells and adult mesenchymal stem cells, as well as, transdifferentiated adult mesenchymal stem cells (36,49,50).

Embryonic stem cells are defined as cells obtained from the inner cell mass of the blastocyst stage of embryonic development and have been shown to differentiate into hepatocytes through the application of cytokines including activin A and hepatocyte growth factor (51–53). In a 2006 study by Soto-Gutierrez *et al.*, murine embryonic stem cells differentiated into mature hepatocytes with significantly higher efficiency through co-culture with human nonparenchymal liver cells and were used to reverse liver failure in 90% hepatectomized mice (54). This study demonstrated the broad plasticity of embryonic stem cells, as well as the degree to which they can be regulated through interactions with their environment. Unfortunately, human stem cells of embryologic origin are unlikely to contribute significantly to the clinical applications of hepatic tissue engineering for two reasons. First, these cells require a great deal of technical expertise to differentiate and even in skilled hands typically result in mixed populations and low yields. More importantly, in the United States the subject of embryonic stem cell research has become so politicized that cells of this type are very tightly regulated. Adult stem cells may prove to be the answer to this critical supply of cells for research in general and hepatic tissue-engineering research specifically.

Adult mesenchymal stem cells (MSC) were originally identified in the connective tissue matrices of several species and have recently been found in human tissues that have a high cellular turnover rate throughout life such as bone marrow, blood, muscle, adipose, and dermis (55–59). Of these human sources, bone marrow-derived MSCs (BMSC) have been the most intensely investigated. It was originally thought that MSC differentiation was limited to the MSC germ layer of origin, but recent studies have called this thinking into question. Several reports have shown that adult stem cells can be "transdifferentiated" into cells not normally associated with their committed state (60). In 1999, Bjornson *et al.* demonstrated the conversion, or "fate switch" of neural stem cells into the hematopoietic lineage (61–67). In a 2004 study, Lee *et al.* demonstrated that BMSCs and MSCs from umbilical cord blood differentiated into hepatocytes with glycogen storage, synthetic capacity for  $\alpha$ -fetoprotein, urea and albumin, and inducible p450 function (68). Other examples include reports by Petersen *et al.* 1999 and Oh *et al.* in 2007 that demonstrated hematopoietic stem cells from bone marrow transdifferentiating from the mesenchymal lineage to an endothelial lineage ultimately expressing markers consistent with an hepatic cell type (60,63,69). These promising preliminary studies indicate the enormous potential of stem cell technology in the field of hepatic tissue engineering. However, both of these cell sources have limitations associated with their use: umbilical blood is only available in small quantities; and the harvest of bone marrow involves a painful, invasive biopsy and has a variable yield (70).

Attention has recently been turned to adipose tissue as a potential source of cells for cellular therapies. It can be obtained in large quantities under local anesthesia with minimal patient discomfort (71). MSCs are present in much greater numbers in adipose tissue than bone marrow and have similar growth kinetics and expansion potential (72). Several studies have shown that MSCs from adipose (ADMSCs) can

differentiate into osteogenic, adipogenic and chondrogenic lineages with equal or greater efficiency than BMSCs (72–75). Seo *et al.* showed that ADMSCs could be transformed into hepatocyte-like cells with morphology, albumin synthesis, urea production, and low-density lipids uptake, suggesting their potential as the primary cellular source for liver tissue-engineering applications (76). These initial efforts to redirect ADMSCs down the hepatic lineage are promising; however, no definitive breakthrough has clearly indicated that these cells will relieve the critical shortage of hepatocytes facing the field of hepatic tissue engineering.

## LUNG

Efforts to develop a tissue-engineered lung have only recently begun to gain momentum despite a significant clinical need. In the pediatric population, there is an unmet need for lung transplantation grafts. In 2006, there were 55 pediatric lungs transplanted, while the current waiting list is nearly three times that at 137 patients (77). In addition to the pediatric population, a tissue-engineered lung structure could have a significant impact in the adult population in which lung disease is a significant source of morbidity and mortality. Development of a tissue-engineered lung would address this issue.

Recognition of the efficient transfer of oxygen and nutrients from the vascular chamber to the parenchymal chamber in the liver scaffold has led to a modification of this concept to create a scaffold for development of a tissue-engineered lung. The lung scaffold consists of a vascular network similar in concept to the liver vascular network. A thin, porous membrane separates the vascular network from an adjacent alveolar chamber, which has continuous oxygen flow. Oxygen and carbon dioxide are exchanged between the blood and the alveolar chamber across the membrane. Like the liver scaffold, this scaffold can be scaled up to achieve a desired total size, in this case the important end goal being surface area for gas exchange.

The gas exchange with this scaffold is significant. A single planar scaffold of this design with a surface area of 0.0018 m<sup>2</sup> can achieve oxygen transfer of 0.17 mL/min and carbon dioxide transfer of 0.16 mL/min (data not published). If scaled up to meet the demands of a resting adolescent or young adult this would result in a lung scaffold with 2.8 m<sup>2</sup> of surface area and oxygen transfer of 250 mL/min and carbon dioxide transfer of 265 mL/min. With this early stage work, it certainly appears feasible to achieve significant gas transfer with a tissue-engineered lung scaffold. Significant optimization will lead to achievement of improved gas transfer to meet the demands of an exercising young individual.

To date these scaffolds have been created using silicone or traditional resorbable biomaterials. These efforts have resulted in the effective scaffolds but the mass of polymer required per unit of tissue is significant. If implanted *in vivo* the scaffolds currently would be too thick to achieve the required lung surface area to support the respiratory needs of an individual. Efforts are currently underway to construct these scaffolds using naturally occurring extracellular matrix proteins, possibly combined with resorbable polymer. Early results suggest

that this approach could reduce the thickness of the scaffolds to a more physiologic size.

The first step in testing these extracellular matrix proteins as scaffold material was to develop a resorbable membrane that could be positioned between the vascular network and the alveolar chamber. A thin collagen membrane has been developed for the lung scaffold. When endothelial cells are cultured on one side of the membrane and pneumocytes are cultured on the opposite side, the membrane is functionally similar to the alveolar membrane or air-blood interface in the lung. When these membranes are tested in an *in vitro* gas exchange model, which utilizes flowing fresh blood past the membrane, oxygen and carbon dioxide transfer occurs across the membrane. Initial testing demonstrates that the P<sub>O<sub>2</sub></sub> in the blood increases after flowing by a collagen membrane with pneumocytes and endothelial cells (data not published). The P<sub>CO<sub>2</sub></sub> in the blood decreased under the same conditions. Although significant optimization is required, the combination of strength and thickness of thin collagen membranes and demonstration of gas transfer through them is an exciting step toward the creation of a tissue-engineered lung.

Other investigators have begun to explore strategies to develop engineered lung tissue. Cortiella *et al.* developed porous scaffolds with polyglycolic acid or Pluronic F-127 (PF-127) (78). These scaffolds were seeded with ovine somatic lung progenitor cells and grown *in vitro* and also implanted on the back of a nude mouse or into an ovine lung wedge resection site. Although the polyglycolic acid scaffold supported the growth of the lung cells in a sponge-like open porous structure *in vitro*, when implanted *in vivo*, the inflammatory reaction, which was generated in response to the polymer. However, when PF-127 and the somatic lung progenitor cells were implanted in these locations tissue developed which was morphologically similar to lung alveolar tissue. These results are promising steps toward the creation of distal lung tissue.

Andrade *et al.* also have achieved “alveolar-like structures” in an *in vivo* lung tissue-engineering model by employing a simple porous scaffold (79). In this work, Gelfoam sponges were seeded with fetal rat pneumocytes and injected into a healthy lung of a rat. The sponges were still present at 60 d with an open porous structure within the sponge resembling native lung alveoli. Angiogenesis occurred in the sponge evidenced by vascular structures, which were in communication with native lung vessels. This study again highlights the ability of native lung cells to regenerate into a morphogenic lung structure.

In similar *in vitro* work, Chen *et al.* achieved alveolar-like structures by growing fetal rat lung cells on a collagen-glycosaminoglycan tissue-engineered scaffold (80). At 2 wk, 50–60 μm alveolar-like structures were present in this open porous scaffold.

These studies have demonstrated that morphogenically characteristic lung tissue can be formed using lung cells and open porous scaffolds. This progress and the demonstration of significant gas exchange in prospectively designed vascularized scaffolds in our lab are exciting advances in this budding area of lung tissue engineering. Future efforts will hopefully

advance both of these approaches and perhaps achieve a tissue structure, which could be used to address the clinical needs of pulmonary failure in the pediatric population.

Unlike the liver, lung support technologies to date have not used cellular material. Thus, the consideration of sources of lung cells for tissue-engineered lung applications has not yet been addressed as widely in the literature. Several possibilities exist including autologous or allogeneic lung cells expanded *in vitro*. Researchers have demonstrated the derivation of type II pneumocytes from human embryonic stem cells (81). This or other stem-cell-based approaches may be feasible.

Perhaps the most significant impact tissue-engineered lung development could have on the pediatric population is the creation of a respiratory device for preterm infants. Utilizing the vascularized scaffold approach, a tissue-engineered lung is being developed as an external temporary lung replacement device. Lung insufficiency and respiratory failure are the leading cause of death in preterm infants. Lungs of preterm infants are underdeveloped and can be significantly damaged with standard ventilation practices, which are currently necessary for the infant's survival.

Our approach is to develop a tissue-engineered lung, which could completely support the infant for weeks or months following birth. This technology would obviate the need to intubate preterm infants at birth. Following birth of a preterm infant, the umbilical vein and umbilical artery would be immediately cannulated and these vessels would be the blood supply and return for the tissue-engineered lung. For this approach, the tissue-engineered lung scaffold would need to include a completely endothelialized vascular network so blood could flow through the scaffold without the need for any anticoagulation of the infant. This would make the therapy available to any gestational age or weight infant. Extracorporeal membrane oxygenation is alternative means of lung support in premature infants but has traditionally not been used in low-birth-weight infants due to the risk of hemorrhage in this patient population. Although recent data suggests that extracorporeal membrane oxygenation can be used in infants with birth weight as low as 1.6 kg, the incidence of hemorrhage and intracranial bleeding is higher in this patient population (82). In theory, an endothelialized tissue-engineered lung will overcome this limitation by avoiding anticoagulation. Many factors surrounding this approach remain unanswered including the persistence of fetal circulation *ex utero*. Despite the technological and physiologic challenges ahead, this approach may be a solution to reducing the respiratory morbidity and mortality of preterm infants.

The field of tissue engineering has the potential to provide a myriad of tissues for future clinical applications. At present, the only tissues in clinical use have low metabolic rates such as bone, cartilage, and skin. However, a vascularized scaffold will likely be required for the clinical replacement of a highly metabolic tissue such as a solid organ. Such a structure should be the focus of research for the development of a tissue-engineered liver or lung.

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