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Eliminating the organ transplant waiting list: The future with perfusion decellularized organs

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In 2008, Ott et al[1] harvested adult rat hearts, stripped away the cells (decellularization), and perfused those same decellularized hearts with neonatal rat cardiac cells. After 8 days in paced culture, the recellularized hearts beat again. Ott et al[1] had developed a novel method to decellularize and primitively recellularize an entire organ while preserving the vasculature and extracellular matrix (ECM) and, therefore, the overall structure of the organ. Termed perfusion decellularization, a new paradigm for bioengineered organs was created.

To address the need for transplantable whole organs, the field of tissue engineering was created, and researchers have been developing methods to bioengineer organs for decades. Fundamentally, an organ consists of cells living within a vascularized ECM. Creating a bioengineered organ requires cellularization of a human-scale scaffold. The scaffold can be made of synthetic and/or biologic polymers but must have the correct functional architecture of the organ of interest. Importantly, the scaffold plays a critical role in the success of a bioengineered organ. By using a xenogenic source, such as a pig, the source, age, size, and quality of the scaffold can be controlled tightly while providing a virtually endless supply.

At a minimum, the scaffold must have the native architecture of the organ of interest, be able to support cell engraftment, and promote cell survival through the delivery of oxygen and nutrients to even the most remote locations within an organ. While the latter point may seem trivial, oxygen and nutrient delivery have been a substantial challenge to bioengineering thick (≥3 mm), dense organs, such as the liver or kidney.

While tremendous progress has been made over the last decade in the field of tissue engineering and regenerative medicine, especially on the cellular level, much of the promise of using these tools to create entirely biologic, 3-dimensional, functional structures for implantation has remained unfulfilled. Other methodologies, such as 3-dimensional printing and transgenic pigs, have made strides but also have encountered difficulties that have kept them distant from the clinic. The invention and successful application of perfusion decellularization and recellularization (decell/recell) has demonstrated its potential as the keystone technology that alters this paradigm and enables the creation of fully biologic human organs for transplant.

The history of the medical device industry is awash with mechanical and electrical constructs aimed at organ function replacement; however, these nonbiologic devices have been largely unsuccessful, because they were intended to mimic one or more functions of a particular organ, though not the organ in its entirety. Humans, unlike medical devices, are not made of metal and plastic. Medical devices have targeted the treatment of a disease, while regenerative medicine has the potential to offer a cure. If one truly wants to replace the function of a human organ, then why not start with a fully functional, healthy human organ? In the thousands of instances in which donor organs are not available for transplant, perfusion decell/recell focuses on a 100% biologic solution to this problem aimed at a full return to health, as opposed to a temporary mechanical repair.

Perfusion decellularization, as first demonstrated in 2008,[1] encompasses a method to decellularize and recellularize whole or partial organs and

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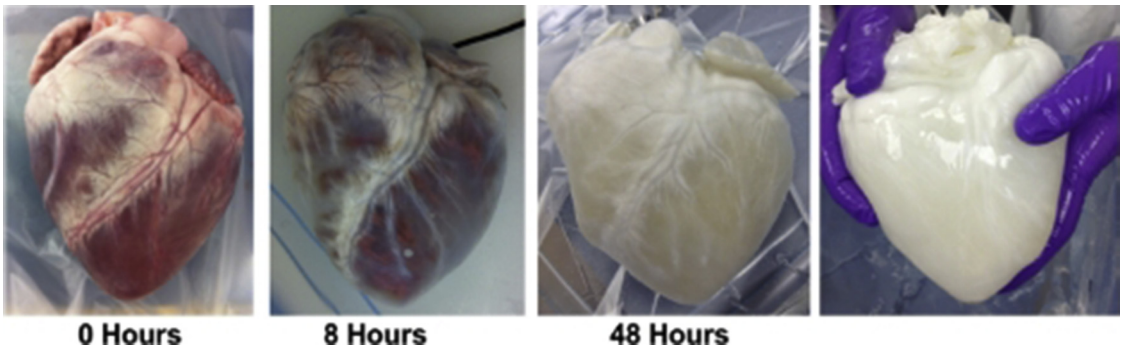

Fig 1. Decellularization of an adult porcine heart over 48 hours. A 6-month-old porcine heart undergoing perfusion decellularization over a period of 48 hours. The native structure and vasculature are preserved after decellularization. After 48 hours, the heart is completely decellularized. This Figure is reproduced with permission from Miromatrix Medical Inc.

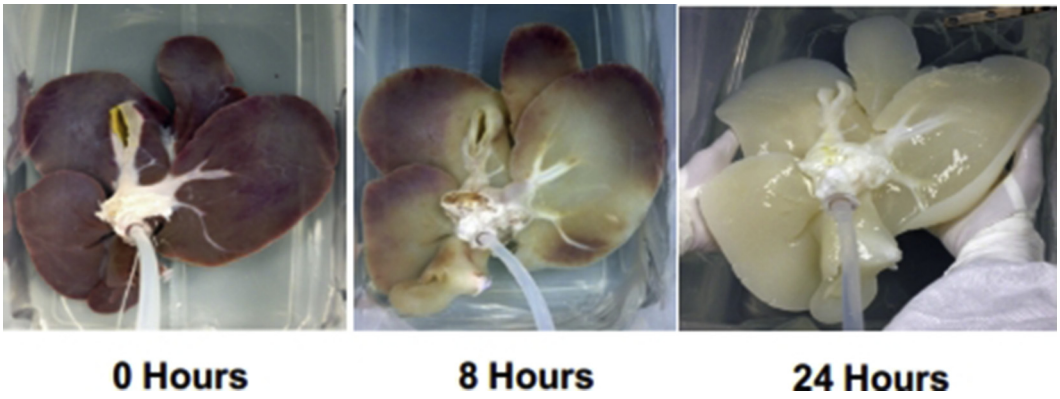

Fig 2. Decellularization of an adult porcine liver over 24 hours. A 6-month-old porcine liver undergoing perfusion decellularization over a period of 24 hours. The native structure and vasculature remain preserved after decellularization. Figure is reproduced with permission from Miromatrix Medical Inc.

tissues. The technology is based on a proprietary method for removing all cells while maintaining an ECM or scaffold with its original architecture, mechanical properties, and a vascular network capable of maintaining physiologic pressures. In the field of regenerative medicine, biologic efforts using porcine, bovine, and human donor material have achieved only limited success, because they are constrained by the available technology.

The most widely recognized method of removing cells in use today is immersion decellularization, in which an organ is placed in a container of detergent. This method is diffusion limited and often employs the additional use of mechanical or enzymatic methods which damage the capsule of the organ. Cells within the organ begin to break down before being exposed to the detergent, releasing various enzymes that also can degrade important functions of the surrounding scaffold. The end result is a partially degraded scaffold with a compromised vascular network and an outer organ capsule that will not maintain physiologic pressures when tested.

Perfusion decellularization is in contrast to immersion decellularization and overcomes the hurdles of immersion by facilitating rapid access to the whole organ through the native vasculature by cannulating the vasculature and perfusing a mild detergent solution through the native blood vessels. Because organs are dense with vascular capillaries, most cells are located within 50–100 μm of a capillary, resulting in an exponential increase in the effective surface area of the detergent and decreased time to dissolve the cellular material as it is expelled through the venous system, as opposed to through the organ wall or capsule. In short, this 1 or more day process, depending on type and size of the organ or tissue, utilizes the organ's natural plumbing to remove rapidly the cells from the inside out (Figs 1 and 2).

More importantly, the end result is a completely preserved native scaffold containing the appropriate microenvironment required for the introduction of organ-specific cells, along with an intact vascular network to reconnect to the patient's blood supply and an outer capsule capable of maintaining physiologic pressures. These components are critical for the later use of perfusion recellularization, which also uses perfusion to repopulate vascular and organ-specific regenerative cells onto the organ, where they migrate to the appropriate microenvironment (via the relevant signaling protein markers that remain within the perfusion decellularized scaffold) as the organs are grown and matured in bioreactors under normal physiologic conditions. The resulting organs then can be transplanted utilizing the same techniques as current organ transplantation.

Scaffolds created by perfusion decell/recell are capable of receiving and incorporating a variety of cell on the organ scaffold utilized. As our understanding of cell isolation and maintenance continues to grow, the fact that scaffolds created with this technology are of a natural, biologic design makes them an ideal template to support the implantation, growth, and differentiation of stem cells into functional tissues, organs, and bioidentical test beds.[2]

In 2008, Ott et al[1] reported the use of perfusion-decellularized rat hearts to create whole bioengineered hearts that, when reseeded with neonatal cardiac rat cells, were able to beat synchronously under electric stimulation. These were groundbreaking results, in that not only were they able to produce a decellularized whole heart scaffold, but they were able to create a beating heart by seeding the scaffold with a heterogeneous population of cardiac cells rather than specific cell types. Specific cell types (such as endothelial cells) were able to migrate to the appropriate tissue (vascular endothelium) autonomously, further highlighting the importance of preserving the native ECM during decellularization to protect any biochemical and mechanical cues that assist with recellularization.

Since this article was published, researchers have continued this work in several types of organs, including heart, kidney, liver, and lung. The ultimate goal is to repopulate a decellularized organ with a patient's own cells, thereby avoiding any issues of immunogenicity. To this end, researchers are examining repopulation of decellularized scaffolds using various cell sources, including stem cells. Decellularized rodent hearts have been repopulated with various stem cells.[3,4]

Human embryonic stem cells and cardiac progenitor cells were used to demonstrate that perfusion-decellularized cardiac scaffolds can direct stem cell differentiation. After static culture, seeded cells demonstrated expression of cardiac-specific markers and expression of myosin heavy and light chains. CD31+ cells also were identified, suggesting differentiation of stem cells into vascular endothelial cells.

Using perfusion decellularized mouse hearts, another group[3] demonstrated differentiation of induced pluripotent stem cell–derived cardiovascular progenitor cells into cardiomyocytes, smooth muscle cells, and endothelial cells. After 20 days of culture, the repopulated hearts exhibited asynchronous, spontaneous contractions and limited contraction force and were responsive to pharmacologic stimulation. Research also has progressed with the perfusion decellularization of clinically relevant porcine hearts, as well as human hearts.[3]

Perfusion decellularized rat kidneys have been seeded with human endothelial cells and rat neonatal kidney cells. After 12 days in culture, evidence of neonatal kidney cells homing to appropriate locations within the scaffold was observed, such as podocytes homing to glomerular regions. In vitro, the bioengineered kidneys were able to excrete urine, albeit at decreased levels compared to cadaveric kidneys. The recellularized kidneys were transplanted orthotopically in rats and demonstrated urine production, but with decreased creatinine clearance with fractional resorption of electrolytes.[5]

Perfusion decellularized rat livers have demonstrated preservation of basement membranes and key ECM constituents of the extracellular matrix (collagen types I and IV, fibronectin, and laminin).[6] These livers were seeded with endothelial cells and primary rat hepatocytes and cultured in vitro for 10 days. Cultured organs revealed repopulation with both hepatocytes and endothelial cells and exhibited synthesis of urea and albumin, although at decreased levels. The livers were transplanted briefly into rats and retained hepatic function and blood flow. Other groups have repopulated liver scaffolds using alternative cell sources, such as human fetal hepatocytes, stellate cells, and human induced pluripotent stem cells.[7,8]

Two groups have perfusion decellularized rat lungs and seeded them with rat fetal lung cells, endothelial cells, and, in one case, pulmonary epithelial cells.[9,10] Histologic evaluation showed the presence of lung-specific cell types distributed appropriately within the scaffold. For example, type II pneumocytes were observed within alveolar

walls. After only 5 days in culture, the lungs demonstrated gas exchange similar to native rat lungs. Bioengineered rat lungs were transplanted orthotopically into rats and demonstrated gas exchange and similar mechanical characteristics to native lung for short periods. Several studies also have been published examining the use of alternative cell sources, such as embryonic stem cells, mesenchymal stem cells, human fetal lung cells, and induced pluripotent stem cells.[7,8]

As highlighted here, great progress has been made in creating whole, transplantable organs. However, creating a whole transplantable organ is not a trivial task, and these various research groups—as well as one commercial company, Miromatrix Medical Inc, the patent holder on perfusion decell/recell—face common challenges, including optimizing decellularization to preserve the native mechanical and biochemical environment, identifying appropriate cell sources, recellularizing the scaffold with appropriate cell numbers, and maintaining the recellularized organ in vitro.

The promising news is that all of these challenges appear to be addressable, with some of these challenges already being addressed today. It is also important to note that while perfusion decell/recell is not necessarily the only method by which the organ transplant waiting list can be eliminated, it likely will be the fastest and most cost-effective. As a reminder, a bioengineered organ requires both a scaffold and cells to be functional. With other tissue engineering methods, substantial time and money is spent developing the scaffold so that it has both the appropriate mechanical and biochemical environment. Perfusion decell/recell achieves this in much less time and with fewer resources, and yields a structurally accurate scaffold with the native vasculature intact, thereby enabling the field to progress toward the clinic at a much faster rate.

As bioengineered organs progress toward the clinic, it is exciting to envision the possibilities this creates. While the end goal of perfusion decell/recell is the ex vivo recellularization of the decellularized matrix with the intended recipient's own cells, thereby creating a transplantable organ and avoiding any immunogenicity issues, there are many clinically useful products that can be created on the way to this eventuality.

When considering a first liver construct, it is important to note that, at the moment, patients in end-stage liver failure have no approved treatment methodologies. There are no devices and no drugs, and death is inevitable within a few months without a transplant. In order to survive, such a patient needs only an estimated 15–20% liver function; and, for excellent health, only 30% liver function is required. In addition, the liver provides a unique (among the solid organs) "assist" to the regenerative process by regenerating its own cells. These cells are invaluable—as long as there is a viable matrix on and within which the cells can survive.

The same sort of development path could be applied to kidney. With the kidney, however, the target patient population would not solely be those awaiting an organ donor for transplant. Rather, a substantial number of individuals currently on dialysis could be candidates for a kidney transplant if the supply of kidneys was unlimited. The current paradigm of organ transplantation as a last resort and admission to the organ transplant waiting list only for the sickest of patients could become a problem of the past. Kidney transplants for all of those in need would mean a "cure," as opposed to years of dialysis and the attendant decline in quality of life and health.

In conclusion, the use of perfusion decellularization as a tool to generate architecturally exact, biochemically active scaffolds with organ-appropriate mechanical properties should change the landscape of regenerative medicine. In the short duration since the first report of perfusion decellularization, this concept has advanced the field of tissue engineering and regenerative medicine from thin constructs to implanting recellularized lungs, livers, and kidneys into small animals. The advances achieved with perfusion decell/recell are making tissue-engineered whole organs a close reality.

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