Commercialization of the DECELL|RECELL Technology Platform

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The goal of cell therapy is to repair, replace, or regenerate diseased tissues and organs. Two key components necessary in the design of new tissue are cells optimized for each application and scaffolds (matrices bioengineered to support the growth of cells into functional tissues and organs). Earlier reports by the authors and others have described the current state of the art with respect to cell types.[1,2] It is this second area, optimal scaffold source and design that is the focus of this summary.

Three key features are required to create a 3D tissue that can successfully substitute for a naturally occurring organ/tissue. These include 3D functionality encompassed by correct geometry and appropriate architecture; the capacity to be perfused to provide nourishment to cells throughout the tissue or organ; and the ability to be populated with the appropriate cell type. While the fabrication of materials to provide 3D templates for cell seeding and growth has been the focus of much research, no single approach has provided a widely applicable solution for perfusable whole 3D tissues or organs.[3-5] At present, engineering a 3D bioartifical tissue, much less organs from cells, remains a fairly crude and unsuccessful process, in large part due to the complexity of the native tissue. In this report, we describe a proprietary technology for both the creation of 'universal scaffolds' that are the optimal solution for 3D architecture and perfusibility requirements, as well as the repopulation of these scaffolds to create nascent functional organs. This universal scaffold, coupled with the cell type of choice, encompasses the DECELL|RECELL technology – a single approach that has broad applications ranging from research tools to organ transplants.

What are the Hurdles in Developing Bioartificial Tissues?

The first hurdle is the lack of a biologically-correct complex organ scaffold that can recapitulate the function of the whole tissue. In most organs, cells are aligned in a precise fashion that impacts function, such as pumping of blood in heart, filtration of toxins in liver or cleansing of blood in kidney. Presently, most tissue engineers use an artificially created mix of biologic or synthetic materials to build matrices to the shape and approximate 'structure' of their organ or tissue. However, the scaffold composition is usually far simpler than the organ of interest. For example, attempts to engineer heart muscle tissue have primarily focused on generating contractile rings and tissue sheets, patches, or even tissue 'cones' shaped like ventricle made from simple off-the-shelf artificial matrices combined with cells.[6-8] These not only lack the organization and structure of the normal tissue, but lack the capacity to support multilayer thick regions of cells. A naturally occurring 3D anatomically correct scaffold would overcome this hurdle.

A second major hurdle to creating a nascent organ is the need for a vascular bed within that tissue to 'feed' the cells within. Again, considering the simple setting of patches or contractile rings, creating 'thick' (>100-200 micron) patches has been limited by an inability to generate a product able to support the high oxygen and energy demands of cardiomyocytes at a depth greater than ~100-200 microns from the surface of the matrix [6]. Building artificial channels into the extracellular matrix (ECM) constructs using biochemical oxygen carriers and stacking thin cardiac sheets have been used to increase thickness but none of these are physiologically relevant. They have however, reinforced a direct relationship between the ability to perfuse cardiac tissue and the ability to generate a larger graft or to increase cell density.[6-8] Again, a cadaver-derived whole-organ scaffold that can be decellularized to give rise to a perfusable matrix would appear to overcome this hurdle.[9]

The third key component is an appropriate cell source. Identifying an accessible and adequate cell source is an equally critical aspect of engineering tissues. Currently, no one source of cells has emerged as the single best, optimal, or widely applicable stem cell type. While the business community believes allogenic (banked lowest cost, immediately available cells) to be the preferred cell type there is, as yet, no clear clinical evidence that allogenic cells can deliver clinically useful outcomes.[2] Autologous cell transplantation has been shown to be both safe and effective, but is applicable only to each individual. In addition, a number of groups are working with what appear to be partially or fully immune privileged cells.[2,10-12] A commercial business model based on a bank of allogenic cells may emerge. However, equally likely is the development of effective, efficient, and economically viable techniques or kits to harvest and purify viable autologous cells as a source for creation of bioidentical tissues and organs. From this and in contrast to the specific biomaterial-cell relationships described in other tissue engineering systems, a key attribute of the DECELL|RECELL technology is the

ability to serve as a platform to be coupled with a variety of potential cell types in the development of a multitude of tissue and organ types.

Nature Provides the Optimal Template in Creating Bioartificial Tissues

Recently, Taylor and colleagues at the University of Minnesota published the characterization of the bioartificial heart construct in which the three major milestones required to engineer a bioartificial organ had been met:[9]

- An engineered construct that provides bioidentical 3D architecture and geometry
- Reperfusion of a bioengineered organ
- Repopulation of this construct with an appropriate cell type

In consideration of the two major limitations in tissue engineering – the difficulty in producing both a perfusable and 3D construct – the authors adopted a novel approach (DECELL|RECELL) to achieve these milestones. Because nature has engineered the optimal scaffold that serves as a basis of a heart, they hypothesized that they would be able to wash out the cellular components of the myocardium to retain the 3D matrix. Then, because the major vascular conduits would remain in place, they could recellularize the decellularized myocardial scaffold to in essence mimic the developmental process. Finally, as the construct matured, the recellularized LV wall would be capable of contractile force with reasonable synchronicity. In this way, Ott *et al*. created a whole heart scaffold from rat containing vascular conduits that can be perfused with blood and/or tissue culture media, and that keep cells alive at thicknesses 5 to 10 times those previously reported.[9] Adult cardiac derived cells have been transplanted into this construct and over a period of days begin to contract in the nascent organ within a bioreactor. This is the basis for the DECELL|RECELL Technology Platform, a system for the generation of 'universal scaffolds' which retain the bioidentical 3D architecture and reperfusion capability coupled to the flexibility to accept any cell type of interest.

DECELL|RECELL Technology Platform

Broadly, the DECELL|RECELL approach encompasses a method to decellularize and recellularize whole or partial organs and tissues. The technology is based on a unique and proprietary methodology to remove all cellular constituents while retaining the extracellular matrix and its biologic cues, organ 3D structure and geometry, and vascular conduits. The resulting 'universal scaffold' created by DECELL Technology can be used to generate functional tissues and organs by using the intact vascular conduits to both deliver cell types of interest RECELL and feed the bioartificial system. By applying the DECELL|RECELL Technology to a human-sized cadaveric heart, Taylor and colleagues have proven this method effective in creating (*See Figure 1*):

- A complex, biocompatible cardiac ECM scaffold with a perfusable vascular tree, valves and a four-chamber-geometry that can be subjected to whole heart physiology (preload, afterload and pacing);

FIGURE 1
Decellularized Pig Heart

- A controlled process that can be manipulated to yield fully or partially decellularized tissues and organs;
- A technology that can be scaled to organs of human size and complexity.

Further the heart construct is recellularized to produce a first-generation organ that:

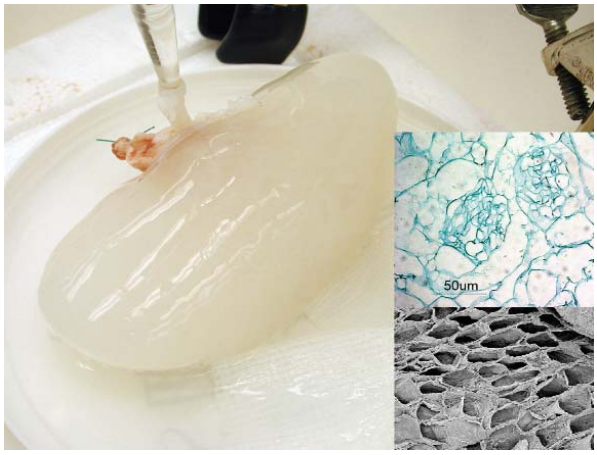
- Provides a biocompatible environment for cell proliferation, migration and differentiation both in the wall of the organ and in the vasculature;
- Creates an environment in which cell-cell interaction occurs readily as evidence by connexin43 expression and synchronous contraction;
- Can be fed via coronary perfusion with media;
- Is superior to two dimensional tissue culture of the same matrix and cells at yielding a beating tissue ring;
- Pumps media *in vitro* against an artificial blood pressure;
- Responds to contractility-inducing drugs such as phenylephrine appropriately;
- Responds to pacing with synchronous contraction *in vitro*;
- Matures *in vitro*, if treated under physiologic cardiac conditions;
- Can be designed with immunocompatible or autologous cells.

The DECELL|RECELL Technology Platform lends itself broadly to diverse product development ranging from 3D cell-based research tools for drug or stem cell discovery and development, to bioartificial tissue and whole organ systems. Although decellularization is not a novel methodology, perfusion decellularization of an intact organ is novel and compelling in that it allows controlled removal of cellular components and results in an anatomically correct organ ultrastructure,

with the complexity, beauty, mechanical properties and to some degree the biology (or at least apparent biologic cues) of the original tissue (*See Figure 2*). In addition, perfusion decellularization can be practiced on whole or partial tissues by localizing catheters into sub-sections of organs or tissue, and can be used for partial removal of cells from any organ or tissue. Finally, by definition perfusion decellularization allows retention of vascular access to the scaffold both for the decell process but also for perfusion of the recellularized tissue and for connecting the nascent organ to a recipient's blood supply. In other words, by using the blood vessels to deliver detergent and wash out cells, perfusion decellularization is a controlled process that yields a 3D, anatomically intact organ scaffold that has a (decellularized) vascular network which can in turn be used both as a pathway to recellularize and to feed the nascent organ and also to ultimately sew that organ into a recipient. Thus 3D geometrically and architecturally accurate whole organ scaffold creation, vascular access, and flexible cell sourcing are three important advantages of the DECELL|RECELL process.

FIGURE 2

Decellularized Pig Kidney Upper Inset: Movat Pentachrome stained light micrograph of decellularized renal cortex. Lower Inset: Scanning electron micrograph of decellularized renal medulla.



Commercialization

A significant and unique benefit of the DECELL|RECELL Technology Platform compared to other approaches in tissue engineering is the uncoupling of the matrix formation (scaffold) with the cell source (tissue/organ selection). The creation of a universal scaffold from virtually any perfusable tissue that can accept a stem cell from any source (including adult, allogenic, autologous, haplotype-specific, or disease-specific) is one the most compelling benefits for the broad application of this foundational technology. Critical to moving the innovative DECELL|RECELL Technology Platform from bench to bedside is a strategic commercialization plan for both the universal scaffold applications themselves, but also timely and calculated partnership(s) for access to appropriate stem cells for tissue and organ creation.

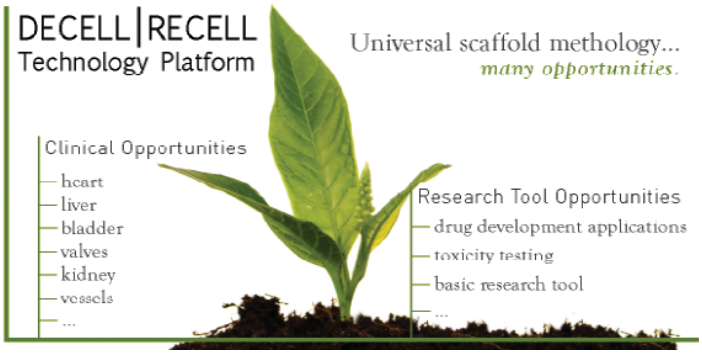
In a recent review, Pearson *et al.*, describe that the medical benefits of engineering tissues fall in three categories:

- The supply of tissues for implantation into a patient to restore a function (e.g., blood vessels, valves, liver, pancreas, bladder, cartilage);
- For the manufacture of extracorporeal devices (i.e., liver, kidney);
- To manufacture complex tissue cultures for toxicological and pharmacological assays.[13]

Fundamental to this diverse set of products would be the ability to utilize the DECELL|RECELL technology to create a set of universal scaffolds that would become the starting material for engineering the breadth of complex tissues and organs – heart, liver, kidney, muscle, pancreas, toxicology tools (*See Figure 3*).[9] The DECELL scaffold is a flexible donor for, potentially, a multitude of cell types – insomuch as the necessity of a dominant design in cell type is not requisite.[1] Moreover, as cell type discovery continues to grow, the development of a scaffold designed by nature and engineered as universal will prove to be an ideal template to support the growth of stem cells into functional tissues, organs and bioidentical test beds. For purposes of this article, we will focus on two products that could be developed from this Technology Platform.

FIGURE 3

DECELL|RECELL Technology Platform



Product: Research Tool

Promising pharmaceutical compounds move into costly clinical trials (or beyond) only to be halted by safety problems that could have been detected in preclinical models. Moreover, as the pharmaceutical industry has begun to adopt a disease-based approach to discovery, in which the understanding and the treatment of underlying human pathology is emphasized, the need for more accurate, efficient, and versatile human safety model systems has emerged. Stem cells have emerged as important new tools for developing unique, *in vitro* model systems to test drugs and chemicals and a potential to predict or anticipate toxicity in humans.[14,15] Hepatotoxicity is the most common reason for non-approval or drug withdrawal by the FDA and currently, there are no *in vitro* liver models that provide safety data in a 3D renewable, versatile human model system. Presently, pharmaceutical safety testing concurrently utilizes hepatocytes derived from cadaveric, animal, and immortalized cell lines to determine drug safety profiles. An increase in pharmaceutical R&D productivity would be gained as potentially one model system would

be used and advantages of this system are as follows:

- Normal, hepatic function depends in part on an organ's 3D structure and this model would allow for more predictive early data thus a better cellular substrate.
- Direct cell contact among cells as well as between cells and the ECM would be achieved in the 3D block. This feature would enhance normal cellular behavior and potentially drive much longer *in vitro* survival and retention of the differentiated phenotype.
- Since cultures can be maintained for longer analysis periods, for the first time the study of chronic conditions, long term drug use and more extensive testing on drug-drug interactions is enabled.
- Human cells would be utilized as they more closely resemble the in vivo environment and the model components would be renewable, versatile and variable.
- High throughput screen for safety using cells from a variety of disease-specific or haplotype specific backgrounds would identify safety concerns early in drug development.

In addition, the utilization of cellular models would be welcomed by the FDA because the current pharmacological section of an NDA provides limited information with regards to the toxicity of the drug. A DECELL|RECELL based model would expand the evaluation of candidate molecules very early in drug development and will identify cell toxicity earlier than functional data.

Product: Bioengineered Heart

According to statistics published by the American Heart Association in 2008, about 57,000 Americans die each year from end-stage Congestive Heart Failure after having exhausted current treatment options. Given the small number of donor hearts available, physicians must apply strict criteria to determine which patients can be listed for transplant. Patients over 70 years of age, or those with severe co-morbidities such as liver failure or cancer are not candidates, although they could receive bioengineered organs. Currently, approximately 40,000 people in the USA under 65 years of age could benefit from a heart transplant while only about 2000 donor hearts become available and are transplanted each year (UNOS). Even among the group of patients that are placed on the transplant list, many die during the long wait for an appropriate donor organ. There is, therefore, a sizeable population that currently has no treatment options. Further and often overlooked, once transplanted, many patients trade their organ failure for a new disease, such as hypertension and long-term renal damage, and in the case of malignancies, diseases resulting from sustained immunosupression.

Scientists and engineers have attempted to fill this need with mechanical and tissue-engineered organ replacements with minimal long-term success in most cases. Thus, the ability to generate a bioartificial organ of the complexity of a human organ is a huge unmet clinical need. A bioartificial heart is a theoretical alternative to transplantation of donor material or mechanical left ventricular support. Generating a bioartificial heart requires engineering of cardiac architecture, appropriate cellular constituents and pump function.

Again, engineering a tissue or organ requires three components:

- Cells that can give rise to the needed cell types within that organ or tissue;
- A biologically-relevant biocompatible matrix into which to place the cells; and
- A method by which the cells can be fed throughout the depth of the new tissue.[16-18]

In proof-of-concept rat studies, the DECELL|RECELL technology has demonstrated that a whole heart scaffold with intact 3D geometry and vasculature can be created and subsequently repopulated with neonatal cardiac cells resulting in the formation of functional contractile myocardium.[9] And finally, while it will take significant effort to achieve, development of a bioartificial heart will dramatically increase the supply of whole or partial organs to meet unmet demand.

Conclusion

Commercializing the innovative perfusion-based whole-organ (DECELL|RECELL) Technology will bring products to market such as bioartificial tissues and organs that can be used either *in vitro* as tools to evaluate the safety and efficacy of bioactive agents in human systems; or *in vivo* as organs or tissues for transplantation. The proof-of-concept studies in rat demonstrating the ability to derive a four-chambered intact perfusable heart matrix, partially recellularize it with cardiac cells, and begin to mature it *in vitro* to generate a beating pumping drug responsive nascent organ construct is a powerful step forward. This technology has recently been expanded to decellularization of human-sized (adult pig) heart, kidney, and liver/gallbladder, demonstrating partial proof of concept. In this straightforward, simplistic and innovative manner the DECELL|RECELL Technology Platform is fundamental to generating universal scaffolds that can be seeded with any and all stem cells desired. The end products will be bioidentical tissues and organs that will allow detection of potentially toxic agents *in vitro*, advance safe pharmacologic agents to market, and create viable therapeutic options for individuals awaiting donor organs.

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