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Organomatics and organometrics: Novel platforms for long-term whole-organ culture

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Abstract

Organ culture systems are instrumental as experimental whole-organ models of physiology and disease, as well as preservation modalities facilitating organ replacement therapies such as transplantation. Nevertheless, a coordinated system of machine perfusion components and integrated regulatory control has yet to be fully developed to achieve long-term maintenance of organ function *ex vivo*. Here we outline current strategies for organ culture, or *organomatics*, and how these systems can be regulated by means of computational algorithms, or *organometrics*, to achieve the organ culture platforms anticipated in modern-day biomedicine.

INTRODUCTION

The empirical sciences have classically used *in vivo* and *in vitro* models to simulate particular aspects of physiology and disease. Through the integration of physics and chemistry into *in vitro* and *in vivo* biomedicine over the last decades, interrogation of phenomena at ever-diminishing scales is possible. We are now able to find cells of interest — such as circulating tumor cells or stem cells — amongst myriad other cells, then capture, culture, and subsequently analyze them at the molecular level^{1,2}. Even visualization of molecular trafficking between organelles is well within our capabilities^{3–5}. Advances at these diminishing scales, however, underline our relative weakness at physiologically relevant tissue scales — the scale that comprises a multitude of cell types in specific three-dimensional arrangements over time⁶. Although the value of *ex vivo* experimentation of whole organs or organ constructs is greatly appreciated, a controlled, sustainable, and spatiotemporally accurate system to recapitulate human pathophysiology is not yet readily available.

The physiological scale assessment of organs or tissue constructs can be considered the unfulfilled promise of *Tissue Engineering*. Thirty years after the term was coined, not only are we not able to create functional organ mimics for clinical use, but with few exceptions even accurate substitutes for use as *in vitro* tissue and organ models remain unreachable. Co-culture of multiple cell types is still a novelty that is rarely very practical, does not necessarily represent natural inter-cell relationships accurately, and neglects crucial cell-

matrix relationships altogether⁶. Comparatively little is understood about how two different cell types communicate and collaborate in the body in conditions of health or disease. The use of microfluidic systems and photolithographic techniques to create more realistic tissue models is emerging, but remains a drastic simplification of *in vivo* cell biology^{7,8}. Attempts at maintaining or recreating the architecture of tissue through the use of biological^{9,10} or engineered¹¹ matrices are gaining ground, but major obstacles still impede their use⁹.

While attempts at building tissues and organs from the ground up remain in infant stages, the diagonally opposite approach of maintaining isolated whole organs is showing potential after years of stagnancy^{12,13}. Although over 100 years has passed since Nobel Laureate Alexis Carrel described his pioneering work on “*the permanent life of tissues outside the organism*”¹⁴, very little progress in sustaining and preserving tissue function for extended durations has been made since. Nonetheless, current demand for improved organ quality for transplantation is pushing towards a paradigm shift in the preservation of solid organ grafts. While hypothermia and specialized preservation solutions have been a sufficient modality for bridging the *ex vivo* period for many years, present endeavors seek to replace conventional static cold storage with dynamic *machine perfusion* techniques^{15,16}. Rather than simply slowing the inevitable deterioration of an ischemic organ, machine perfusion aims to create an extracorporeal pseudo-body that supports and retains organ viability during transportation¹⁷, or attempts to recover function during a short pre-transplant treatment phase¹⁸. This applies predominantly to organs from untapped sources. Marginal organs, such as those donated after cardiac death, are often unusable when conventionally preserved, as this preservation adds additional injury. Ultimately, the objective of restoring these rejected organs and using them for transplantation will result in lower discard rates and diminution of transplant waiting lists^{19–21}.

While those developing machine perfusion techniques have the primary focus of expanding the donor organ pool for transplantation, metabolically supportive organ preservation may have significant secondary benefits for biology and medicine. These systems and protocols may offer viable long-term culture of isolated organs, making this a practical and economical alternative to cell culture. Standardized, sustainable, and functional *organ culture* systems, which we refer to as **organomatics**, will allow us to keep an organ under physiological conditions for days to weeks or longer, dynamically analyze its function across the scales of omics, evaluate perturbational responses, and ultimately manipulate this function with the aid of advanced computational algorithms — an endeavor we term **organometrics** (Fig. 1). Here, we will summarize the current state-of-the-art technologies that shape organomatics and organometrics and form the foundation of organ culture. The potential applications of organ culture as a standard operating procedure will be discussed as well as the challenges that need to be resolved. The hierarchy of needs to approach organomatics and organometrics as a platform technology is illustrated in Fig. 2.

THE ORGANOMATICS PLATFORM

The first challenge in establishing a model of any biological phenomena has always been to sustain the biological functions with adequate fidelity in a controlled environment. For isolated organs, this translates to the need for hardware that will accomplish the equivalent

of blood perfusion through the tissue, providing adequate oxygenation and nutrition and clearance of waste products. These requirements have provided the framework for typical *ex vivo* machine perfusion systems, which will need to be improved to the point that the organ function can be retained with minimal deterioration for extended periods, during which the organ can be considered stable in a relevant functional state.

Machine perfusion of organs has been used as an isolated whole-organ model for nearly a century²² as well as the organ preservation method for transplantation prior to the introduction of specialized cold storage solutions^{23,24}. Whole-organ perfusion for transplantation has typically been investigated at three temperature ranges: hypothermia (0–10 °C), subnormothermia (20–28 °C) and normothermia (37 °C). All modalities have their advantages and disadvantages. Hypothermic perfusion was the first to be translated to clinical organ preservation as it comes with minimal risks when the machinery malfunctions and has shown promising results for kidney preservation¹⁶ and pilot studies in the liver^{25,26}. The risk of accidental warm ischemia has led to a cautious approach to normothermic perfusion. However, sustained function of the organ at warm temperatures allows the organ to be more accurately tested for viability prior to transplantation and is one of the reasons that normothermic perfusion is gaining interest in the transplantation field. Subnormothermic perfusion offers an intermediary alternative, benefiting from a still significant metabolic rate while negating some of the complexities and risks of normothermic perfusion.

Despite what this field has taught us, functional normothermic organ perfusion remains feasible for durations measured in hours rather than days or weeks²⁷. This highlights our poor understanding of whole-organ regulation at fundamental physiological levels and our inability to mimic this *ex vivo*. The result of using deteriorating organ models limits the evaluation of isolated organs to short-term studies, which are likely laden with artifacts. To date, the science and engineering aspects of achieving practical, non-degradative and comprehensively functional organ culture for extended durations are yet to be fully explored. Alternative motives, including the required practicality in the organ transplant setting, have also driven *ex vivo* systems in the opposite direction. For instance, a short duration of machine perfusion is often employed end-ischemically to recover organs from preservation injury just before transplantation, and many new clinical applications are taking this form^{28,29}. Extended machine perfusion has been reported for various organs. Of the few studies that have demonstrated perfusion of organs *ex vivo* for multiple days, the effect of extended perfusion on more fragile organ functions was not investigated. Porcine livers perfused at body temperature for 72 hours showed continuous basic synthetic functions with minimal evidence of biochemical or structural injury, but were not transplanted to assess function in an *in vivo* system³⁰. Hypothermically perfused organs have been successfully transplanted after multiple days of perfusion. Canine kidneys perfused hypothermically for up to 5 days were successfully transplanted with 63% survival³¹. Similarly, canine livers proved transplantable after 72 hours of hypothermic machine perfusion. Again, these studies did not closely examine changes in organ function over time. Although hypothermic perfusion has delivered transplantable organs after numerous days, it does not offer an ideal platform for organ culture. The most important limitation to hypothermic preservation is the poor representation of physiological organ function at highly suppressed metabolic rates and

potential adaptations that take place over the course of hypothermic preservation that changes the behavior of the organ.

Perhaps more promising is the observation that machine perfusion enables recovery of marginal organs for transplantation^{20,32–34}, the premise being that if the system is good enough for the organ to repair itself, it should be good enough for it to sustain itself. Of course, there are fundamental differences between short-term recovery and long-term survivability.

Creating a complete body mimic requires the use of an organ-appropriate culture system, a suitable perfusion medium (perfusate), an oxygen carrier, and for some organs, hormonal and biophysical stimulation as reviewed below.

Organ culture system

The need for constant supply of nutrients renders *ex vivo* machine perfusion the only feasible approach for organ culture of all highly vascular organs that require vascular anastomosis to the recipient, thus *organ culture* and *machine perfusion* will be used interchangeably. Nutrient- and oxygen-rich perfusate can be actively pumped into the tissue through an existing vascularization to ensure adequate supply. This is in contrast to less vascular, often two-dimensional tissues such as cartilage³⁵, skin³⁶, gastrointestinal mucosa³⁷, blood vessels³⁸ and heart valves³⁸, which can be cultured in mixed reactors.

Reliable and accurate temperature regulation is essential in most perfusion systems. Culture within an incubator^{39,40} using a water-jacketed reservoir^{41,42} or heat-exchangers^{15,43} has been used to achieve temperature regulation. Modern perfusion devices developed for transplantation provide a good combination of temperature and hemodynamic control as well as an ease of access to the organ for analyses^{44–46}.

A variety of pump systems exist to act as the heart of the pseudo-body and circulate the perfusate through the graft. The choice of pump depends mostly on the perfusate of choice, with cellular perfusion media requiring atraumatic pumps to minimize damage to circulating cells during long-term perfusion^{13,47}. For acellular media peristaltic roller pumps can be used⁴⁸, which are generally easier to prime, but can exhibit unwanted pulsatility at the organ inflow if pulses are allowed to propagate unabsorbed after the pump. Specific hemo- and hydrodynamical properties have not yet been fully defined for the various machine perfusion systems. Near physiological flow rates and pressures are the common and logical first approach for normothermic perfusion. Results with pulsatility in arterial flow have produced inconsistent results and may differ between organs^{49,50}. Hypothermic perfusion requires a lower flow and pressure to prevent shear stress and damage to the endothelial lining^{51,52}. For instance, 25% of physiological pressure was deemed ideal during hypothermic perfusion of the rat liver⁵³. However, even with reduced flows at hypothermic temperature, shear stress resulting in endothelial dysfunction may be observed^{54,55}. Significant endothelial dysfunction early during organ culture, which is most apparent as increased vascular resistance, warrants flow and shear analysis. Finally, to prevent heterogeneous perfusion of the organ, imaging studies would be a worthwhile addition to machine perfusion studies⁵⁶.

In addition to a “heart”, the pseudo-body may need other organ analogues. The metabolic demand for oxygen is $40.5 \text{ nmol O}_2 \cdot \text{min}^{-1} \text{ mg}^{-1}$ protein for beating cardiomyocytes⁵⁷ and $0.8 \text{ nmol O}_2 \cdot \text{min}^{-1} \mu\text{g}^{-1}$ DNA for cultured hepatocytes⁵⁸, both of which cannot be met by diffusional supply of oxygen across thick tissues. Therefore, an oxygenator as an artificial lung is needed for normothermic perfusion to avoid sustained hypoxia. The most common approach involves gassing the perfusion medium with an oxygen/carbon dioxide mixture in a membrane oxygenator, such as hollow fiber oxygenators used in cardiopulmonary bypass machinery. In our experience with the liver, normothermic perfusion requires additional oxygen delivery by means of oxygen carriers. Examples include artificial oxygen carriers (AOC) such as perfluorocarbons (PFC)^{59,60} and hemoglobin-based oxygen carriers (HBOC)^{61,62}; or packed¹³ or freshly isolated⁶³ red blood cells and whole blood^{64–66}. The most ideal carrier is likely to be either packed/fresh red blood cells or newer AOCs, as some classic HBOCs tend to get oxidized quickly⁶⁷, and whole blood introduces immune-components that contribute to an inflammatory response⁶⁸. Dilution of whole blood or erythrocytes is preferred, as a higher hematocrit may result in harmful hemolysis^{65,69}. For isolated erythrocytes, a hematocrit of 15–25% is recommended⁶³, and for whole blood a 50% v/v dilution with perfusion media may be used, which corresponds to protocols used by others¹³. Normothermic machine perfusion of the lung forms an obvious exception in the discussion on oxygenation. Ventilated lung perfusion requires deoxygenation and carbon dioxide addition, which can be achieved with high CO₂/low O₂ gas-exchange⁷⁰.

One alternative for oxygenation that involves retrograde perfusion of gaseous oxygen through the organ is well worth mentioning^{71,72}. Persufflation has yielded surprisingly successful results in oxygenating and replenishing ATP supplies, particularly in ischemically injured organs^{73–75}. Although controversial as it seems to introduce air embolisms, persufflation provides an alternative or complementary approach to oxygenation. Effects of persufflation on restoring an organ’s energy status are rapid, and more long-term effects are not well known. Since gaseous perfusion does not seem easily compatible with relevant models of organ function, persufflation might be primarily applicable in short-term organ recovery in a transplantation setting.

Additional artificial organ analogues, while rarely employed, may be necessary for long-term stable function. In our experience with normothermic rat liver perfusion, a dialyzer is useful to maintain transplantability of perfused rat livers, by maintaining pH and electrolyte homeostasis, clearance of toxic byproducts such as bilirubin from the perfusion circuit, as well as replenishing the nutrient feed using a two-circuit system⁶³. Electrical stimulation or mechanical stretch for heart perfusion may be necessary for functional testing^{42,76}, and similarly, ventilation for lungs is necessary for oxygenation as well as determining lung oxygenation capacity as a measure of viability^{70,77}. Finally, incorporation of biological organs to assume functions necessary for long-term culture of the target organ may be a feasible alternative to artificial organ replacements, as was demonstrated in a study adding a kidney to a porcine liver perfusion system⁷⁸. A brief summary of perfusion protocol alternatives is presented in Table 1, and Fig. 3 depicts the organ analogues discussed above.

Finally, the *sine qua non* of organ culture is the perfusion media or perfusates, which must take on the role of blood as the communicative vehicle for exogenous and endogenous regulation.

Perfusate

The fundamental challenge in designing the perfusate is the combinatorial complexity caused by the sheer number of possible ingredients necessary and their dynamics. The basic expectation from the perfusate is to provide the necessary biochemical ingredients to maintain function, which requires optimization of metabolites, hormones, and cofactors. While starting with a blood-like composition is rational, maintaining those levels in a dynamic system requires replenishment of deficits or, in some cases, removal of produced excesses. Regulating hormonal control complicates perfusate design even further and is an underappreciated topic. For instance, typical serum-free protocols only employ insulin, whereas a choreographed insulin-glucagon combination may be desirable to more closely mimic the *in vivo* situation⁷⁹. Thus, aside from providing for the organ metabolically, the ideal perfusate needs to assume various endocrine roles to achieve long-term organ culture, as very few organ functions are not influenced by hormonal regulation from other organs^{80,81}. Notably, different organ preservation approaches warrant different regulatory interventions. For instance, while insulin supplementation is essential in warm organ perfusion, if the goal is hypometabolism, stimulating carbohydrate and fat metabolism may be undesirable⁸².

In current organ preservation, antithetical tactics are followed that have produced two subsets of preservation solutions. The first are cold-preservation solutions, most commonly Belzer's University of Wisconsin (UW) solution, which have been classically employed for static preservation in organ transplantation. These solutions are minimal in components, the combination of which was initially designed to support hypometabolism, prevent cold-induced functional and structural deterioration, and assist the recovery on reperfusion, with the goal of facilitating static cold preservation of limited duration⁸³. The rationale for the use of these solutions in dynamic, often hypothermic, perfusion systems is simply an extension of this biostatic approach⁸⁴. The additional benefit of using clinically applied solutions is the simplification of regulatory processes by building on prior approvals. By comparison, warm culture of organs requires a perfusate that can support a higher metabolic rate. These solutions are by necessity richly supplemented media or sanguineous solutions that support the needs of constituent, organ-dependent, cell types^{39,85}. This approach has generally shown promise, enabling culture of engineered constructs in the order of weeks, compared to hours or at best days³⁹. Theoretically, the use of donor blood would offer a physiologically relevant perfusate. Friend *et al.* developed a normothermic porcine platform based on supplemented whole blood that required porcine blood donors⁸⁶ and op den Dries *et al.* in Groningen was able to acquire blood bank products for their normothermic human liver perfusions¹³. However, unless organ procurement techniques allow for effective simultaneous blood donation, the cost and availability of blood limits its use for widespread organ culture. Our work has demonstrated that perfusion of human livers with a cocktail solution based on Williams' medium E enables viable preservation and bile synthesis, as opposed to perfusion with UW solution. Although whole blood is naturally designed to be

universally supportive, this cannot be assumed for culture media, which are often designed to provide for a specific cell type. Hence, the perfusate must meet the organ-specific needs of the resident cell types just as cell-specific media must be tailored for the culture of specific cells.

Although culture media may arguably be superior to hypothermic storage solutions for warm organ culture, their optimality is doubtful. For instance culture media, such as the minimal essential media (MEM) and Williams' medium E, have been in use for decades with few changes and are the *de facto* gold standards for hepatocyte culture. Regardless, within a few weeks of *in vitro* culture, hepatocytes start to lose their hepatocyte-specific functions such as CYP450 activities⁸⁷. Further, these media have non-physiological levels of some supplements (e.g. insulin is supplemented to ± 1000 fold of *in vivo*), and are likely to create aberrant results. Preconditioning in hyperphysiological levels of insulin has been shown to lead to hepatic steatosis⁸⁸. On the other hand, in our metabolic analysis of perfusion media we noted that several metabolites are exhausted rapidly and may need to be replenished more frequently or continuously through infusion via a secondary circuit³³. Nutrient composition should be monitored closely and tailored in close agreement with hormonal regulation.

A number of unpredictable donor factors influence an organ's nutritional requirements and hormonal sensitivity. For instance, the effect of feeding on the condition of the organ, the liver in particular, cannot be neglected⁸⁹. Dietary hepatosteatosis can be induced by just a few days of a high-carbohydrate diet or binge alcohol consumption^{90,91}. Moreover, in our work we found that fasting of rats results in significantly decreased ATP content of the liver. On the other hand, the restoration of normal energy metabolism and ATP content appears to occur within 2–3 hours in perfused rat livers, provided the media is supportive²⁰. As a result, the use of human organs with a multitude of unpredictable factors requires sophisticated regulatory or servo control of perfusate composition with a multi-input multi-output control scheme to correct for the variability of donor characteristics.

Finally, the perfusate design task is complicated by I/R injury itself, which inevitably occurs during the procurement of the organ, back table preparation, and oxygenated reperfusion. The energy status and glycogen content of the ischemic organ is rapidly altered, but can be improved effectively during perfusion^{20,92,93}. Recovery from I/R injury may benefit from pharmacological interventions as well. Increased vascular resistance is observed during cold ischemia⁹⁴, and organ preservation seems to benefit from the addition of vasodilators and thrombolytics⁹⁵. Moreover, addition of anti-inflammatory agents such as hydrocortisone or dexamethasone may ameliorate the immune response of reperfusion. It should be noted that these ischemic disturbances require time to reequilibrate, and a steady state of the organ cannot be assumed in the early period of organ culture.

SENSORS, ANALYTICS AND AUTOMATION: ORGANOMETRICS

The most underexplored aspect of the human body continues to be its dynamics. With a constant circadian rhythm and sporadic, fluctuating external and internal perturbations, our bodies never operate at a truly steady state. The limited knowledge of physiological

dynamics is largely the consequence of technological limitations. First, there are only a few scenarios where it is ethically feasible to obtain time-response data from a human for extended durations, such as intensive care units. Even in these cases, it is rarely feasible to go beyond macro/clinical vitals to study more in-depth aspects of the physiome, such as the metabolome and transcriptome. Moreover, in such contexts it is almost uniformly impossible to analyze the function of any organ or system in isolation from the rest, or indeed design an experiment in a truly controlled fashion. Nevertheless, developments in omics techniques are rapidly reducing the costs and speed of analysis. For instance, as the threshold for mass spectrometry gets lowered and run times improve⁹⁶, its use for on-line analysis of organ dynamics becomes feasible. Similarly, mRNA and protein array chips, which were for a long time limited by cost in dynamic experiments, have become a mainstream method now within reach of researchers. Combining such analytical power with a well-controlled environment that is conducive to observing organ responses is a revolution similar to cell culture itself, with a much higher clinical relevance. Achieving organometrics, however, requires a well-designed and controlled environment as described in the previous section, combined with a tandem deployment of sensors, data analytics, and automation beyond what perfusion devices in development today have to offer.

Sensors — Data and observed variables

The observable data in an organ perfusion system can be classified according to the ease of measurement. At the most basic level, measuring variables such as pH, temperature, oxygen uptake, air pressure for lungs, and hemo/hydrodynamics can all be done via commercially available and relatively economical, fast, and accurate sensors. This is currently measured in the in- or outflow tubing, ensuring the perfusate has the desired composition. However, more advanced perfusion systems might make use of novel microsensors that allow accurate intra-organ measurements of these parameters^{97,98}. These variables can be used real-time for a superficial indication of function⁹⁹. As a consequence, these variables are fairly easy to regulate and are both well measured and usually under feedback control in most clinical perfusion devices.

The second layer of data involves cellular and tissue processes that have relatively fast dynamics and are possible but difficult to measure real-time, such as metabolic responses that occur within minutes to seconds. Examples include anaerobic shifts in metabolism during ischemia, or effects of rapid-acting drugs. Observing such phenomena with fidelity requires on-line analysis. The ideal solution would be rapid mass spectrometry, although such approaches perform better when targeted to a few molecules of interest. The last decade has seen the development of portable point-of-care (PoC) microfluidic devices that can analyze a variety of disease-relevant metabolites, such as creatinine and liver enzymes, in under 15 minutes with enhanced automation and easy integration¹⁰⁰. Commercially available devices offer panels for blood chemistry and biomarkers¹⁰¹, while newly developed immunoassays provide solutions for high-sensitivity multistep processes¹⁰². These technologies are excellent starting points that could be integrated to the organometrics platform immediately, but still do not offer the desired real-time analysis. Similar simultaneous detection of multiple cytokines would also appear feasible through multiplex

technology, although a rapid on-line solution is not commercially available at the moment¹⁰³.

Control algorithms — Soft sensors

Since it does not appear likely that comprehensive metabolic or inflammatory data will be available in actual real-time speed, one approach to complement the available data is using mathematical models in tandem to predict missing information, either to interpolate between actual measurement time points or to obtain estimates of data that is difficult to measure directly from the media^{104,105}. Examples of such approaches can be readily found for metabolism, where techniques such as metabolic flux analysis (MFA) enable the prediction of intracellular reaction rates (that would otherwise require destructive assays) from rate of metabolite uptake/release into media⁸⁸. In more sophisticated embodiments, such as flux balance analysis or cybernetic modeling, it is possible to develop a model with more predictive power by employing an assumption such as optimality of the organ metabolism^{106,107}. Soft sensor predictive algorithms are a common technique in process industries as part of chemometrics and statistical process control¹⁰⁸. Notably, a major strength of statistical process control approaches is the ability to test and tune the mathematical model on-line, whereas MFA-type models are typically stuck with a defined model. On the other hand, statistical methods like multivariate partial least squares (MPLS) usually rely on purely black box models, which offer little scientific insight or ability to extrapolate to other systems. Therefore the ideal solution would apply the validation and reconciliation techniques found in statistical process control methods to more fundamental models of the metabolism or inflammatory cascades.

A natural extension of mathematical predictive models is their use in controlling the perfused organ. The most obvious application is regulation of the perfusate composition to ensure that the observed dynamics are not due to non-experimental changes in media constituents. Of particular note is that model predictive control (MPC) can be done in multi-input multi-output. It is quite possible to consider and regulate not just one component at a time (e.g. increasing temperature), but the entire system at the same time by considering what will happen to other elements when one component is changed (e.g. increasing temperature + anticipating metabolic responses). For instance, rather than blindly compensating for consumed metabolites, MPC employing a dynamic MFA model could make minimalistic changes, driving the organ to the desired metabolic state to reach an ideal perfusate steady-state. An interesting application would be to perform MPC of hormonal supplements to maintain stable function — for instance glucose regulation — where using a predetermined insulin/glucagon dosage would prove inexact because of inter-organ variations, such as sensitivity to hormones or time of last feeding. Moreover, in an application like this, the MPC would in effect be recapitulating the complex and multi-tissue *in vivo* hormonal regulation. It would therefore be possible to analyze the responses of the MPC to gain insights into how such regulation is performed in an *in vivo* setting. Perturbing the system in an experimental setting will lead to a change in the steady-state of the system, which will be accompanied by both responses from the organ and built-in feedback mechanisms, which coordinate to achieve a new steady-state condition.

Finally, the slowest responses occur at the genome and transcriptome levels, on the order of hours or longer. Such analysis can be used to observe phenomena such as viral spread (e.g. Hepatitis C), cancer, or genetic engineering at an organ level, provided the organ can be maintained in a functional state for days. Similar to the metabolomics discussed above, genomic, epigenomic, and transcriptomic models can be constructed to create soft sensors and employ a more slow-moving MPC. Since the cost and lag in obtaining data for such tests are much longer than other variables discussed, the ideal approach would be to combine models at multiple omics levels such that data with higher frequency of observation can be used to estimate others. Figure 4 below outlines the proposed organometrics approach.

APPLICATIONS

We expect that *ex vivo* organ perfusion practices will naturally evolve into the distinct fields of organomatics and organometrics that will form. The two will be synchronized to create a platform that allows both the preservation and the empirical observation of organs *ex vivo*, supported by the mathematics that predict and regulate the responses of tissues, organs, and organ systems, ultimately enabling computer-aided engineering of physiological responses. Potential applications of these technologies can be conceptually categorized into those that culture organs for improved preservation to facilitate transplantation, which are generally of short duration, and those that culture organs for experimental observation, where longer preservation is generally desired.

Organ culture for the preservation and treatment of donor organs for transplantation is under rapid development, and a paradigm shift in the *ex vivo* preservation of organs has been taking place over the last few years^{12,28}. The posited advantages of warm preservation are substantial. Avoiding hypothermic injury and allowing for internal recovery processes to take place are just the beginning.

For transplantation purposes, treatment of the donor is ethically difficult, making the *ex vivo* treatment of a diseased organ a logical tactic. Treatment of a hypothermic/hypometabolic organ is difficult, as the results are often only seen on warm reperfusion, making warm *ex vivo* perfusion the method of choice for manipulation of the organ. Warm perfusion offers a platform for diagnosis, treatment, and prognosis of organs on an individual basis.

Organometric systems closely monitor the organ in perfusion, analyzing input from hemo-/hydrodynamics, biochemistry, dissolved gas analysis, and even imaging to gain a comprehensive appraisal of organ quality. This assessment may subsequently drive the treatment of the organ. Organ perfusion systems provide a platform for the treatment of: (1) pre-existent conditions such as nephropathy, hepatosteatosis and fibrosis, pneumonia, and pulmonary aspiration of gastric content; (2) injury sustained during procurement or preservation, including warm ischemic injury and brain death-induced injury¹⁰⁹; and (3) organ pretreatment to ameliorate reperfusion injury and immunomodulation for improved allograft tolerance. If *ex vivo* treatment of liver steatosis proves effective, a large pool of currently discarded steatotic organs may become available for transplantation^{110,111}.

Similarly, with more than 85% of donor lungs discarded for reasons such as pulmonary

edema as a result of brain death, gastric aspiration, and infection¹¹², an enormous potential for *ex vivo* recovery of these grafts can be realized^{113,114}.

A particularly exciting development for organ repair is the use of gene therapy. Cold preservation prevents effective expression of transduced genes until rewarming¹¹⁵. Circumvention of ethical complications of donor pretreatment, easy isolated-organ delivery of the gene vectors, as well as an absent host immune system that reacts to the commonly used adenovirus vector makes isolated organ perfusion an attractive vehicle¹¹⁶. The current primary focuses are the reduction of ischemia/reperfusion (I/R) injury and preventing rejection of the donor organ in transplantation. Clinical efforts have reduced I/R injury through introduction of anti-inflammatory IL-10 into donor lungs¹¹⁷ and modulation of the alloresponse by inhibiting T-cells¹¹⁸.

The ability to test the viability of the organ is one of the main motivators from the transplantation field. Numerous parameters have already been identified to reflect organ quality and potentially predict transplant outcome. The parameters are often organ specific, such as lung oxygenation capacity (PaO₂), hepatic clearance (urea production, ICG clearance)^{119,120}, or renal excretion (creatinine clearance). Organometrics will take this assessment to a next level; combining input from the organometrics platform over time and using algorithms to provide a score of organ quality of prediction of transplant outcome¹⁰⁴. In situations where recovery *ex vivo* is possible, organometrics may even provide the required duration of perfusion to recover the organ to a transplantable condition.

Mention should also be made of autotransplantation techniques that may benefit from organ perfusion. Oncological patients with unresectable liver tumors due to the anatomical localization of the lesion may be treated by *ex vivo* (*ex situ*) resection followed by autotransplantation¹²¹. The tumors are excised *ex vivo* following a total hepatectomy along with surgical reconstruction of compromised vascular structures. Since these procedures can take as long as 19 hours, this technique benefits from a period of *ex vivo* perfusion to maintain organ viability in a manner very similar to allotransplantation^{122,123}. Similarly, it is imaginable that pharmacological or radiotherapeutical treatment of solid organ tumors can be done *ex vivo* with doses that cannot be achieved by systemic therapy.

After decades of culturing cells and experimenting in whole organisms, the applicability of *in vitro* and *in vivo* experimentation is becoming more and more limiting. Spatiotemporal orientation of various cells in a complex extracellular matrix plays a large role in maintaining physiological function and regulation; conversely, *in vivo* testing makes assessment of a single organ difficult. The benefit of isolated organ perfusion is not unrecognized and has been used extensively for a variety of purposes. The field of imaging is one that benefits greatly from the observation of an isolated organ. Intravital microscopy, for instance, can be performed conventionally for only a short duration of time limited by how long the animal can be anaesthetized and opened. Isolated organ perfusion would simplify long-term intravital imaging, which is currently a challenging and invasive procedure^{124,125}. Conventional radiological imaging modalities are improved by removing the organ from the surrounding organism. Additionally, isolated organ perfusion allows us to reach high concentrations of fluorescent dyes and contrast agents that cannot be achieved

in vivo, due to either limitations to delivery or toxicity of the agent¹²⁶. An organonomics platform that maintains tissue viable for long periods of time and allows on-line monitoring via integrated imaging technologies such as ultrasound, μ PET, μ CT, and optical imaging will also be an excellent tool for regenerative medicine following the onset and development of disease processes^{56,127,128} as well as their resolution. Organonomics with integrated imaging could also be used as a new tool to study the results of various regenerative medicine treatments, such as stem cell infusions to repopulate and/or replace injured, untransplantable organs with viable cells¹²⁹. Besides the advantages of imaging, isolated organ culture logically enables direct observation of the organ as well, which is especially beneficial when studying the heart, where myocardial contractility and valvular motion are of particular interest^{130,131}.

Isolated organ perfusion is a well-established experimental model for the study of organ-specific metabolism. Naturally, an isolated organ is the sole contributor to changes in metabolite concentrations, which allows these changes to be attributed to organ-specific influences. This can be exploited to study the effects of certain interventions in basic metabolic pathways¹³² or the metabolism of a target compound^{133,134}. Additionally, the ease of in- and outflow sampling allows for metabolic flux analysis over the organ and the evaluation of metabolic pathways over time and between groups^{33,135}. Studying the (patho-)physiological response of an organ is similarly complicated by interaction with other organ systems. By designing the organ perfusion system to mimic a diseased state, the response of the target organ can be accurately observed — for instance, in the examination of the pancreatic response to a hyperglycemic state¹³⁶ or the erythropoietin production of the isolated kidney in response to anoxia¹³⁷.

Finally, organonomics and organometrics may have substantial benefits for the development of novel pharmaceuticals, screening assays, and efficacy and safety testing, while simultaneously reducing the reliance on animal testing. Numerous pharmaceutical compounds show excellent results *in vitro*, but prove ineffective in clinical trials after substantial investment. As a result, better models to predict effect, interactions, and toxicity are in high demand. Standardized organonomics and organometrics applied as described above will allow us to study whole organs in a controlled fashion rather than their representations as isolated cells. Clinical relevance is significantly improved with use of human grafts, which puts organ culture in the position to become a key test phase on the interface between pre-clinical and clinical testing.

CHALLENGES AND CONCLUDING REMARKS

Achieving true long-term organ culture will require interdisciplinary collaboration and coordination between surgeons, engineers, and scientists. All major components of this process and of the technology are in place today, and advancement depends on their integration and refinement into a cohesive and standardized platform (Table 2). Platforms for organ perfusion have been extensively developed, and the systems for regulation and automation are well under way. Organ culture as a whole has benefited significantly from advances in the transplantation field. However, repurposing machine perfusion as a long-term organ culture platform requires redefining our expectations. The ability to successfully

orthotopically transplant an organ has rightfully served as the ideal metric of viability when testing machine perfusion approaches. Although this gives insight into the majority of the organ's functions and achieves the absolute requirement of fulfilling the body's demands, it may not be the metric over which we assess our ability to culture organs long-term. Characterization of cells for culture has taken a standardized form and it is to be expected that similar metrics will be developed for whole organs. The requirements for long-term maintenance of delicate organ function remain undetermined, yet it can be expected that developing organomatics and organometrics in tandem will provide a platform for a better understanding of the permanent life of organs *ex vivo*.

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Figure 1.

Development path and the impact of *organomaterials* and *organometrics*. Achieving an automated stable organ culture system requires an integrative effort to bring together sensors, new imaging methods, and a mathematical framework to develop a capability to sustain whole organs *ex vivo* in a stable but fully functional fashion. This previously unattainable technology of organ sustenance will then enable the analysis of biological phenomena and relationships in a totally new perspective. With a well-defined, precisely-controlled and stable environment, we will be able to acquire high quality time-series of genomic, proteomic, and metabolomic data that can be translated using novel mathematical models of high physiological relevance with direct clinical impact. With intra-organ cellular relationships and transport and reaction phenomena preserved, this platform will be ideal for studies of drug metabolism and disease mechanisms at the organ scale, the reconditioning of discarded donor organs, and also tissue regenerative processes triggered by new cellular therapies.

Figure 2.

Organ culture platform hierarchy of needs. The *ex vivo* machine perfusion system needs to provide nutritional support as well as adequate gas exchange, hormonal regulation and biophysical stimulation. For assessment of the organ in perfusion a combination of sensors are needed, including flow dynamics, dissolved gas analysis, point-of-care biochemical analysis, post-hoc biochemical analysis and imaging. Proper sensing provides a substantial multi-source data set that requires advanced analytics for modeling of function and subsequent feedback for automation of the culture process.

Figure 3.

In vivo analogues and organ culture unit operations. Accurate *ex vivo* organ modeling requires recapitulation of selected other tissues in the form of (bio-)artificial analogues to sustain function and viability.

Figure 4.

Organometrics platform. At the most basic level, classical feedback control systems are used to regulate environmental factors such as temperature and perfusion flow rate. With the development of practical continuous metabolic analysis, it is possible to use mathematical models (such as Metabolic Flux Analysis) to predict the organ trajectory quantitatively at a metabolic level, hence precisely back-calculating the optimal intervention for homeostatic regulation. As multiplex omics devices become faster and cheaper to enable online use, it will be possible to combine the omics data at multiple levels (e.g. metabolomic, proteomic, transcriptomic, epigenomic) to predict the long-term functional state of the organ, and regulate or induce desired responses at this more fundamental level, such as initiating regeneration in a perfused liver.

Table 1

Perfusion protocols.

Temperature	Hypothermic (0–10 °C) ^{26,51,138–141} Subnormothermic (20–28 °C) ^{20,32,89,93,148–152} Normothermic (±37 °C) ^{13,39,41–43,46,47,64,70,77,143–145,154–159}	Perfusate	Rich composition (blood/cell culture medium-based solutions) ^{13,78,142–147} Minimal composition (UW/HTK/Celsior solution) ^{12,16,26,32,44,77,150,152,153}
Flow	Continuous ^{20,32,52,60,141} Pulsatile ^{16,17,42,45,48,138,161,162}	Oxygenation	Red blood cells ^{13,30,47,64,145} Artificial oxygen carriers (PFC/HBOC) ^{146,160}
Flow rate	Physiological ^{13,39–42,86} Subphysiological ^{51–55}		Active oxygenation ^{20,32,39,42,89} Passive oxygenation ^{16,17,48} Persufflation ^{72,73,163–165}

Table 2

Summary.

	Hardware platform: <i>Ex vivo</i> perfusion	Analytical integration: Sensors	Automation: Mathematical modeling
Challenges	Clinical and physiological relevance Standardized platform Practicality	Real-time data acquisition Multiple scales of physiome Non-destructive	Interactions in a complex network Causal analysis Identification of therapeutic targets
Approach	Apply sophisticated engineering tools and concepts to enable <i>ex vivo</i> perfusion of an organ. Organ stability and function over time will be monitored by a number of methods including transplantation	Point-of-care devices for use in tandem with the organ culture system, enabling detailed metabolic and genomic analysis on-line Dynamic imaging methods, such as μ PET, μ CT*, <i>in situ</i> fluorescence microscopy	Model-Predictive Control: Dynamic flux models to predict organ trajectories. Utilize control theory to identify targets for manipulation An <i>in silico</i> organ model that can be interrogated to identify unknown interactions practically, with limited data, as was done for microorganisms previously ^a
Tools	Normothermic Organ Perfusion integrating analogues for the heart, blood, lungs, kidney and liver	Microfluidic devices for monitoring organ responses at mRNA and metabolic levels Imaging instruments, radiolabeled and fluorescent probes	Metabolic Engineering Toolbox: Metabolic Flux Analysis ^b , Cybernetic Modeling ^a Process Control
Expected outcomes	A stable and functional organ culture platform	Real-time data acquisition from a dynamic organ, enabling causal analysis of perturbations	A framework to integrate the data obtained at multiple scales Soft-sensors, algorithms to predict information that cannot be measured real-time from limited data Comprehensive, dynamic functional maps of the organ
Broader impact	Extended organ preservation Increased donor organ pool by marginal organ transplantation	Organ scale research model Viability testing of organ grafts Pharmacodynamic testing platform Physiological data to develop mathematical methods	The basis for a new mathematical science of systems physiology Computer-aided engineering of tissues, organs, organ systems

^a Predicting responses that postulate that the response is optimal with relation to a biologic goal, such as homeostasis or proliferation. Also referred to as Flux Balance Analysis.

^b Mass-balance based analysis for calculating intracellular metabolites changes from extracellular measurements.

* Positron emission tomography, computed tomography.