NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCEINCES
Division of Extramural Research and Training

NATIONAL ADVISORY ENVIRONMENTAL HEALTH SCIENCES COUNCIL
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Concept Clearance
For
Engineered Tissues Systems for the Environmental Health Sciences

Overview:

This program is intended to foster the development of *in vitro* experimental and computational models replicating major organ systems to be used for increased throughput and high data content screening of the mechanistic and toxicological effects of potential environmental toxicants. The proposed model systems will incorporate the inherent complexity of biology including the interactions between cells and cell types and the multi-scale connections between macromolecules and phenotypic outcome. An emphasis will be placed on developing systems critical for understanding the mechanisms and risks of environmental exposures, such as route of exposure (skin, lungs, gastrointestinal tract) and metabolism/clearance (liver and kidneys), as well as key target organs (respiratory, nervous, vasculature, and reproductive systems) or functions (immune or hormonal signaling). In many cases, these engineered tissues can be generated using biopsy, explanted, or excess transplant tissue or differentiated human stem cells and, therefore, the screening systems can be more directly relevant to human health than models based on *in vivo* animal experiments. Given the current state of the art for these engineered tissues systems, the program will use multiple mechanisms to develop both 'wet' and computational tissue models and to integrate these into a screening paradigm. The awarded projects will build on expertise in toxicology, tissue engineering, lab-on-chip, and computational modeling to develop these virtual tissues. A long-range goal of the program would be to integrate the products developed in the individual grants in order to create a 'virtual human' that will advance the field of predictive toxicology and environmental risk assessment.

Background:

Over the past decade, the National Institutes of Health have made a significant investment in integrating the engineering sciences and biomedical research. A cornerstone of this effort has been the evolution of tissue engineering science and the creation of architectures which faithfully replicate aspects of the structure and function of particular organs. Much of this tissue engineering work has focused on the development of replacement organs as well as devices that can be used therapeutically to replace organs either as direct transplant material or as extracorporeal functional replacement. A relatively smaller but emerging focus has been on the engineering of macro- or microscale tissue model systems that can be used for moderate to high throughput screening for drug discovery and toxicity assessment.

Cell culture systems exist in a range of scales, each of which offers benefits for biomedical research. Single cell assays or two-dimensional (monolayer) cell culture systems offer a setting which is relatively simple yet involve a significantly wider range of interactions than an isolated enzyme system. These systems allow for a study of subcellular interactions that may influence the system and pathway level

response to a toxicant. However, these relatively simple *in vitro* cell systems lack the multiple cell-cell or cell-matrix communications that are known to influence the tissue level response to toxicants and, as such, often fail to replicate *in vivo* responses.

The past two decades have seen an emergence of three-dimensional culture systems in which multiple cell types from an organ are co-cultured on a matrix support. In many cases, these culture systems have been shown to replicate tissue level structures such as the liver lobule, as well as tissue level functions such as metabolic capacity (liver cytochrome P450) and injury (release of markers such as ALT) that cannot be faithfully replicated in more simple culture systems. Furthermore, these 3D culture systems have been shown to maintain their structural viability and functional activity for extended periods of time, in some cases for several months. This longevity allows for an assessment of the chronic effects of toxicants. In addition, given that some of these systems appear to be conducive to microscale lab-on-chip formats and that several such bioreactors can be established from relatively small samples of tissue, these systems are amenable to moderate to high throughput screening in a highly cost-effective manner compared to *in vivo* studies using animal models. The availability of transplant organs that are healthy but which for various reasons are not used and the recent development of complex cell culture systems based on differentiated human stem cells also allows the creation of 3D-culture systems using human tissue rather than animal models; increasing the relevance of the results to human health.

Parallel to this effort in engineering of organ systems has been an effort to develop computational models enabling prediction of organ and organism function and response to toxicants. Much of this effort have been in 'top-down' approaches such as physiologically based pharmacokinetic (PBPK) modeling which allow predictions of toxicant distribution to target organs as well as clearance. Conversely, increasing efforts have been in 'bottom up' modeling efforts to predict the molecular effects of toxicant exposure on protein-protein interactions and gene expression changes. In recent years a new emphasis has been placed on multi-scale modeling which seeks to bridge the gap from molecular response to organ and organism effect. This is enabling a new generation of virtual tissues that have the potential to be used as a completely *in silico* screening system for predicting the consequences of exposure.

Extensive discussion of these topics has occurred through conferences supported by the NIH Bioengineering Consortium (BECON) and Biomedical Information Systems and Technology Initiative (BISTI), the NIH Roadmap, the Interagency Modeling and Analysis Group, and the US-EU biotechnology partnership. A number of solicitations in tissue engineering and computational modeling have been released across NIH including a focus on 3D models in the roadmap Transformative R01 program; however, few have solicitations focused specifically on tissue engineering for environmental health applications. NIEHS supports a very small portfolio in this area. Of particular note, however, NIH held a Roadmap sponsored working group on engineered 3D model systems in support of an inclusion of this topic in the Transformative R01 program and the EPA hosted a US-EU biotechnology partnership workshop on virtual tissues that included leadership from NIEHS and the European Commission. The deliberations and interagency partnerships formed in these meetings have supported the development of this concept developing and applying engineered tissue models in the environmental health sciences.

Project Plan:

Much of the work on 3D engineered and virtual (computational) tissue models for drug discovery and drug toxicity screening has resulted in the development of models of significant interest to the environmental health sciences (e.g., liver, skin, lung). These models appear to reasonably well replicate the function and response of these major organ systems. Other tissue/organ systems (e.g., kidney, neurons, breast) have been the focus of developmental work and show promise but have not yet been demonstrated to adequately model the corresponding *in vivo* systems. Still other systems are proving difficult to model and will require extensive developmental work; these include immune and endocrine functions. This program will, therefore, be implemented in a comprehensive approach through multiple solicitations each with distinct goals and investments spanning from development through application.

These programs will be lead by the NIEHS but will involve significant participation both programmatically and potentially financially. These include the National Toxicology Program, other NIH Institutes such as NIBIB, NHLBI, NIDDK, NIAMS, and NIGMS, the EPA, and the European Union.

Development of 3D Engineered Tissue models (R21/R33):
Through a 'phased innovation' program this concept will support the development and refinement of *in vitro* 3D culture systems for tissues of importance to the environmental health sciences, but for which validated model systems have not yet been developed. This would include systems such as vascular, neural, renal, gastrointestinal, and reproductive organs, for which model systems of limited utility appear to have been developed, and systems that are more challenging such as immune and endocrine systems. This effort will include the engineering of 3D culture systems to replicate these functions and their validation in comparison to appropriate *in vivo* model systems. In some instances this will be refinement of systems such as breast tissue mammospheres. In other systems it will include *de novo* engineering of systems such as immune cell signaling and migration or the linkage of systems such as immune cells into an existing lung model.

The phased innovation award allows an 'innovative/exploratory' phase in which a high risk/high reward project with little preliminary data will be taken over a one or two year period to the proof of principle stage. This is followed by an administrative and advisory council review and conversion to an R33 validation phase in which, through increased financial support, the system is refined and validated against gold standard environmental exposures.

Potential foci of applications to this program could include, but are not limited to:

- The development of scaffold architectures and cell culture systems that allow for physiologically representative architectures of the combination of cell types found in the target organ.
- The use of human stem cells (including embryonic or induced pluripotent cells) or excess human tissue samples to create humanized systems in parallel to animal models.
- The use of gold-standard toxicant responses to provide proof of principle and validation of the system. Validation of the ability of the developed models to mimic *in vivo* biology at the molecular and physiological level.
- Adaptation of systems to enable moderate to high-throughput screening through the miniaturization of existing macroscale 3D culture systems to smaller scales and the creation of multiplex format bioreactors.

- Adaptation of the system to high content data analysis through the coupling of 3D culture systems to multiple outputs including histological endpoints, analytical chemistry analysis of eluents, and 'omics technologies on recovered cell samples
- Adaptation of the system to longitudinal studies, potentially including the development of imaging technologies for tracking system outputs over time.
- Integration of multiple tissue interactions to more closely replicate *in vivo* conditions

Development of Computational/Virtual Tissue Models (R21/R33):
Parallel to the 'wet' engineered models; it is desirable to develop computational models that can mimic a broad spectrum of the responses of a real tissue. These models are not likely to replace 'wet' experimentation; however, they can be a significant tool to both supplement and inform environmental health research. Multi-scale Virtual Tissue models offer a bridge between the simplified abstract representation of current statistical models and the complex reality of 'wet lab' models. This linkage of molecular interactions and responses to cell, tissue and organ behavior allows for a prediction of responses to environmental perturbations. For example, such models have already been used to predict an increased metastasis from anti-angiogeneic chemotherapy which has subsequently been validated in clinical studies.

The goal of the virtual tissue development program is the creation of multiscale computational models that can be used to enhance, interpret, and guide the experimental analysis of toxicants in an engineered model system. Projects supported by this program will again follow the 'phased innovation' format with an initial one to two year developmental aspect followed by a potential scale up to validation and expansion. In addition to developing multiscale models relating molecular, cell, tissue, organ and potentially organism effects of environmental exposures, the models developed should establish an architecture for tissue modeling that is:

- Representative – able to replicate key features of the biological system modeled
- Predictive – able to demonstrate emergent behavior that is not explicitly modeled
- Flexible – able to incorporate differing levels of granularity
- Adaptable – able to incorporate new information as it becomes available
- Compatible – able to interact with other tissue models
- Reusable – able to be reconfigured to allow testing of different hypotheses or experimental conditions

Application of Engineered and Virtual Tissues in the Environmental Health Sciences (R01/U01):
The largest investment and primary focus of the program is the application of existing *in vitro* and virtual tissue model systems to probe the response to environmental factors. The projects will be interdisciplinary research project grants (R01) modeled on the NIH Bioengineering Research Partnerships in which teams of bioengineers, computational biologists, and toxicologists will work to refine their model systems and apply them as screens of the response to exposures. This effort will include significant staff (including NTP, EPA, and EU) involvement on issues including prioritization of toxicants for study and endpoints of interest and opportunities for cross-project collaboration towards the development of integrated systems. The projects will include both mechanistic and functional endpoints as well as developmental activities to refine the 3D culture systems to more faithfully reproduce what occurs *in vivo*. The awardees will refine model experimental and computational systems to develop a unified mechanistic understanding of toxicant action and the phenotypic responses to exposure. This multi-scale effort will allow a more comprehensive output that will:

- Use 3D tissue models as a screening tool to rapidly and cost effectively identify injuries induced by toxicants and their mechanistic underpinnings.
- Cross-validate systems with *in vivo* response in animal models including cross-species extrapolations of response.
- Integrate 'wet' models with '-omics technologies to identify candidate biomarkers of exposure and response including biomarkers at the pathway and network level.
- Use computational modeling approaches to develop strategies for predicting the risks associated with toxicant exposures and generate novel hypotheses for testing in 'wet' models.
- Develop strategies for manipulating the genetic background of the culture system to study alterations in susceptibility to environmental factors resulting from genetic variation.
- Integrate across the experimental *in vitro* and *in silico* computational efforts to evaluate the risks and mechanisms of real world exposures including chemical mixtures and chronic, low-level exposures.

A critical aspect of this program, in keeping with a long-range goal of this effort, is the integration of these virtual tissue models across organ systems so that the impact can be seen not only at the isolated organ level but across organ systems and ultimately to the level of the 'virtual human.' This effort to link the developmental aspects of the program across grantees will be supported by the establishment of an 'opportunity fund' type supplement program, and will be limited to the grantees of the program with possible outside collaborators. Through linking, for instance, models of barrier function (lung and skin) with metabolism and clearance (liver and kidney) with target organ damage (neuron) we can gain a more complete understanding of how the human responds to toxicant exposure. This integrated capacity will also allow for modeling of systemic affects through the inflammatory and endocrine systems which cannot be adequately represented in isolation. Possible aspects of this effort could include integration of lab-on-chip devices from multiple grantees to study cross-organ system interactions and the integration of modeling strategies such as PBPK to make the effort more directly relevant to the risk assessment field.

Program Management, Implementation, and Budget:

The NIEHS will provide overall scientific leadership for this program and will coordinate the interactions between the partnering agencies; awards issued through the NIH solicitations will be either transferred to another participating Institute or jointly funded depending upon the alignment with that Institute's mission and their financial contribution. Awards from other agencies (EPA and EU) will be solicited through parallel solicitations reflecting those agencies priorities and will follow their funding policies and guidelines. Memoranda of Understanding will be established with these agencies to delineate the contributions of the partnering agencies.

All grantees will be expected to participate in an annual grantee meeting to be held at either a grantee Institution, a Funding Agency's facilities, or at a major topical meeting of relevance. In addition, the grantees will be encouraged to for cross-program topical working groups to discuss, prioritize, and standardize issues of cross project importance. These work groups will be facilitated by funding agency staff but will be lead by the grantees. It is possible that the level of staff mediated coordination of projects may necessitate the use of a cooperative agreement mechanism; this will also allow a more complete integration of partnering groups such as the NIH Chemical Genomics Center and the

Interagency Coordinating Committee on the Validation of Alternative Methods to enhance the translation of products developed within this program.

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Due to the developmental nature of this program, it is intended that the projects will have a duration of up to five years. For the phased innovation awards this includes up to two years as an R21 and up to three years of validation activity as an R33, pending administrative review by program staff and the appropriate advisory council. We anticipate funding a minimum of 10 awards for developmental activities; approximately five in each program. These awards will have direct costs of up to $150,000 for the R21 phase and $300,000 for the R33 phase. The R01/U01 'application' program is also expected to have a minimum of five awards which will have a five-year duration due to the interdisciplinary and integrative scope of the projects. These projects will have direct costs of up to $750,000 per year. Finally, we propose the establishment of an 'opportunity fund' to facilitate the integration of technologies and their validation. This set aside for administrative supplements will be $1M in years 2 through 5. While not ideal for the pace of science; it could be possible to phase the implementation of this concept with a staged implementation of the developmental projects and a delayed implementation of the R01/U01 application projects until the third year. The investments are expected to be as follows, potentially expanding with increased partnering commitments:

Total Costs (thousands)

Program	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**
Wet' model phased innovation	$1,125	$1,125	$2,250	$2,250	$2,250
Virtual tissue phased innovation	$1,125	$1,125	$2,250	$2,250	$2,250
Application R01	$5,625	$5,625	$5,625	$5,625	$5,625
Opportunity Fund	$0	$1,000	$1,000	$1,000	$1,000
Total	$7,875	$8,875	$11,125	$11,125	$11,125