Commentary

Programming microphysiological systems for children's health protection

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Impact statement

This 'commentary' summarizes research needs and opportunities for engineered MPS models for developmental and reproductive toxicity testing. Emerging concepts can be taken forward to a virtual tissue modeling framework for assessing chemical (and non-chemical) stressors on human development. These models will advance children's health research, both basic and translational and new ways to evaluate complex embryological and reproductive impacts of drug and chemical exposures to inform safety assessments.

Abstract

Microphysiological systems (MPS) and computer simulation models that recapitulate the underlying biology and toxicology of critical developmental transitions are emerging tools for developmental effects assessment of drugs/chemicals. Opportunities and challenges exist for their application to alternative, more public health relevant and efficient chemical toxicity testing methods. This is especially pertinent to children's health research and the evaluation of complex embryological and reproductive impacts of drug/chemical exposure. Scaling these technologies to higher throughput is a key challenge and drives the need for *in silico* models for quantitative prediction of developmental toxicity to inform safety assessments. One example is cellular agent-based models, constructed from extant embryology, that produce data useful to simulate critical developmental transitions and thereby predict phenotypic consequences of disruption *in silico*. Biologically inspired MPS models built from human induced pluripotent stem (iPS)-derived cells and synthetic matrices that recapitulate organ-specific physiologies and native tissue architectures are providing exciting new research opportunities to advance the assessment of developmental toxicity and offer the possibility of deriving a full 'human on a chip' system, or a 'Homunculus.'

Keywords: Developmental, toxicology, systems, computational, conceptus, organ

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Introduction

Every 4.5 min a baby is born with a structural birth defect affecting one or more body parts and/or systems (http://www.cdc.gov/ncbddd/birthdefects/index.html). While prevalent in the USA (about 1 in every 33 babies), the etiology is unknown for two-thirds of these cases. The incomplete understanding of the causes and risk factors of human birth defects, and the desire for actionable solutions that ensure and protect children's health and well-being, are major drivers of basic and applied developmental toxicology.

Recent investments by the Defense Advanced Research Projects Agency (DARPA), the US Food and Drug Administration (FDA), the National Institutes of Health (NIH), the US Environmental Protection Agency (EPA), and other federal agencies in the US and Europe to support the development of human organ-on-chip and microscale tissue constructs are providing direction on research furthering the ability to assess drug efficacy and chemical toxicity.[1,2] Engineered as microphysiological systems (MPS), these *in vitro* models provide an environment for empirical testing of chemical safety or drug efficacy in human cell-based tissue constructs using microfluidics that impose realistic fluid-flow and overall complexity of the system to better recapitulate normal biology.[3] Target-level molecular/cellular data from high-throughput screening (HTS) assays, virtual tissue computer simulation models constructed from extant knowledge of embryology, and MPS technologies form a powerful triumvirate for characterizing potential developmental hazards in drug development and chemical safety.[4–8] This overview of opportunities and challenges in programming MPS models for children's health protection research is focused on novel testing strategies for developmental and reproductive toxicity. It is intended on one hand to the MPS community to highlight challenges and opportunities related to the developmental basis of health and disease, and on the other hand to the developmental biology community to note that validation

criteria are being discussed in the MPS community that put these technologies on the horizon.

Testing strategies for developmental and reproductive toxicity

Current testing guidelines for developmental and reproductive toxicity testing are based largely on descriptive end points or 'apical outcomes' from animal studies. For example, the OECD 414 guideline (prenatal developmental toxicity) describes a standard testing protocol for fetal growth, development and viability in pregnant rats or rabbits exposed during organogenesis.[9] A precautionary strategy is taken whereby a developmental hazard is assessed by the observation of increased rates of fetal malformations in an animal study. However, extrapolating findings to human physiology and susceptible populations comes with uncertainty, particularly for low-dose exposures that vary by lifestage, lifestyle, and genetics.[10] Traditional testing strategies also place a high demand on animal resources, making it impractical to test the universe of thousands of environmental chemicals with the potential for human exposure with traditional methods.[11]

Alternative test platforms that reduce, refine, or replace animal studies (3Rs) with comprehensive *in vitro* data and holistic *in silico* models can transform toxicity testing to a more predictive science.[12] The recently reauthorized Toxic Substances Control Act (the Frank R. Lautenberg Chemical Safety for the 21st Century Act) advances the need for high-throughput alternatives to animal testing that would generate equivalent or better information, giving new impetus to alternative predictive toxicology models. In assessing developmental toxicity, alternative models currently include platforms utilizing human stem cells or differentiating cell lines, observations of development in small model organisms (e.g. zebrafish, amphibians, chick embryos), mammalian organ culture, and embryo culture.[13] To date, embryonic stem cell cultures and zebrafish embryos appear to be the most promising vertebrate models for rapid and cost-effective screening of chemicals for developmental toxicity. MPS models contribute the advantage of a human cell-based system with the potential for self-organization and realistic physical constraints of a spatially dynamic system. Programing MPS platforms for developmental toxicity should give consideration to the fundamental principles of abnormal development based on the chemical nature of the exposure (i.e. pregnant mother as the exposure unit), dosimetry (i.e. placental dynamics and transport change during pregnancy), initiating mechanisms (i.e. chemicals interacting with biological systems wired for change), genetic factors (i.e. species or individual), and gestational stage (i.e. precisely timed orchestration of cellular dynamics). Ideally, these most basic observations from animal studies, that examine the effects of drugs/chemicals on developing systems/organs within the fetus, should keep in mind a refined profile in microscale tissue chips that mimic critical aspects in each system's development, such as controlled fusion of opposing surfaces or epithelial-mesenchymal transition during morphological development. These additional considerations should be kept in mind when designing specific MPS.

Tactical benefits of MPS for developmental and reproductive toxicity testing

One utility of MPS models for developmental and reproductive toxicity testing is the elucidation of mechanistic targets and key events linking a molecular perturbation with its predicted phenotypic consequences. Conceptually, a biological pathway for developmental toxicity or adverse outcome pathway (AOP) would commence with a molecular initiating event that sets off a cascade of key events to culminate in a measurable adverse outcome of relevance to health effects assessment.[14] An AOP basically says ''here is a biological perturbation that can lead to a specific adverse outcome, and here is how we think it happens'' (D Villeneuve, US EPA). The adverse outcomes are catalogued by utility for regulatory purposes and decision-making, and the pathway describes how a molecular lesion could be propagated through a tissue to invoke that endpoint. A compendium of AOPs with demonstrated relevance to the mode-of-action for specific drugs/chemicals is maintained in a knowledgebase [http://www.aopkb.org]. To date, few AOPs have been derived on adverse pregnancy outcomes. MPS models of human embryology and development could stimulate activity in this regard by providing an intermediate level of biological complexity for investigating how a molecular perturbation propagates through a population of cells to invoke an adverse response at the tissue/organ level.

Another potential utility of MPS models is the potential reduction of uncertainty in regulatory decision-making. Many factors contribute to uncertainty when extrapolating outcomes from animal studies to human populations. Two underlying factors are differential susceptibility across exposure scenarios (e.g. acute exposure to high doses in animal subjects versus long-term exposure to low doses more likely to occur in humans) and the incomplete understanding of mechanism-specific developmental toxicity between species.[10]

Furthermore, the etiology of most birth defects is multifactorial. Women of child-bearing potential may be exposed to hundreds of chemicals with potentially different modes of action on the conceptus,[15] and cumulative effects on different embryological pathways may create a combination of deleterious circumstances that disrupt development. MPS platforms that consider earlier toxicological impacts on human embryology and development can set the stage for assessing cumulative exposures, including interactions with non-chemical stressors for early life exposures (including pregnancy).

The types of cells selected for use in MPS models is another important consideration. If embryonic stem cells or other pluripotent cell types are the cells of choice, they may represent a rather limited genomic diversity reflecting their heritage, and/or only a limited perspective on the totality of human heritage. One could argue that this shortcoming may be overcome by increasing the diversity of cells in a mixture so that many genomes are included, but this

raises issues such as cost and availability of cells from a wide enough range of individuals to be appropriately representative. Induced pluripotent stem cells (iPSCs) that are patient-derived from individual human donors and reprogrammed to specific cellular phenotypes now raise the possibility of engineering personalized organs-on-chips that could guide individualized therapy regimens or environmental assessments to protect children's health. MPS models simulate the normal condition of human physiology, which is not always the case in drug development or chemical safety assessment, but can also be used to recapitulate abnormal development.

Quantitative outcome measures are essential to the overall success of the MPS approach if they are to be used in regulatory evaluations such as drug safety assessments (e.g. FDA) or risk assessment of environmental chemicals (e.g. EPA). Attrition in drug development due to efficacy failures or adverse drug reactions may be due in part to animal model imperfection (e.g. discordance between animal and human responses); whereas with environmental health protection the problem is too many chemicals in production or in the environment to assess by traditional animal-based methods. For example, EPA's inventory currently lists about 85,000 chemicals (www.epa.gov/tsca-inventory) that would take decades or more to test by conventional animal test methods. Growing practical (cost, time) and social pressures to move away from animal testing further incentivize a course of action for *in vitro* data-driven approaches. Toward evidence-based medicine or evidence-based toxicology, quantitative outcome measures in MPS models that reproduce normal functioning of human tissues offer a more direct understanding of chemical–biological interactions invoking human developmental toxicity. For this purpose, the model systems will need to reflect an appropriate disease state or developmental stage in order to provide a valid prediction. To aid FDA in the assessment of drug safety, MPS models that reflect clinical trials could be used to gather new information about drug safety and efficacy for children or used first to predict toxicity, where they would have the greatest impact at phase I. For environmental exposures, the Lautenberg Chemical Safety Act requires EPA to identify groups of individuals potentially at most risk in assessing the safety of a chemical and to assess risks to those populations. To aid EPA's current review processes for chemicals management, MPS models that reflect sensitive populations (children) could be used to gather information on pesticides, drinking water contaminants (e.g. halogenated organic chemicals), persistent chemicals (e.g. dioxins and flame retardants), and chemicals found in plastics, pharmaceuticals and personal care products, including those used in children's products.[16]

Testing the 'Homunculus'

Standard procedures will need to be developed and widely accepted to assess reproducibility of the findings and to integrate them with HTS and *in silico* models for AOP elucidation. Reproducibility is a big issue and there is no single evidence in MPS indicating its reproducibility across labs and systems. A series of mutually agreed upon quality control measures, including positive and negative test agents, will be useful to ascertain functional status and reproducibility. Multi-center trials will be necessary to confirm reproducibility and the ability to perform the assays in many different environments. Towards this end, the NIH recently established two university-based Tissue Chip Testing Centers to ensure availability of tissue chip technology and promote adoption by the research community by validating tissue chip platforms and their qualification for use in the regulatory-decision process as a path forward (https://ncats.nih.gov/news/releases/2016/tissue-chips-testing-centers-funding).

An important consideration of simulating human outcomes is the quantification of chemical exposure and internal dosimetry. Absorption, distribution, metabolism and elimination (ADME) are features that need to be taken into account.[2] Systemic absorption of compounds in the small intestine, distribution by blood, metabolism by the liver, and renal excretion are key determinants of ADME for drugs and chemicals. These characteristics are mostly lacking in current *in vitro* screening models. Modular MPS models that mimic microphysiology of the hepatic lobule,[17] transport barriers to evaluate drug and xenobiotic metabolism,[18] and microvascular networks for realistic distribution systems[19] provide organ-specific structural and functional characteristics for translating local dosimetry into a quantitative response.

An initial motivation for creating MPS was that neither *in vitro* monolayer cultures of immortal nor primary cell lines can adequately recapitulate the dynamics of an integrated human toxicological response.[20] For example, an MPS maintaining the functionality of four organs with realistic fluid-to-tissue ratios established a system for *in vitro* microfluidic ADME profiling and repeated dose systemic toxicity testing of drug candidates over 28 days.[21] As progress continues with well-constructed and well-tuned fluid dynamic systems integrated by microfluidic control,[20] the linking together of organs-on-a-chip to simulate an intact human ('Homunculus') appears plausible.

Horizons and challenges in MPS models for children's health research

The ability to recapitulate the spatial-temporal kinetics and dynamics of a biological system provide challenges and opportunities for advancing alternative, more public health relevant, and efficient chemical toxicity testing methods. Alternative testing methodologies that are faster, less costly, and more scientifically robust than many currently available methodologies for assessing how organs and tissues respond to environmental chemicals, coupled with the rigorous requirements of contemporary toxicology screening, are critical to informing implementation of the amended Toxic Substances Control Act. As noted above, the reauthorized Toxic Substances Control Act (2016) drives the need for alternative predictive toxicology models. It explicitly requires consideration of impacts to pregnant women and children as susceptible populations. And yet, the complexity of human development poses a

critical challenge for understanding how MPS methodologies can fit into the new line of thinking, when it comes to safety assessment and regulatory toxicology. Tailoring human microscale tissues and MPS as mechanistic models for developmental and reproductive toxicity testing would ideally recapitulate apical outcomes manifested during pregnancy (intrauterine growth restriction, prenatal loss, preterm labor), at birth (low birth weight, structural malformations), and into postnatal life (functional delays, subfertility, chronic disease).

Gender-based testing

Increased knowledge of individualized therapy responses and their importance in designs of human clinical trials has renewed interest among preclinical researchers in understanding the role of gender on the potential for a drug or chemical to produce toxicity. These sex-related susceptibility differences could be examined with microphysiological technologies when appropriate consideration to using male vs. female cells and, just as important or perhaps even more important, to using media that mimics the hormone balance of the appropriate sex. In the same way that racial and ethnicity differences can be elucidated by using cells from several populations of donors, the variable of gender can also be systematically evaluated. By carefully employing the use of MPS models, many biological obstacles can be overcome and a great deal of fundamental biology and physiology will be elucidated along the way, much to the benefit of our understanding of human health and disease processes. In the future, MPS models built from patient-derived iPSCs may recapitulate the complex systems science of biological susceptibility for predictive toxicology to translate data from epigenetics/epidemiology. Currently, many iPSC lines tend to be fetal- or neonatal-like in their differentiation. This may be a problem for assessing adult reactions, but at the same time offers hope to consider fetal–juvenile–adult life stage progression in the same individual genotype.

The development of female and male reproductive microfluidic systems is critical to gender-based *in vitro* toxicity and drug testing. Preclinical early testing in animals of environmental and pharmaceutical chemicals in females is challenging because of complications introduced by hormone changes throughout the menstrual cycle and pregnancy. The recapitulation of the 28-day menstrual cycle and pregnant-like hormone control has been achieved through integrating multiple tissues into an MPS microfluidic culture system called EVATAR™.[22] A microfluidic organ-on-chip model of the uterine endometrium (EndoChip) has been engineered to reassemble the endometrium (immune, vascular, stromal and epithelial components) to individually compartmentalize each cell type with control over endocrine and biomechanical stimuli (e.g. shear stresses).[23] Potential applications of EndoChip are to assess pre-pubertal impacts of exposure to endocrine active chemicals on longer-term uterine health and disease. Microphysiological modeling of hormonal balance has been presented in concept for the male reproductive tract.[22] Although there are no MPS data yet available on this challenge in a human MPS model, a three-dimensional co-culture model of rat testis development has shown promise for incorporating local metabolic capability and pharmacokinetics for phthalate male reproductive toxicants.[24] When anchored to developmental regulation of the testicular transcriptome the changes will capture critical processes in puberty such as the peak in steroidogenesis and increase in meiosis and spermatogenesis-related pathways during the first wave of spermatogenesis.[25]

Another complex process that advances from fetal through pubertal stages and pregnancy is mammary development. Mammary microbioreactors, hollow mammospheres coupled with microfluidics, provide novel research models to probe disease pathways in a human system.[26] Mammosphere formation requires diverse cellular functions, including extracellular matrix remodeling. Introducing various drugs/chemicals into the fluid supply system provides an approach to probe a developmental response to chemicals such as endocrine disrupters that may increase the risk of breast cancer and other disease outcomes dependent on the timing of exposure.

Neurodevelopment

In vitro models of human brain development have emerged from human pluripotent stem cell-derived three-dimensional organoid culture system (organoids) that develop interdependent regional features such as the cerebral cortex and that recapitulate aspects of human cortical development. These organoids have been used to model microcephaly, a complex human birth defect.[27] Human organoid models have shown pragmatic use in demonstrating neuroprogenitor cell-specific susceptibility to adverse consequences of the Zika virus.[28,29] Human brain mimics have been used for developmental neurotoxicity screening, such as a 3D human brain model derived from iPSCs for the purpose of testing drugs and chemicals.[30] The model recapitulates the complex interactions between different types of glial cells and neurons and reflects critical developmental windows of vulnerability. Even more complex human iPSC-derived neural tissue constructs, including interactions with vascular networks and microglia, were produced with high sample uniformity by combining precursor cells on synthetic hydrogels.[31] Machine learning was then used to build a predictive model from changes in global gene expression for neural constructs exposed to 60 toxic and nontoxic training chemicals. Yet another novel use of 3D *in vitro* platforms is shown in the Brain MAPs project that synthetically engineers morphogenesis from human neural stem cells (hNSCs) in microscale tissues and prints them into organoid arrays that can be used for phenotype-specific, quantitative high-throughput developmental neurotoxicity studies.[32] Chemically defined microenvironments utilizing tunable synthetic matrices have been shown to promote the formation of complex 3D organoids that recapitulate key steps in early neurogenesis.[33] The approach is applicable to the mechanistic understanding of complex cell–matrix interactions that coordinate growth and differentiation in various organoid systems.

Cardiovascular system

Cardiovascular development, physiology, and vascular function are important considerations for assessing the toxicity of drugs and environmental chemicals. MPS models for these parameters offer new insights into the predictive modeling and mechanistic understanding of xenobiotics, including those affecting cardiac physiology and valvuloseptal morphogenesis. Microscale tissues built from iPSC-derived cardiomyocytes show promise for *in vitro* assessment of multiple cardiomyocyte physiological parameters (e.g. Ca++ flux, beat rate, amplitude, width, raise, decay, and regularity) using automated data analysis.[34] Human MPS models that recapitulate endocardial cushion development, for example, provide a potential resource to investigate impacts of drug and chemical exposure on the signaling between endothelial and cardiomyocyte cells that initiate formation of the heart valves and septa that are targets for mechanistically diverse teratogens. Engineering microscale devices that impose morphogenetic constraints, including fluid-flow kinetics, provide novel research models to investigate underlying pathways of developmental toxicity for matters pertaining to the heart.

Placenta

During human pregnancy, the placenta mediates the transfer of metabolites and drugs/chemicals between maternal and circulatory systems. A microfluidics device that mimics the structural and functional complexity of the placenta was engineered to reflect the dynamic flow conditions of maternal–fetal exchange, and to reconstitute expression and physiological localization of membrane transport proteins such as glucose transporters, at the maternal–fetal interface.[35] The 'placenta-on-a-chip' platform closely approximated glucose exchange similar to an *ex vivo* model, and could potentially be incorporated into integrated toxicity assessments of human pregnancy with regard to the complexity of maternal–fetal exchange across multiple tissue layers.

Blood–brain barrier

Perfusable organoids and MPS models that recapitulate functional vascularization have the potential to enable real-time data acquisition of molecular, biochemical, and cellular responses during brain development. Sophisticated MPS models have been engineered to recreate exchange across the blood–brain barrier (BBB), a complex structure that has remained difficult to model faithfully *in vitro*.[36] A human 'neurovascular unit-on-a-chip' was engineered to evaluate drug and chemical penetration to the brain. Many previous models have failed to support all the diverse cell types involved in the BBB formation and/or lacked the fluid-flow shear forces needed for mature tight junction formation. Microfabrication of the human NVU has both vascular and neural chambers separated by a porous membrane that enables cell-to-cell communication for various analytical measurements. Drug/chemical interactions with the BBB may perturb brain development and are therefore an important, yet understudied aspect of developmental neurotoxicology. Various *in vitro* and *in silico* models established for assessing human BBB function have not been assessed for their potential utility in this regard and remain an area of opportunity for research and technology development.

Integrative simulation

Although technologies are now in-hand to measure molecular components of cellular and tissue-level phenomena in great depth and detail, computational (*in silico*) approaches such as machine-learning and computer simulation are required to assemble the pieces into an integrated system.[4,6,8] As methodologies continue to evolve for the purposes of developmental hazard assessment, they must be tied to maternal/fetal physiology and toxicity of the developing individual across early life stage transitions, from fertilization to birth, puberty and beyond. Three main organizing principles to include are: (1) novel *in vitro* platforms with human cells configured in nascent tissue architectures with native environments yield mechanistic understanding of developmental and reproductive impacts of drug/chemical exposures; (2) novel *in silico* platforms with HTS or other modes of data collection, biological models of complex adaptive systems, and chemical structure information yield predictive understanding of developmental and reproductive impacts of drug/chemical exposures; and (3) a combination of technologies is necessary for analytical (to understand) and theoretical (to predict) applications for probing the relevant biological processes and toxicological mechanisms to inform safety assessments.

Further opportunities for computational approaches cycle back to key determinants of life-stage vulnerability, genetic susceptibility, and dose response.[15] Additionally, many human birth defects are mechanistically linked to complex multicellular processes in the embryo such as fusion of opposing tissues, epithelial-mesenchymal transition, vascularization, biomechanical shaping, and fluid-flow remodeling. Deconvoluting these complex multicellular dynamics *in silico* has been possible using cell agent-based modeling and simulation.[37] These computational approaches have successfully been applied to reconstruct a morphogenetic series of events from the bottom-up, cell-by-cell and interaction-by-interaction, as a heuristic approximation of mechanism-specific developmental toxicity.[38–40] Each simulated cell in an agent-based model, as with a biological cell, interprets local cues from its microenvironment and behaves according to its own blueprint or history. An appealing aspect of an integrative MPS-computational platform is the translation of human cell-based HTS data into theoretical phenotypes or 'cybermorphs'[40] that reflect tissue-level predictions of fetal development based on extant knowledge of the embryology and correlating the prediction to outcomes from empirical observation. Elucidating a tipping point between adaptive and adverse responses in MPS models and simulating dose–time response relationships has potential application for predictive modeling of points of departure in response to drug or chemical exposure to enable a virtual reconstruction of developmental toxicity.

Final thoughts

Advantages of MPS models as an approach to solve the prediction dilemma of substance testing have been discussed by many other reviews, whereas this commentary summarizes advantages for developmental and reproductive toxicity testing. A side-by-side overview of specific case examples with human MPS and animal models remains to be done as proof-of-concept that such approaches can provide robust predictions of developmental processes and toxicities. Coupling of MPS and computer modeling is an important step to bridge emerging concepts in bioengineering and synthetic toxicology with human embryology. Interdisciplinary collaborations are needed to integrate information on embryology with novel *in vitro* data and *in silico* models. Scaling these technologies to higher throughput is a key challenge and drives the necessity of *in silico* models for quantitative prediction of developmental toxicity able to inform safety assessments relevant to children's health research.

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DECLARATION OF CONFLICTING INTERESTS

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