Minireview

Microphysiologic systems in female reproductive biology

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Impact statement

This review discusses existing microphysiologic systems technology that may be applied to study of the female reproductive tract, and those currently in development to specifically investigate gametes, fertilization, embryo development, pregnancy, and diseases of the female reproductive tract. We focus on the clinical applicability of these new technologies in fields such as assisted reproductive technologies, drug testing, disease diagnostics, and personalized medicine.

Abstract

Microphysiologic systems (MPS), including new organ-on-a-chip technologies, recapitulate tissue microenvironments by employing specially designed tissue or cell culturing techniques and microfluidic flow. Such systems are designed to incorporate physiologic factors that conventional 2D or even 3D systems cannot, such as the multicellular dynamics of a tissue–tissue interface or physical forces like fluid sheer stress. The female reproductive system is a series of interconnected organs that are necessary to produce eggs, support embryo development and female health, and impact the functioning of non-reproductive tissues throughout the body. Despite its importance, the human reproductive tract has received less attention than other organ systems, such as the liver and kidney, in terms of modeling with MPS. In this review, we discuss current gaps in the field and areas for technological advancement through the application of MPS. We explore current MPS research in female reproductive biology, including fertilization, pregnancy, and female reproductive tract diseases, with a focus on their clinical applications.

Keywords: Microphysiologic systems, microfluidic systems, organ on a chip, female reproductive tract, reproductive biology, personalized medicine

***Experimental Biology and Medicine* 2017; 242: 1690–1700. DOI: 10.1177/1535370217697386**

Introduction

''Microphysiologic systems (MPS)'' is a broad term encompassing a number of recently developed devices that attempt to incorporate physiologic features into *ex vivo* tissue or cell culture. More often than not, this includes microfluidic flow of media within the system, leading many to use the term ''microfluidic systems'' interchangeably with MPS. Additionally, ''organ-on-a-chip'' is used when these devices involve whole or parts of organ systems, such as the gastrointestinal (GI) or hepatic system. Many advances have already been made in organ systems other than the female reproductive tract, even though MPS technology could expedite research pertaining to the complexities of female reproductive biology and related diseases. Likewise, though not addressed in this review, MPS research in other organ systems may greatly benefit from incorporation of the female reproductive tract, and in particular the ovary, which secretes hormones that are known to have systemic effects in many tissues.[1] In this minireview, we discuss features of current MPS technologies that are complementary to female reproductive biology studies, including devices that improve tissue or cell culture and add-on technologies that streamline additional assays or endpoints. The advantages and possible applications of MPS are discussed from gametes to fertilization to the embryo and pregnancy, with implications for human infertility, assisted reproductive technologies (ART), drug testing, and prenatal diagnostics. Finally, this summary concludes with the sparse literature on female reproductive tract diseases modeled in MPS, including ovarian cancer (OC) and endometriosis, and offers ideas on potential future applications. The promise of MPS is far reaching, ranging from new insights into the basic biology of the female reproductive tract to directly applicable point-of-care diagnostics in the clinic.

New technologies

The female reproductive system is an interconnected series of organs that support the production of steroid hormones, production of ova, union of egg and sperm, and development of the fetus. Each organ of the reproductive system is tightly connected to the others via the complicated crosstalk of endocrine signals, such as the sex steroid hormones, estrogen and progesterone. There are currently no MPS that investigate the female reproductive system as a whole.

A wide range of potential MPS applications exists from modeling the entire reproductive system and menstrual cycle to testing the effect of therapeutics on reproductive health. As the field evolves, advances in other biological and organ systems can be adapted to probe reproductive physiology. The following section highlights emerging technologies that would be particularly useful for modeling the female reproductive system.

Chamber organization

The chamber configuration of an MPS can be customized for the system of interest. The physiology of individual organs can be studied alone,[2,3] or the interactions between cells and tissues can be monitored using interconnected multichambered devices.[4–7] The complexity of the reproductive system, with multiple organs communicating via temporally fluctuating molecular signals, is best modeled using multichambered devices. A challenge for multichamber MPS is the need for simultaneous coordination of multiple organs and tissues during system set-up. This can be a significant hurdle for systems relying on sporadic access to patient samples or immortalized cells that grow at different rates. To address this issue, Loskill *et al.*[6] developed μOrgano, a lego-like customizable plug-and-play device where individual tissues or organs can be cultured in independent chambers, then plugged into the multiorgan MPS. With the flexibility to optimize each part, the whole system functions more reproducibly to model *in vivo* physiology.

Analytics and biosensors

The small amount of media or specimen samples available to analyze after an MPS experiment pose a challenge to traditional analytical techniques that require large volumes or concentrated molecules. To overcome the small volume problem, analytical devices have been adapted for these micro-volume systems. To probe the metabolites of cells grown in individual chambers, Lima *et al.*[8] developed an in-line multianalyte microphysiometer to detect up to four analytes in six electrochemical cells in real time. As a proof of principle, Lima *et al.* monitored glucose, lactose, oxygen, and pH levels to assess the metabolic rate of primary neural and glial cells in co-culture. Following completion of an MPS experiment, in-cell Western blotting can also be used to monitor the expression of individual proteins.[9] Some point-of-care devices, intended for low-cost, rapid, and sensitive diagnostics in the field, have achieved ultrasensitive detection using immunoassays.[10,11] The downside to these techniques is that only one or a few targets can be monitored in a single experiment at a time. Alternatively, on-chip microfluidic mass spectrometry allows the exometabolome, the biomolecules secreted or excreted from cells, to be dynamically probed throughout the time course of an experiment by sampling the media.[12] Marasco *et al.*[12] developed a microfluidic device containing an online desalting and mass spectrometry module to monitor the differences in drug metabolism in naïve and treated Jurkat cells. A new generation of analytic technologies and biosensors have been incorporated into MPS technology and adapted to handle small sample volumes or provide real-time detection. Future collaborations between groups developing specific MPS biosensors may yield multimodal panels of assays run in parallel, capable of providing diverse data-sets from single experiments.

In regard to the female reproductive tract, these biosensors could potentially be applied to study the dynamic physiologic changes associated with the female menstrual cycle. Throughout the 28-day female menstrual cycle, the organs of the reproductive system, as well as non-reproductive organs like the liver and kidney, alter production of biomolecules in response to the fluctuating endocrine hormone feed-back and feed-forward signals. For example, many reproductive organs respond to estrogen secretion from the ovary: the fallopian tube secretes glycoproteins that help support the growth and transport of sperm and eggs, the uterus proliferates and increases production of the progesterone receptor, and the cervical mucus thins to allow sperm to migrate into the uterus from the vagina.[1,13] Because microfluidics provide continuous flow of media through the system, the changes in secreted biomolecules throughout the menstrual cycle, such as hormone levels of estrogen and progesterone or the production of secretions in response to the hormones, can be monitored. These fluctuations can and should be monitored throughout the menstrual cycle when evaluating, for example, female-specific effects of drug metabolism. Many of the technologies developed for the study of other organ systems could be applied to the study of female reproductive biology, including biosensors to monitor dynamic changes throughout the menstrual cycle.

Imaging

In many studies, researchers use MPS to observe the cells and tissues in chambers repeatedly throughout an experiment. Kim *et al.*[14] and Zhang *et al.*[15] have developed MPS connected to mini-microscopes using off-the-shelf materials to record observations in real time. Using a mini-microscope, Kim *et al.*[14] monitored the migration of mouse 3T3 fibroblasts in the presence of varying FBS concentrations. These micro-cameras could be used to monitor the effect of drug treatment on fallopian tube epithelial cilia beating or the movement of the sperm and oocyte through the fallopian tube mediated by the beating of cilia. Observation of cilia beating usually requires removal of the device from the incubator to use a standard microscope. However, use of an in-line microscope and camera would prevent researchers from altering the environment, such as the temperature and CO_2 levels inside the chambers, every time observation or an image is required. Real-time imaging of embryo development during *in vitro* fertilization (IVF) would also benefit from an on-device microscope camera, avoiding disturbance of the device during sensitive developmental time points and potentially improving the success of embryo selection.[16]

Fluorescent mini-microscopes have also been incorporated into microfluidic devices.[15,17] Using a mini-fluorescence microscope, Akagi *et al.*[17] monitored angiogenesis in a transgenic GFP-labeled zebrafish embryo. Fluorescence microscopes could be used to observe

co-cultures of different cell types tagged with distinct fluorophores. For instance, the migration of cancer cells through the reproductive tract can be monitored by fluorescent tagging. Alternatively, metabolite or gene expression changes can be tracked using fluorescently tagged proteins. Eventually, all of the on-device cameras and analytics could be monitored or controlled from the researchers' own home using Google Glass.[18]

The sampling of potential applications described above represents just a few of the advances in the field that will be valuable additions to MPS probing female reproductive systems. Different chamber configurations may provide insights into the effects of endocrine signaling in the female reproductive tract. Emerging plug-and-play systems may allow for the independent culture of primary human tissues or cells, easing the technical difficulty of setting up such experiments. In-line analytic technologies and imaging systems have been developed in other organ systems, which could be incorporated into future female reproductive tract MPS. Subsequent sections of this review summarize already existing systems created to culture gametes, embryos, and reproductive tract tissues in order to study biological phenomena like fertilization, pregnancy, and disease.

Gametes and fertilization

The study of fertilization naturally involves mechanical considerations since sperm must travel through a viscous and highly intricate environment to reach the oocyte for reproduction. The oocyte must escape from the ovary through ovulation and meet the sperm in the fallopian tube. Confinement in limited fluid volume, medium composition and viscosity, fluid movement, and temperature all impact the normal biology, maturation, and union of gametes both in the female reproductive tract and in the clinical preparation of gametes for assisted reproduction. MPS have been developed to incorporate physiologic factors in the study of sperm and oocyte biology. These new technologies may streamline ART and improve future success rates for subfertile couples.

Sperm motility

In the field of fertilization, an understanding of the basic mechanisms that govern sperm movement toward the oocyte in the female reproductive tract is still being elucidated, especially as they differ between species. For example, one explanation of how the sperm finds the egg is chemotaxis, or the process of the sperm moving toward the egg based on concentration gradients of chemical signals like peptides and lipids.[19] Changes in temperature in the reproductive tract have also been proposed as a factor influencing the ability of the sperm to find the egg,[20] but studies in multiple species suggest that perhaps this is not the major driving force. The process of sperm swimming against a flow stream is a third possible mechanism. The importance of chemical gradients, temperature, and fluid flow on sperm movement and fertilization could all be tested with the implementation of different MPS that are capable of changing flow strength, factors in the media, and even media temperature. Studies on bull sperm, for example, have revealed that these sperm prefer to swim upstream (against the flow) and that fluid shear stress and the wall shape of whatever container they are confined in (MPS, reproductive tract, etc.) are important factors contributing to swimming direction and ability to locate the egg.[21] Not only can these systems be used to study aspects of the sperm, but of more complex environments, including those with other cell types. MPS created with special microgrooves and fluid flow to simulate the female reproductive tract also reveal that sperm prefer to swim upstream. However, pathogens like *Tritrichomonas foetus* were swept downstream, illustrating how biophysical features of the female reproductive tract may serve dual roles, facilitating sperm migration while protecting against pathogenic invaders.[22] Many physiologic factors that were previously difficult to study *ex vivo* can now be simulated in MPS technology.

Sperm selection

MPS can be used to help sort sperm and ensure that those of the highest quality are used for ART. Emerging MPS devices have been created that incorporate fluid flow and separate spermatozoa based on morphology, motility, or various other endpoints that may impact gamete health, such as DNA content.[23] Integration of sperm quality monitoring into MPS improves throughput by allowing investigators to image multiple sperm in different experimental settings, such as in the presence of a progesterone gradient, at the same time.[24] In addition, the design of such devices allows for the most motile sperm to be captured for fertilization. Results of studies selecting sperm based off their ability to bind hyaluronic acid (HA), which may reflect their ability to bind the zona pellucida, show that HA-bound sperm have lower percentages of apoptosis and aneuploidy.[25] Sperm motility studies performed with methyl cellulose, which more closely mimics human cervical mucus than traditional media, suggest that this is not only a better method to characterize motility, but also a way to identify oligozoospermic semen (low sperm concentration).[26] The isolation of sperm with methyl cellulose sorted more viable sperm as compared to traditional methods, based upon not only the sperm's ability to swim slower in a more viscous solution but also in a straighter trajectory. Alternatively, MPS are being developed to isolate high-quality sperm with reduced DNA damage by electrophoresis.[27] Existing materials, such as HA and methyl cellulose, or technologies, like electrophoresis, for motility testing and sperm selection may be incorporated into MPS.

Oocyte preparation

Similar to sperm preparation, oocytes must also be analyzed and selected for ART. In a single IVF production run, oocytes may be cultured in large volumes of media in static conditions and are handled and moved several times. The stresses of such a culture system include the physical interaction of cells, their mechanical manipulation, the chemical composition of the medium, and the accumulation of wastes. To address this, existing MPS have the

ability to trap and transport oocytes for single-cell manipulation[28] and use microfluidic flow to refresh small volumes of media, eliminating built-up wastes.[29] Studies have shown that microfluidic culture of murine oocytes, versus traditional static culture techniques, can improve rates of oocyte maturation to metaphase II, fertilization, and blastocyst formation, while potentially decreasing damage caused by reactive oxygen species.[29] Computer-assisted polarized light microscopy systems, like Oosight™ (Hamilton Thorne, Beverly, MA, USA), have existed for many years to identify mature, metaphase II oocytes by the presence of birefringent cytoplasmic meiotic spindles and could be potentially incorporated in future automated MPS.[30] Systems also exist which incorporate removal of supporting cells from around the oocyte. These MPS chemically remove the zona pellucida[31] and mechanically denude the oocyte of associated cumulus cells, using combinations of specially designed narrowed channels and cumulus cell removal ports, acting through the application of mild suction.[32] Once the highest quality sperm and oocytes are selected and prepared, IVF may occur.

Fertilization

MPSs are able to incorporate new techniques like gamete immobilization, which are important for ART, especially for intracytoplasmic sperm injection (ICSI). ICSI is a treatment for male factor infertility caused by poor sperm quality, such as abnormal morphology or motility. Sperm are isolated from semen, immobilized and injected directly into the cytoplasm of the oocyte. Some MPS boast reduced ICSI treatment time by improving sperm selection methods in samples with low sperm count and motility.[33] These systems may also eliminate the need for centrifugation steps that can cause physical damage to gametes. Additionally, automated systems have been created to immobilize sperm,[34] which is necessary in ICSI as sperm tail movement within an oocyte may damage intracellular components and reduce successful fertilization rates. Immobilization is a lengthy and difficult technique, requiring highly trained ICSI technicians and embryologists when performed manually. However, automation of sperm immobilization may standardize performance across clinics, reduce failure rates due to steep technician learning curves, and decrease treatment times. MPS have also been developed for oocyte immobilization and microinjection of sperm into the oocyte.[35]

Other MPS have the flexibility of design so that they can position gametes in particular orientations. The ability to tune this property can be manipulated to reduce polyspermic penetration, which can lead to aberrant embryonic development and decreased IVF success rates. Clark *et al.*[36] developed a system whose channel design mimics *in vivo* structure in order to ''park'' the porcine oocyte in space, which allows for continuous visualization and the ability of the sperm to flow freely throughout the media.[36] This occurs naturally via mucosal secretions and mechanical, anatomical constraints of the oviduct (analogous to the fallopian tube in humans). Techniques employed by Clark *et al.* increased the rate of monospermic penetration and number of potentially viable embryos without sacrificing typical production efficiency. Excitingly, systems that incorporate each step in IVF have been developed. Ma *et al.*[37] created a system, called ''IVF lab-on-a-chip,'' with a circular fencing structure large enough to contain and position the oocyte with gaps that allow for sperm entry and fertilization. Their chip takes into account sperm motility screening and medium replacement, and even allows for dynamic monitoring of early embryonic development for selection of healthy embryos for implantation.

New MPS technologies streamline the process of gamete selection and preparation, with many showing new gains in efficiency, treatment times, and fertilization rates. They allow for reduced manipulation and damage to gametes and improved culturing techniques to maintain *in vitro* health of these cells. Incorporation of new technologies, such as improved real-time imaging and determination of gamete quality, allows for inclusion of only the highest quality gametes for use in ART and may reduce issues, such as genetic abnormalities, poor sperm motility and morphology, and polyspermic fertilization, in the future. The field of ART may be reinvigorated by MPS technology, which has the capability to increase consistency across clinics, reduce the amount of technician training time and error, and increase success rates for subfertile couples.

Pregnancy

Microfluidics have been implemented to improve on the current technologies for embryo development, prenatal diagnosis, drug testing, and to study placental biology. Platforms have been specifically designed to accommodate a more physiological microenvironment for the developing embryo and to provide a quantitative measurement of embryo metabolism with minimal handling in small sample volumes. Microfluidics have been used to improve upon the PCR technology in order to provide a more specific, sensitive, and faster detection of fetal DNA in the maternal serum. Drug testing on non-human embryos has been performed in MPS that allow for more rapid and automated analysis of drug effect on the developing embryo. MPS have also been developed to model placental function, which may provide further insights on the ability of drugs to cross the maternal–fetal barrier. These emerging MPS technologies have forged a new frontier in facilitating our understanding of human pregnancy.

Embryo development

In ART, MPS have been employed for studying embryo development in culture, which has been recently reviewed.[38] Usually, microfluidics are designed to provide a mimic of the *in vivo* environment or to streamline processing with minimal disruption. Research studies using mice embryos demonstrate an improvement in embryo development and quality, with better strategies for monitoring embryo quality. Various forms of microfluidic platforms have been constructed from a number of different materials, including PDMS and silicon glass.[38] Platforms involve channels in various shapes and sizes that allow for a controlled flow of media and are designed to hold a single

embryo or group of embryos. With such devices, the entire preimplantation stage of the embryo in culture can be monitored.

Huang *et al.*[39] developed one such device to more closely mimic the *in vivo* microenvironment of the developing embryo. They developed a digitalized microfluidic device powered with electrowetting on a dielectric (EWOD). The EWOD device was designed to mimic the *in vivo* fluid flow that occurs during embryo development. In order to do this, the movement of a single droplet of liquid that harbors the embryo is possible through varying the wettability of liquids on a dielectric solid surface by altering the electric potential.[39] As a result, the rate of mouse embryo cleavage to a hatching blastocyst with this system was significantly greater than that with a traditional static culture ($P < 0.05$). Embryo transfer to pseudo-pregnant female mice produced live births, demonstrating viability and potential for live birth after culture in EWOD device. The force of flow[40] and the pulsatile nature of the flow[41] can directly affect embryo quality.

The ability to monitor the biochemical actions or metabolism of developing embryos in real time has been made possible with some devices. Heo *et al.*[42] built a platform that was able to monitor in real time glucose consumption by embryos at the blastocyst stage. This automated program used a modified ''gated injection'' scheme in which sample was injected, reagent mixed, and an enzyme reaction allowed to occur, followed by sample detection with fluorescence every hour. With this system, measurement of time-dependent nutrient consumption by live mouse blastocyst-stage embryos was possible. Urbanski *et al.*[43] built a platform that non-invasively measured glucose, pyruvate, and lactate. Samples and enzyme cocktails were aliquoted, reagents mixed, data acquired and analyzed without intervention. Oxygen consumption has also been measured in microfluidics during the development of the zebrafish embryo by tracking the oxygen concentration of the medium over time via phase-based phosphorescence lifetime detection.[44] MPS that are better able to control features of the microenvironment, decrease manipulation of embryos, and monitor embryo quality in real time may improve IVF for our patients.

Finally, MPS are being developed that allow for the preservation of embryos through automated vitrification of zygotes.[45] Pyne *et al.*, for example, created a digital microfluidic device, which allows for the manipulation of embryos within liquid droplets via electrodynamic force and improves imaging capabilities to monitor embryos. Devices such as this may allow for the development of better cryopreservation techniques for human embryos in IVF.

Prenatal diagnosis

Steele and Breg[46] were the first to culture and karyotype fetal cells in amniotic fluid for diagnostic purposes. Technologies testing fetal cells for chromosomal abnormalities continue to improve by becoming less invasive with earlier diagnostic potential. The detection of fetal cells in maternal serum has been made possible with new technologies that are sufficiently sensitive to identify a significantly limited number of cells. MPS has emerged as a method superior to conventional processing of serum to isolate fetal cells.[47]

MPS have been used to detect and amplify cell-free fetal DNA using PCR. With this technology, less DNA and reagents can be used and a higher sensitivity is achieved. A microfluidic chip was built to perform a digital PCR that allows for simultaneous assessment of thousands of PCR reactions in a tiny droplet. Thus, the use of multiple dilutions of each DNA sample in multiple compartments results in the quantification of low abundant DNA. Such devices are now commercially available[48,49] (Life Technologies, Carlsbad, CA, USA) and offer analysis of up to 36,000 parallel reactions. In one study, detection of fetal aneuploidy of trisomies 21, 18, and 13 using maternal serum was possible using microfluidic digital PCR as early as 14 weeks of gestation.[50] Digital PCR was used to identify male fetuses carrying the X-linked mutation for hemophilia as early as 11 weeks of gestation.[51] In another study, sickle cell anemia was detected in male and female fetuses (82 and 75%, respectively).[52] Fetal Rhesus D (RHD) genotyping has been done by microfluidics digital PCR from two alloimmunized women with the variant RHD(IVS3 + 1G > A) allele.[53]

Microfluidics have also been used to enrich or separate the DNA of interest in a more refined and sensitive way from maternal plasma.[54,55] Enrichment of fetal nucleated red blood cells has been possible with a high-throughput and highly efficient microfluidic device where nucleated blood cells from the maternal plasma were sorted through an intricate system of micropillars.[56] This device was able to process 5–20 mL maternal blood in 2–6 h, effectively eliminating 99.99% of red and white blood cells. Cases of trisomy 21 and 18 were detected in the 11th to 21st week of gestation and trisomy 13 was detected in the 16th week of gestation. Other systems of similar concept used various materials, such as PDMS or silicon, and different microchannel designs to separate fetal cells from maternal plasma.[57–59] Ho *et al.*[60] developed a microfluidic system that performed FISH analysis within 3 h. This system was faster in terms of processing time, compared to the conventional 24–48 h for FISH analysis, and reduced the cost by half. MPS currently have the capability to detect genomic abnormalities prenatally using minimally invasive procedures, such as maternal blood draw, with high efficiency and improved processing considerations, including decreased time.

Drug testing and safety

The lack of appropriate technologies and model systems has made drug testing and screening for pregnant women a major challenge. Zebrafish embryos have been used as a model for drug toxicology and one group developed a miniaturized chip-based device for *in situ* analysis of the zebrafish embryos.[17,61] This device trapped and immobilized embryos for a more rapid and automated analysis of the developing embryo. Continuous micro-perfusion of drugs and toxins was possible and used small quantities of compound.[62] Compounds tested included copper sulfate, phenol, ethanol, caffeine, nicotine, and dimethyl

sulfoxide.[62] There are few studies that implement microfluidics for drug safety testing on embryos. Improvement in this area would have a significant impact in pharmaceutical safety testing of embryos.

Placenta

Thus far, two groups have reported the development of a ''placenta-on-a-chip'' micro-device[63,64] in order to simulate placental function for the ultimate use in drug testing, as well as investigating functional mechanisms of the placental barrier. Both groups created an *in vitro* placenta mimic of the architecture of the human placental barrier by co-culturing human trophoblast cells and human fetal endothelial cells. Blundell *et al*.[63] built a device with upper and lower microchannels separated by a thin, semipermeable membrane. The BeWo b30 human trophoblast cell line was cultured in the upper microchannel on the apical side of the membrane, and villous endothelial cells were grown in the lower microchannel on the basal side of the membrane. Trophoblast cells were induced to fuse and form a syncytialized epithelium that resembled the syncytiotrophoblast. Cultured trophoblasts formed dense microvilli under dynamic flow conditions and expressed glucose transporter proteins, which are physiological membrane transport proteins that play a role in barrier function of the placenta. Physiological transport of glucose across the microengineered maternal–fetal interface was demonstrated by this group.

Another group built a ''placenta-on-a-chip'' device using soft elastomer-based microfabrication also known as soft lithography.[64] This device made use of microfluidic channels separated by a thin extracellular matrix membrane. On one side of the membrane a human trophoblast cell line (JEG-3) was cultured and on the other side, human umbilical vein endothelial cells were cultured with a constant dynamic flow. Glucose transport function over time was investigated in this device as a test of physiologic function. Placental MPS models may provide new insights for researchers studying the complex microenvironment of this tissue and additionally open the door for the development of devices to safely test drug effects and toxicities in pregnancy *ex vivo*.

MPS technology has a variety of applications in embryo development and pregnancy. Systems that are built to better mimic in vivo physiology could reduce the physical manipulation of embryos and improve culture techniques to yield higher quality embryos for implantation in ART. Non-human embryo culture in MPS, such as those being developed in zebrafish, may improve the efficiency of drug testing and development. Furthermore, ''placenta-on-a-chip'' systems may help us identify drugs that may cross the placental barrier and cause toxicities in pregnant women. Finally, MPS, such as those incorporating PCR-based technologies, have potential to improve prenatal diagnosis of fetal genetic abnormalities. Overall, MPS technology involving pregnancy-related tissues and cells, such as embryos and the placenta, hold great translational promise.

Reproductive tract disease

Most of the existing literature on female reproductive tract diseases using MPS centers around OC, and in particular the especially lethal high-grade serous ovarian cancer (HGSOC). A handful of additional studies focus on endometriosis. Considering the fact that MPS can be widely applied from basic biological studies to the creation of point-of-care diagnostic devices, the field is currently wide open for expansion of this technology to other female reproductive tract diseases.

OC

Though relatively rare, HGSOC is the female reproductive tract disease most extensively studied in MPS for a number of reasons. First, the dramatic decline in relative survival in early versus late stage disease (92% relative five-year survival in early stage disease versus 29% in late stage) combined with the current unmet need for early detection methodologies creates fertile ground for the technological advances promised by MPS.[65] A number of examples exist in the literature exploring MPS's diverse capabilities, including platforms that are capable of screening panels of biomarkers in blood samples using in-chip multiplexed immunoassays.[66] Some MPS are capable of enriching for tumor antigens in exosomes, which are abundant in cancer patient blood and may be involved in disease progression and metastasis.[67] Other platforms have been created for the development of new biomarkers, such as one system designed to screen phage display libraries for HGSOC-specific oligopeptides,[68] another MPS made to examine the cancer glycome (or global protein glycosylation) for new glycan biomarkers,[69] and a third system that uses cell-based systematic evolution of ligands by exponential enrichment (called Cell-SELEX) for OC-specific nucleotide-based aptamer development.[70] The latter group incorporates the SELEX process, a popular method for affinity screening, into their MPS in order to greatly improve processing efficiency and throughput and reduces the amount of time, reagents, and sample size required.[70] Finally, MPS have also been used to enrich for tumor cells isolated from ascites fluid of cancer patients in order to perform molecular analyses, including biomarker identification.[71] All of these systems improve upon currently existing techniques by streamlining the process within a single device and decreasing processing time to minutes or hours and sample volume down to microliter amounts.

One predominant goal of MPS has been to improve the process of drug development and to provide personalized medicine to patients currently battling disease. In HGSOC, though current first-line treatments are initially effective, most patients relapse or develop chemoresistance, at which point no widely accepted second-line therapies exist.[72] To illustrate this, Das *et al*.[73] developed a microfluidics-based multiplex platform wherein spheroids were subjected to chemotherapeutic treatment with or without specific microenvironmental factors. Compared to traditional culturing techniques, spheroids in the MPS were more chemoresistant, and the addition of extracellular matrix and high serum concentration further augmented

the chemoresistance observed. Astolfi *et al.*[74] have developed a process for creating uniformly sized micro-dissected tissues (MDTs) from small clinical samples, such as biopsies, for use in a microfluidic device capable of extended culture and drug treatment. Their system standardizes the processing of primary human tissues in order to improve treatment success rates for individual patients or screen new potential chemotherapeutics in the future. MDTs are comparable in size and ability to grow without continuous perfusion to spheroids, which have become increasingly popular due to their ability to form 3D structures. The use of primary tissue in the form of MDTs is ideal since they encapsulate a more complex tissue architecture and represent the specific patient's disease better than spheroids developed from cancer cell lines. Systems are also being developed that can rapidly isolate cancer cells from patient samples. For example, Lien *et al.*[75] developed a system that can isolate over 90% of OC cells using immunomagnetic beads from samples of relatively large volume (~1 mL) in just 10 min. The system has the built-in capability to lyse the tumor cells and perform a panel of PCR reactions, which may have promising clinical applications for diagnosis and identification of specific mutations in each individual's disease. Additionally, the aforementioned ascites tumor cell MPS could be adopted to monitor individual patients for drug response or relapse of disease through the minimally invasive collection of ascites fluid.[71] The development of new model systems and devices for use in OC brings us closer to fulfilling the promise of MPS, and the field awaits data on the successes of their translation to the clinic.

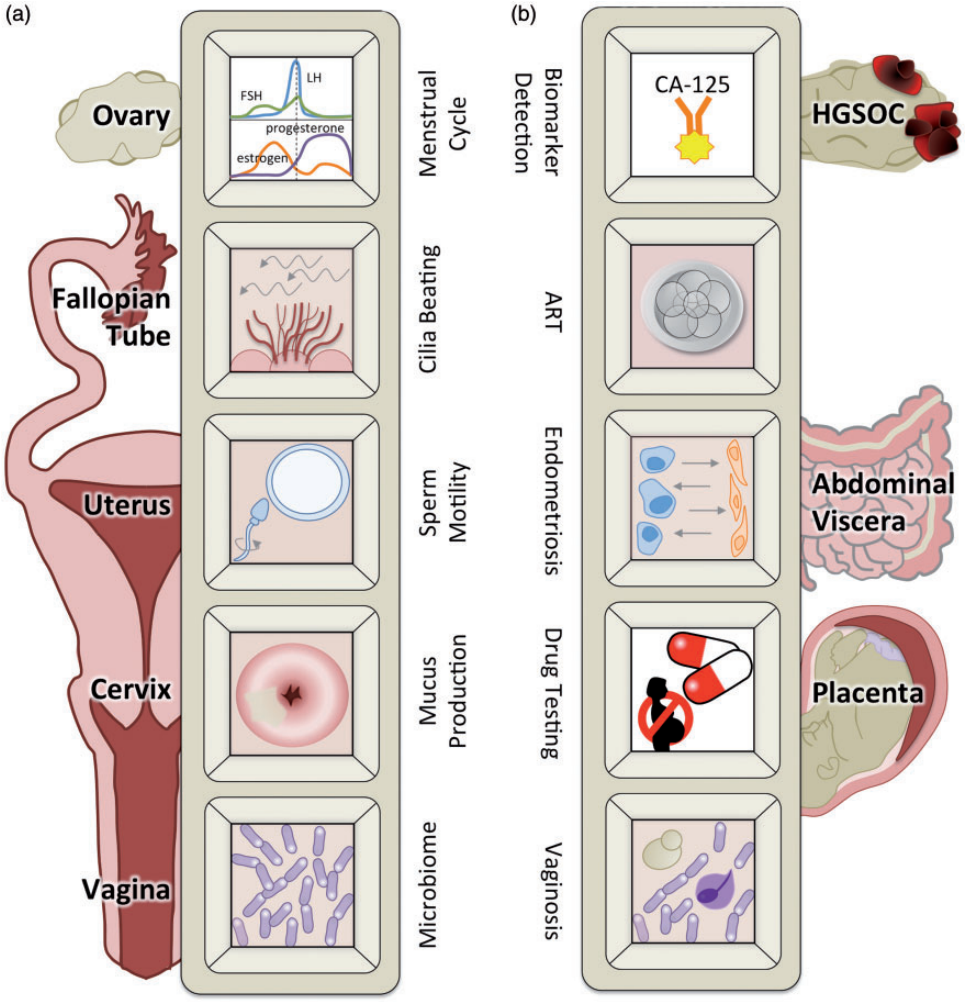

Figure 1 Examples of MPS technology to model and study the female reproductive tract and related clinical applications. (a) The tissues of the female reproductive tract are depicted paired with examples of physiologic phenomena that may be investigated in MPS. These technologies include monitoring metabolic and reproductive biomolecules and hormones secreted by reproductive organs using in-line biosensors, ultrasensitive immunoassays, and/or mass spectrometry. Cilia beating and gamete selection and fertilization can be detected by on-device micro-cameras, which enable monitoring of cell movement and interaction in real time. Cervical mucus and other materials for sperm selection may be produced or incorporated in MPS. Detection of the complex interactions between organs and microorganisms can be monitored with MPS-based PCR. (b) Examples of the clinical applicability of MPS with co-culture of additional tissues are illustrated. Point-of-care MPS are being developed for the detection of ovarian cancer biomarkers or pathogenic microorganisms. IVF labs-on-a-chip promise to streamline and improve success rates for assisted reproduction. Complex interactions between endometrioid and intestinal cells may yield insights into the processes of cell migration and invasion in endometriosis. Incorporation of human placental tissues into microfluidic drug testing may reveal potential toxicities in pregnancy. IVF: *in vitro* fertilization; MPS: microphysiologic system; PCR: polymerase chain reaction

Endometriosis

Endometriosis is hypothesized to occur when endometrial epithelial and stromal cells travel from the uterine lumen through the fallopian tubes and into the peritoneal cavity through retrograde menstrual fluid flow. Outside the uterus, endometrial cells may cause significant damage to tissues they invade, leading to a range of sequelae, including pain and infertility.[76] Endometriosis' combination of fluid dynamics, cellular migration, cell–cell interaction, and invasion makes this disease especially well suited for study in MPS. Chen *et al.* created a small platform with microfluidic channels to co-culture endometrial stromal cells (ESCs) and human peritoneal stromal cells (HPMCs) from endometriosis patients versus healthy controls. The platform allows for separate cell populations to grow, migrate and interact with one another, and for the cell–cell interactions to be monitored in real time. The group showed that normal HPMCs are capable of resisting invasion by both normal or endometriotic ESCs, but endometriotic HPMCs cannot resist such invasion from either type of ESC.[76] While many endometriosis studies focus on endometrial tissues and the immune system as instigators of disease, this study brings new focus to the microenvironment of pathogenic sites. While some groups like Chen *et al.* are developing devices to culture and study tissues and cells, others are using these versatile technologies to monitor specific endpoints. For example, another group used their MPS to discover decreased activity of various matrix metalloproteinases, which have been implicated as important enzymes in endometriosis, in peritoneal fluid of endometriosis patients versus healthy controls. Their droplet-based microfluidic platform is especially notable in its ability to simultaneously analyze hundreds of protease activity reactions in biological or clinical samples of limited quantity ($<20\,\mu L$).[77] These two studies highlight the versatility of MPS and embolden us to consider the possibilities of uniting combinations of these systems in future studies and collaborations.

Future potential

Few, if any, studies exist looking at female reproductive tract diseases apart from OC and endometriosis, though many could benefit from application of this technology. For example, great progress has been made in GI MPS, including the successful co-culturing of GI epithelial cells and bacterial flora.[78] The vaginal epithelium has its own unique microbiome, which is vitally important in maintaining the reduced vaginal pH, among other characteristics, that protects the vagina and reproductive tract from potentially pathogenic organisms.[79] Alterations to microbiomes throughout the body, including the vagina, have been associated with a number of factors important for female health, including obesity, sex hormones, and cancer.[80] For example, the composition of the vaginal microbiome is significantly different in premenopausal patients versus peri- and postmenopausal patients.[81] MPS could be used for similar co-culture experiments to those conducted by our colleagues in GI to study any number of microbiome-related phenomena. Though new culturing techniques may need to be developed for the study of specific pathogens, there exists extensive space for research into bacterial, viral, and other infections of the female reproductive tract, including bacterial vaginosis, yeast infections, sexually transmitted diseases, pelvic inflammatory disease, HPV infection, and cervical cancer.

Though literature on MPS research in diseases of the female reproductive tract is sparse, existing studies on OC and endometriosis are forging a new path with exciting results. HGSOC has proven to be a formidable cancer, and decades of study have offered little progress in early detection methodologies and targeted treatments. MPS may provide the technological advances needed to isolate and detect biomarkers or inform diagnostic decision-making from small-volume patient samples. Some systems are even being developed for the discovery of new biomarkers with improved sensitivity and specificity. Other groups have ventured into the realm of personalized medicine with the creation of high-throughput systems to test new chemotherapeutics on primary human samples. MPS researchers in endometriosis have focused on foundational studies elucidating cell-to-cell interactions and enzymes important in the microenvironment of the disease. Many other diseases affecting the female reproductive tract could benefit from incorporation of MPS, including those whose pathogenesis involves microorganisms. As MPS technology evolves and becomes more common, new insights may be gained in these and other female reproductive tract diseases.

Conclusion

MPS technologies, including microfluidics and organs-on-a-chip, are formed through the union of extensive foundational science and advances in bioengineering. They are aspirational technologies, driven by a researcher's need to create increasingly realistic physiologic microenvironments for scientific study, while maintaining the level of experimental manipulation they are accustomed to *ex vivo*. MPS benefit from ongoing progress in making existing technologies better, faster, more accurate and, of course, more ''micro''. Many fields have benefitted from the addition of these technologies to existing studies, though female reproductive tract research is lagging on this front. The anatomy, physiology, biological signaling, and mechanical factors that impact the female reproductive tract are highly amenable to study in such MPS, and many devices engineered for use in the study of other organ systems could be adopted or modified to fit the field's needs. Studies on the female reproductive tract can be complex, but MPS offer the capability to overcome such complexity through the incorporation of different tissues (ex. reproductive tract organs, placenta) or cell types (ex. gametes, tumor cells), features of the media (ex. hormones and signaling molecules, viscosity), fluid flow, and assays and technologies used for endpoint analysis (ex. micro-cameras, mass spectrometry, immunoassays, etc.). The translational promise for some of these systems is on the horizon, with applications in assisted reproduction, drug testing, and point-of-care diagnostics. The possibilities for MPS technology are truly

endless in the field of female reproductive biology and related reproductive tract diseases.

Authors' contributions: All authors contributed by writing sections of this minireview: GM-H was responsible for the ''New Technologies'' section, JEB the ''Gametes & Fertilization'' section, and JJK the ''Pregnancy'' section. AY wrote all other sections, compiled, and edited the final manuscript.

ACKNOWLEDGEMENTS

The authors wish to acknowledge financial support through grant UH3 TR001207 from the National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH), Bethesda, Maryland.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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