Vagina-on-Chip Model for Elucidating Underlying Mechanisms of Vaginal Cell Interactions

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Abstract

The vagina is a critical organ that remains understudied due to the insufficiency of current methods to replicate tissue interactions. No current three-dimensional (3D) models exist to model the vagina's dynamic, multi-layer physiology thus limiting the development of targeted therapeutics. The emerging field of Organ-on-chip (OoC) technology utilizes advances in tissue engineering and microfluidics to reproduce tissue and organ- level functionality of living organs, but none have been developed to model the vagina. Therefore, the aim of this study was to design the first vagina-on-chip (VoC) device to mimic native vaginal tissue physiology and allow for predictive drug response. A novel device was created, which includes a cell culture chamber for the encapsulation of vaginal cell types in layered hydrogel scaffolding, a fluidic component for sustained nutrient delivery, and a platform for real-time, high-throughput data collection. Prototypes were created using CAD and 3D printing techniques. Fluidic components were assessed using COMSOL Multiphysics software for convection and diffusion modeling. Such techniques revealed 2 mm hydrogel depth as the optimal thickness for maintaining a 0.16 mM oxygen concentration after 1 hour of media delivery. The final design incorporates these parameters for enhanced cell viability and is multiplexed to allow for the culturing of eight tissue constructs supplied by a single media port for high-throughput experimentation. The development of this device serves as a crucial first step in the creation of a novel platform to study dynamic vaginal tissue physiology and accelerate the development of therapeutics to improve female healthcare outcomes.

Keywords: vagina, organ-on-chip, microfluidics, tissue engineering, reproductive health

Introduction

The vagina is a female reproductive organ that serves a multitude of functions throughout a person's lifespan. This organ plays many vital roles in female health, serving as a canal for menstrual fluid and tissue to leave the uterine cavity, a receptacle for sperm, and a vessel for childbirth. Additionally, the vagina serves as a line of immune defense, protecting the body against harmful pathogens via its acidic pH, local flora, and chemical signaling.[1] Recent evidence also indicates the vagina may have potential as a drug delivery route for both systemic and local therapy due to the ability to avoid first-pass metabolism, the ease of administration, and high permeability for low molecular weight drugs[2]. Despite these critical roles for female health, the vaginal organ remains relatively understudied with many key cellular interactions to investigate. Although developments in gynecologic health research continue to advance, relatively few groups specifically focus on vaginal tissue research, especially in areas such as wound healing, device development, and drug toxicity.[3] As a result, there is insufficient data for researchers to use for the development of targeted therapeutics, thus leaving vaginal conditions such as the atrophic effects of menopause, the fibrotic tissue effects of pelvic radiation after gynecologic cancer treatment, and the dysbiosis of the vaginal flora without treatments.[4–6] Therefore, there is a major need to create novel research methods to study this organ and model its dynamic physiology, including layers of predominantly

three cell types: the squamous epithelial layer that lines the inner vaginal cavity, a connective tissue layer primarily composed of fibroblasts, and a smooth muscle layer.[1]

Current methods of studying vaginal tissue are limited but still provide critical insights on isolating controlled variables to study the complex and dynamic composition of the vagina and its microbiota.[7] The most common method of studying vaginal tissue is two-dimensional mammalian cell culture; unfortunately, this technique provides an unrealistic representation of the cellular microenvironment since these cells have been separated from their cellular and physical contacts, propagated through weekly divisions, and maintained on flat plastic. Thus, these cells are missing the cell physiochemical microenvironment, three-dimensional tissue specific architecture, and dynamic fluid flow of native tissue. Recent advances in organoid culture allow for some of this 3D dimensionality, but rely on the spontaneous self-assembly of cells to achieve complex tissue and organ-level organization and function.[8] Finally, animal models such as mouse, rat, rabbit, minipig, and sheep are commonly used in vaginal research, but these models do not fully encapsulate the complexity of the human vagina due to structural and physiological differences.[3] Although animal models can provide valuable insights into the complex biological responses needed to assess the safety and efficacy of therapeutics, these species-specific differences often lead to inaccurate data extrapolation that further increase the expenses of preclinical testing.[3,9] Therefore, innovative methods to culture cells *in vitro* and test new compounds are therefore necessary to properly replicate tissue properties and provide a platform to test under biomimetic physiological conditions to measure therapeutic effects. Developing a system to effectively study physiologically relevant vaginal tissue will provide a platform for elucidating critical mechanisms of vaginal wound healing for increased understanding of female physiology and the development of much needed therapeutics.

Organ-on-a-chip (OoC) technology utilizes advances in tissue engineering and microfluidics to enable the design of customized cellular microenvironments with precise fluidic, mechanical, and structural control.[8] These emerging technologies facilitate the creation of 3D models that exhibit functional hallmarks of native tissues, a more precise recreation of species-specific biology. While organoid culture relies solely on the self-assembly of cells, OoC devices can incorporate multiple cell types, guide spatial confinement of cells, as well as integrate sensors and microfluidic channels.[8] Lab-on-a-chip systems can then be developed by connecting multiple OoCs to enable dynamic and precisely controlled interaction between organs, a critical step in toxicology and therapeutic discovery.[10] Therefore, these systems can replicate key aspects of human physiology for the understanding of drug effects, improving preclinical safety, and efficacy testing. OoC devices have recently been developed for many organ systems, including the liver, heart, lung, and even the female reproductive system, yet no such platform exists to study the vagina.[11]

Thus, we propose to address this critical limitation by applying OoC techniques to develop a design for the first vagina-on-chip (VoC) device that will aptly mimic the dynamic and layered structure of the vagina and enable predictive human response to novel treatments in high-throughput. Therefore, the aim of this study is to design, iterate, assess. and optimize an organ-on-chip solution to be implemented into vaginal research for elucidation of key mechanisms into the vaginal wound healing process that will lead to the downstream development of novel therapeutics. The design will be developed on the basis of three design criteria: the device must allow for the recapitulation of the multi-layer vaginal tissue physiology, incorporate a fluidic component for sustained nutrient delivery, and provide a platform for real-time, high-throughput data collection.

Results

Development of Design Concept

To begin the design iteration process, a conceptual schematic was generated to illustrate the essential components to be included for the incorporation of the design criteria (Figure 1). Recent organ-on-chip literature was examined to reveal methods of integrating various cell types in a single fluidic circuit that allows crosstalk while preserving the individual cell functionality. Yu *et al.* revealed the technique of vertically stacking two or more

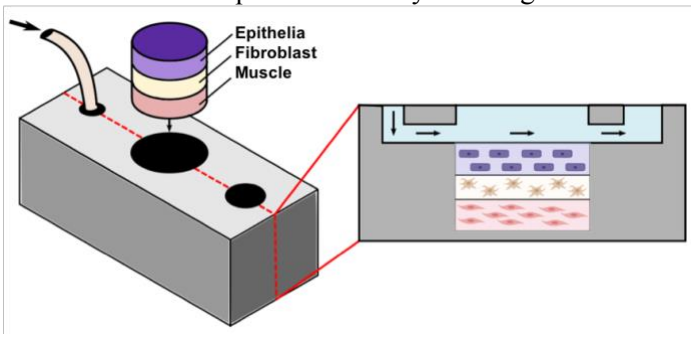

Fig. 1. Overall device schematic. An overview of the design concept and cross-sectional view of its components: a cell culture chamber to incorporate vertically-stacked hydrogels with three vaginal cell types, a port for tubing attachment to supply media through a microfluidic channel across the surface of such chamber, and a reservoir to collect the excess fluid after delivery.

Fig. 2. Preliminary CAD models. (2A) Primary iteration of the vertical design, including a port for tubing attachment, a singular cell culture chamber for vertical hydrogel stacking, and fluidic channel that empties into one reservoir for media collection. (2B) Primary iteration of the horizontal design, including a port for tubing attachment, three ports leading to individual chambers for hydrogel insertion, and two reservoirs for media collection. (2C) Inside view of the horizontal design, illustrating the three adjacent channels separated by cylindrical phase guides and surrounded by two channels for fluid delivery that empties into the separate reservoirs.

cell culture compartments to model tissue interfaces with different cell types.[12] Therefore, to best model the multi-layer organization of the vagina, we decided to use this vertical connection strategy to best culture the three vaginal cell types. In this configuration, each of the vaginal cell types would be integrated with its own layer of hydrogel scaffolding and individually inserted into the cell culture chamber. The smooth muscle layer would be inserted first, followed by the fibroblast and then epithelial layer. This scaffolding will allow the cells to co-cultured in close proximity to each other and thus grow into the 3D, layered orientation of native tissue. Additionally, this schematic includes a port for tubing attachment so that cell media can be delivered through a microfluidic channel across the surface of the cell culture chamber, allowing for diffusion of media through to the bottom of the tissue construct. Finally, this design includes a reservoir on the other side of the chamber to collect the excess media to be tested for different metabolic compounds, growth factors, or other components that would provide valuable data for studying vaginal cell culture. Figure 1 illustrates this overall schematic, providing a cross-sectional view of the chamber and fluidic compartments.

Design Constraints

The incorporation of fluid flow is essential for the dynamic modeling of cells to facilitate the administration of nutrients and waste discharge. At the microscale level, the fluid acts primarily as laminar flow, resulting in a stable gradient of biochemical molecules with spatial and temporal control[13]. Thus, microfluidics can be used to simulate complex physiological processes in the human body by altering flow velocity and channel geometry to achieve stable 3D concentration gradients and is therefore a critical component for VoC design. Thus, design constraints included the incorporation of consistent fluidic delivery to the tissue culture in order to replicate the dynamic conditions of the cell microenvironment. Additionally, adequate media delivery is critical for the maintenance of viable cell culture within the device for extended periods of experimentation. Therefore, the VoC system must be developed to meet the metabolic requirements of vaginal tissue culture over time. The application of studying vaginal scar formation poses additional constraints, since the wound healing process occurs over the span of multiple days, and thus our device must maintain cell viability for at least a week to allow for relevant experimentation and long-term observation. Finally, the multi-layer physiology of the vaginal organ presents additional constraints for VoC design, as the device must allow for the culturing of all three vaginal cell types in a way that allows them to keep their distinct morphologies but allows the cell-cell communication that occurs in native tissue. This has yet to be achieved by current methods but is critical for elucidating the underlying mechanisms of vaginal cell physiology.

Iteration Development

Initially, two different CAD models were generated to meet these design requirements. The first design is similar to the schematic in Figure 1, allowing for the vertical stacking of three layers of hydrogel containing the vaginal cell types. This design includes a port for tubing, a cell culture chamber with an open top for hydrogel loading, and a microfluidic channel leading into the reservoir (Figure 2A). The central chamber will host the three hydrogel layers, with cell media flowing over the top. Because this design relies on the diffusion of media through to the bottom of the

chamber to promote cell viability, we decided to investigate an alternative design to enhance nutrient delivery. This design includes the hydrogel loading of three cell types in a horizontal orientation (Figure 2B). Thus, instead of a single cell culture chamber, this design includes three ports for hydrogel insertion into adjacent chambers separated by staggered cylindrical barriers to act as phase guides (Figure 2C). These phase guides were implemented to prevent overflow of the gels into the incorrect chambers, but still allow room for the different cell types to interact as they settle into their 3D physiology. This design similarly includes a port for tubing attachment, but has multiple channels for media delivery on both sides of the chamber construct that lead into two separate collection reservoirs.

Stereolithography printing demonstrated the successful replication of the designs' micro-scale features and provided information for design selection. Agarose was loaded to mimic the incorporation of tissue culture biomaterials to determine the feasibility to incorporate three distinct yet interacting hydrogel layers. Figure 3A illustrates the efficacy of such distinction in the vertical design, with three visibly distinct colored layers stacked on top of each other. Meanwhile, the horizontal design failed to maintain the agarose within their individual regions. The geometries of the cylindrical phase guides did not effectively delineate the three layers. The enclosed layout of this device was not conducive to including such hydrophobic regions, thus making agarose loading within the specific regions rather difficult. Therefore, 3D printing and agarose loading revealed the vertical design as the more viable option for further device development.

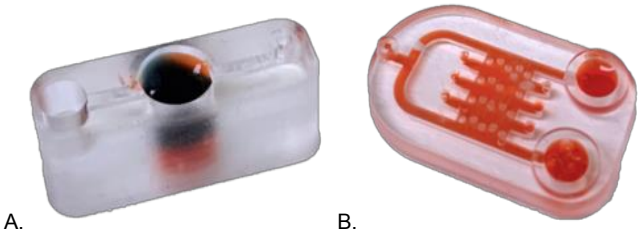

Fig. 3. 3D printed prototypes. (A) Printed vertical design with the successful layering of three agarose samples in the cell culture chamber. (B) Printed horizontal design with unintended leakage of agarose to all the channels. Both prototypes were loaded with multi-colored agarose to test hydrogel loading.

Assessment of Microfluidic Delivery

Selection of Microfluidic Components

Literature review informed the selection of a syringe pump as the method of fluidic delivery for this device. We decided that the attachment of a syringe pump would be most feasible for this application since it is the easiest and most common way to deliver precise, accurate, small amounts of fluid in a programmable manner.[14] Additionally, literature demonstrated the use of a syringe pump in a connective tissue model utilizing fibroblasts to study stretch/strain[15]. Since our device will include a fibroblast layer and may eventually be used to study these mechanical properties as well, the syringe pump revealed to be a reasonable selection. Since a syringe pump can be set at any desired value, we selected a velocity that is known to be sufficient based on previous COMSOL experience for our first attempt, 100 μm/s.

Velocity Profile Generation

In order to demonstrate that the fluid delivered through the microfluidic channel to the cell culture chamber would reach across the entire surface of the hydrogel, we created a top-view model of the design in COMSOL Multiphysics (Figure 4A). This simulation allowed for the generation of a velocity profile to visualize the flow distribution across the diameter of the chamber (Figure 4B). With the input velocity of 100 μm/s, the maximum velocity at the center of the well revealed to be 37 μm/s with lower velocities, illustrated by the darker blue color, at distances further from the center. Ideally, the velocities towards the edges of the chamber would be closer to the maximum value to provide

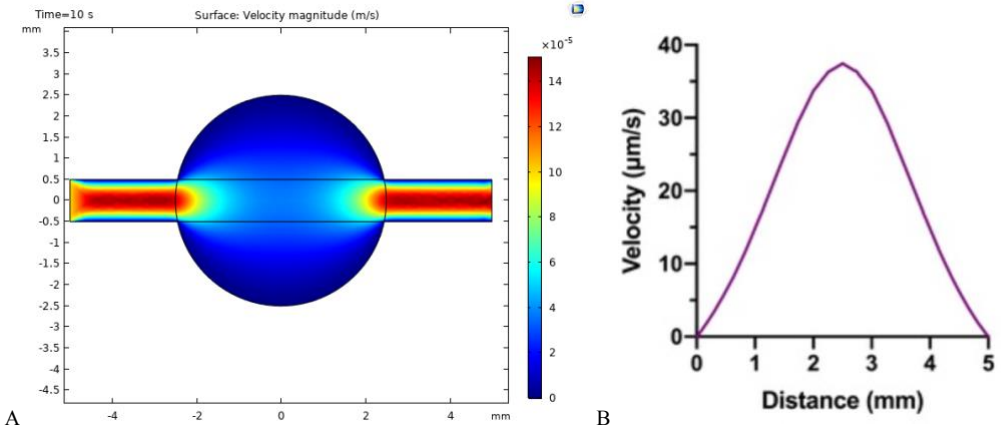

Fig. 4. Fluid flow simulation and velocity profile generation. (4A) Illustrates the velocity profile generated from the simulation of fluid flow across the surface of the hydrogel in the vertically-stacked design. The peak velocity reaches 37 μm/s. (4B) Shows the top-view model of system created in COMSOL Multiphysics software with a color gradient to visualize the different velocities across the area.

more equal media distribution and induction of shear stresses; future work may be done to adjust the chamber geometries in order to improve this. Overall, this analysis revealed the creation of a parabolic flow profile across the chamber surface, a valuable insight for microfluidic development.

Hydrogel Optimization

Assessment of Metabolic Requirements

The metabolic requirements to sustain cell culture in a microfluidic device were evaluated to determine the limiting factor for optimizing the chamber depth. Ideally, the cell culture chamber would be as large as possible to allow ample room for hydrogel incorporation; however, the supply rates for cell nutrients are limited by the diffusion distance, and thus the chamber depth. Therefore, to ensure the proposed system will be able to maintain cell viability of all three tissue layers, we must first determine the necessary rate of nutrient delivery. We analyzed literature to determine that oxygen would be the limiting factor, since it has the lowest exchange time compared to metabolic compounds such as glucose, lactate, and ammonium.[16] We found the optimal concentration to be 0.2 mM and the diffusion coefficient to be 2.4 x10^{-9} m^2/s (Figure 5). Finally, we determined our cutoff oxygen concentration would be 0.1 mM based on the physiological oxygen concentrations of several tissues.[17]

Compound	Metabolic Rate	Concentration	Exchange Time	Diffusion Coefficient
Glucose	250 $\frac{amol}{cell\,s}$	25 mM	28 h	2.4×10^{-9} $\frac{m^2}{s}$
Lactate	490 $\frac{amol}{cell\,s}$	20 mM	11 h	1×10^{-9} $\frac{m^2}{s}$
Ammonium	11 $\frac{amol}{cell\,s}$	2 mM	50 h	1.9×10^{-9} $\frac{m^2}{s}$
Oxygen	20 $\frac{amol}{cell\,s}$	0.2 mM	2.7 h	2.4×10^{-9} $\frac{m^2}{s}$

Fig. 5. Selection of oxygen concentrations. Bunge et al. comparison of metabolic compounds for a cell density of 1 x 10^6 cells/mL that illustrates oxygen as the compound with the lowest exchange time of 2.7 hours. Additionally, this study provides 0.2 mM as the optimal oxygen concentration and 2.4 x10^{-9} m^2/s as the diffusion coefficient.

Oxygen Diffusion Simulation

Next, we created a side-view model of the cell culture chamber in COMSOL Multiphysics software and simulated the diffusion of oxygen through the hydrogel layers over time (Figure 6). We repeated this simulation for gel thicknesses of 1, 2, 3, 4 and 5 mm, and time points 0.1, 0.5, and 1 hour. Figure 6D compares the oxygen concentration profiles for each of the gel thicknesses; based on this, we determined 2 mm to be the optimal thickness, since the oxygen concentration remains consistent at 0.16 mM, a

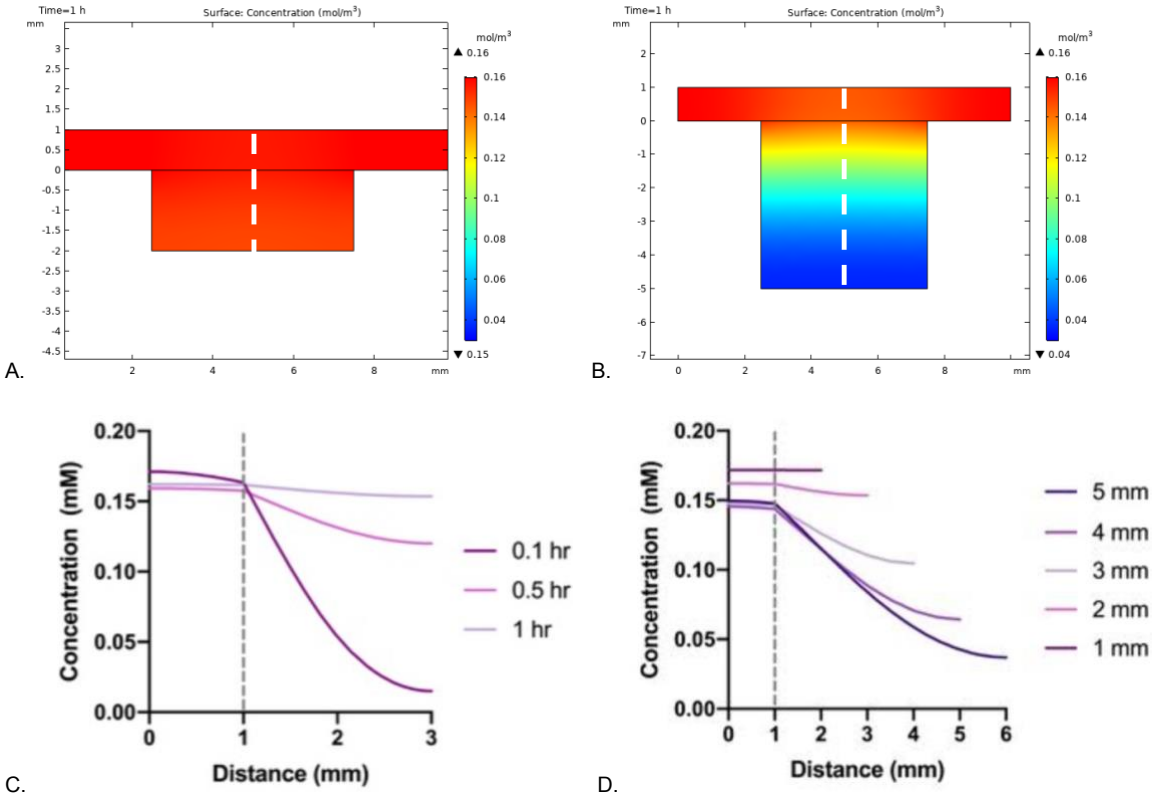

Fig. 6. Oxygen diffusion modeling for hydrogel thickness optimization. (A) Side-view model of 2 mm gel with a color gradient to visualize the range of oxygen concentrations. Dashed white line represents the point of velocity measurements. (B) Side-view model of the same experiment in the 5 mm gel. (C) Oxygen concentration profile throughout the 2 mm gel at time points 0.1, 0.5, and 1 hour. Dashed gray line represents the end of the channel and beginning of chamber. (D) Comparison of the oxygen concentration profiles for the models at different hydrogel thicknesses.

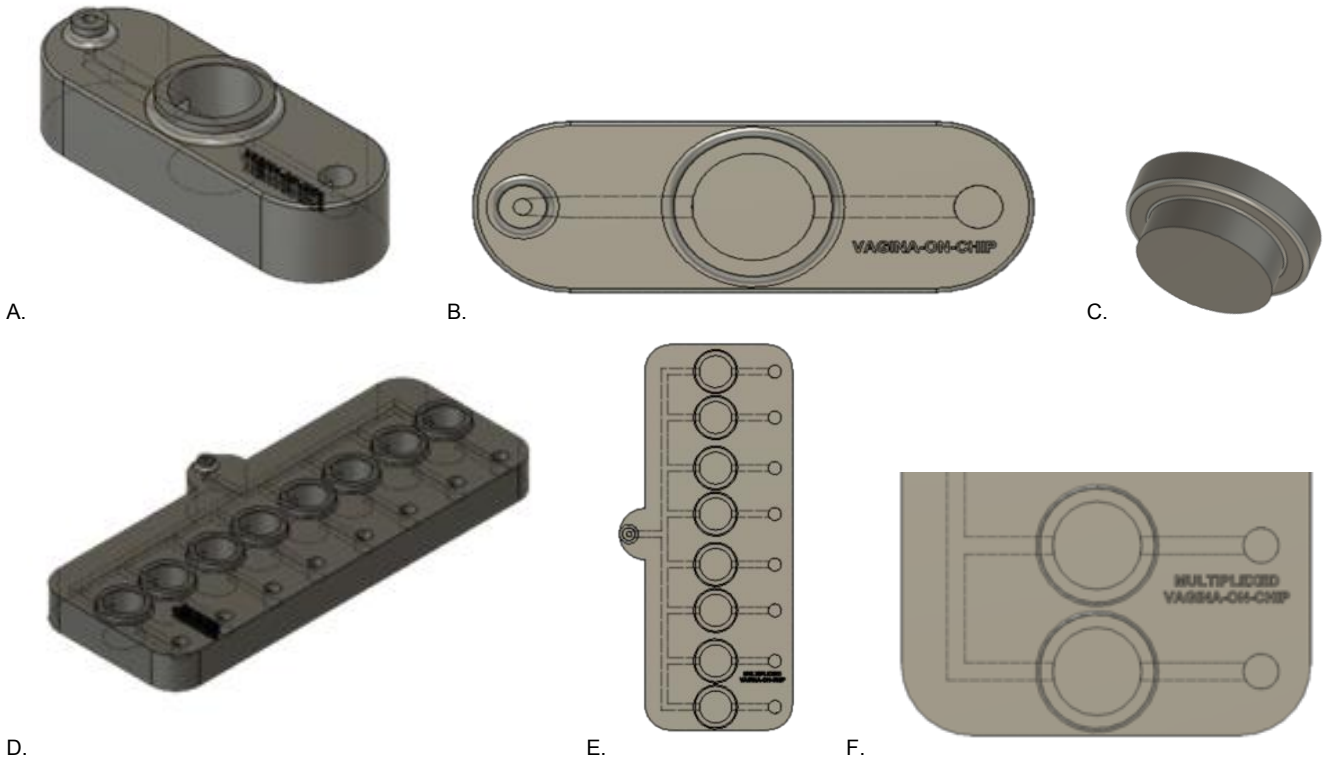

Fig. 7. Final design & multiplexed platform. (7A) Final CAD model of the vertical design including adjusted chamber dimension and a raised port for lid attachment. (7B) Top view of final CAD model, illustrates "VAGINA-ON-CHIP" inscription (7C) CAD model of removable lid (7D) Final CAD design of multiplexed platform including eight cell culture chambers supplied by a single port for tubing attachment (7E) Top view of final multiplexed CAD model (7F) "MULIPLEXED VAGINA-ON-CHIP" inscription

value within the determined range, throughout the construct after one hour. Although the 1 mm gel also remains at a high oxygen concentration, the 2 mm gel provides more room to incorporate the three different cell layers. Additionally, while the oxygen concentrations of the 3 mm gel also fall within the selected range, there is higher variability throughout the gel depth which is less ideal for maintaining consistency in cell culture. Therefore, the relatively even distribution of oxygen throughout the 2 mm gel presents a more ideal selection. Figure 6C illustrates this oxygen concentration profile in the 2 mm gel at the three different time points, demonstrating the ability of the entire gel construct to reach a stable oxygen concentration of 0.16 mM after 1 hour.

Final Design & Multiplexed Platform

The insights revealed from these assessments were applied to adjust and finalize the design. Since the diffusion modeling revealed 2 mm to be the optimal gel thickness, the dimensions of the cell culture chamber were altered to reflect this depth (Figure 7). Additionally, we created a removable lid for the cell culture chamber to improve oxygen retention and sterility during future experimentation. Lastly, we adjusted the rim of the cell

Fig. 8. Final printed designs. (8A) 3D-printed multiplexed device (8B) 3D-printed final single device with agarose to simulate hydrogel loading (red) and water (blue) to simulate cell media delivery through the channels and across the surface of the cell culture chamber.

culture chamber to provide a platform for the lid to rest on and added a "VAGINA-ON-CHIP" inscription to the device surface (Figure 7).

Finally, this design was expanded to create a multiplexed platform with eight identical cell culture chambers supplied by a single media port (Figure 7). This design allows for high-throughput experimentation as multiple experiments can be conducted at the same time. Figure 8 illustrates the final prints of these devices, with red-dyed agarose loading to simulate hydrogel incorporation and blue-dyed water to act as the cell media through the channels.

Discussion

The results of this project provide a novel method for studying the multi-tissue interactions of vaginal tissue that meets the three design requirements. Firstly, the chamber design allows room for the incorporation of three hydrogels to recapitulate the 3D physiology of native vaginal tissue. Secondly, the device includes an optimized fluidic component for sustaining cell viability throughout the construct. Finally, the multiplexed platform and open-top design with removable lids enable the collection of real-time, high throughput data readouts.

Next Steps

The accomplished design is limited as there is still work to be done in testing and demonstrating its efficacy as a tissue culture method. Firstly, the COMSOL simulations completed for the single chamber design must be repeated for the multiplexed device to evaluate the velocity profile through the eight channels and adjust the flow rate accordingly. Additionally, since the velocity profile generation revealed relatively low velocities towards the sides of the cell culture chamber compared to the center, future work can be done to alter the chamber geometry in order to produce a more even distribution.

Next, alternative 3D printing resins must be investigated to increase biocompatibility. The current resin was selected due to its availability and accuracy with which it can replicate geometries of microfluidic devices, however, this material will likely not be biocompatible with the introduction of cell culture. Thus, future work will involve printing the device with alternative resins or otherwise modifying the current material with a biocompatible spray. The Pompano laboratory is currently investigating the use of Parylene C Coating to improve the biocompatibility of their microfluidic devices. Once its efficacy has been demonstrated, this coating may be used to modify our device while remaining in its current resin.

Finally, although agarose was a useful tool for preliminary demonstration of hydrogel incorporation within the device, it will not be the biomaterial we ultimately select for scaffolding the layers of vaginal cell culture. Agarose forms a stiff, inert hydrogel that does not carry and biological information, and thus human cells do not adhere well to it. As a result, we plan to investigate alternative materials, such as collagen or polyethylene glycol (PEG) that can be tuned to match the mechanical properties of healthy and scarred vaginal tissue. Since the original diffusion simulations were conducted utilizing the parameters of agarose gel, these will have to repeated with different values once the new material is selected. Therefore, we acknowledge this design is limited in the way that it uses agarose as the baseline of the dimension optimization, and future iterations may differ from our original model.

Future Work

Once these steps are completed, the finalized device will be ready for incorporation of vaginal tissue samples for viability testing. According to OoC literature, vertically organized designs utilizing common cell culture medium to support multiple cell types are limited to tissues that are already mature and phenotypically stable.[18] Thus, it is critical to include human tissue samples for each vaginal cell type, incorporate them with mechanically-matched hydrogels to support their growth, and monitor viability within the device for at least a week in continuous perfusion culture. Confocal microscopy will be used to image the three layers to examine their formation into a 3D tissue construct.

The success of organ-on-chip devices depends on validation to ensure that the biological functions reproduced in the chip are representative of the native tissues.[18] Thus, further work will need to be done to refine the VoC to accurately parallel the mechanical, molecular, and physiological features of vaginal tissue. Hence, we plan to introduce a hormonal component that will model the physiological changes that the human vagina experiences throughout the 28-day estrus cycle. Such inclusion will present the ability to assess the physiological and pathological vaginal states, an essential feature for the development and evaluation of novel therapeutics.

Alternatives

The design that incorporates three culture chambers for horizontal layering of the three vaginal cell types was

rejected due to the failure of the cylindrical phase guides to adequately separate the different agarose samples. Although this was not further investigated, additional work can be done to optimize this design approach. For example, the dimensions of the phase guides can be adjusted to allow less room for the gel to move through and optimized so that the surface tension of the agarose prevents flow into adjacent chambers. Additionally, the nature of the material used for loading may have contributed to such overflow; a stiffer gel may better confine to its specific compartment and thus can be investigated for development of the horizontal design. Future work could include adjusting the stiffness of the agarose by increasing the gel concentration or using an entirely different material, such as a photo-curable hydrogel. Finally, the phase guides can be further modified by utilizing hydrophobic patterning to direct hydrogel insertion.[16] Therefore, although the horizontally-oriented device was not further examined in this project, there is potential for this design concept to be explored and developed as a novel platform for studying vaginal tissue.

Challenges & Limitations

The limited research on vaginal tissue presented many challenges in the development of the VoC device. Without adequate data on the biomechanical properties of vaginal tissue, we could not select mechanically relevant biomaterials for testing within the printed designs. Thus, our diffusion modeling may not provide entirely accurate representations of the oxygen concentration profiles in future tissue constructs. Furthermore, the lack of data on vaginal tissue physiology affected our selection of oxygen requirements for such modeling. Since the physiological oxygen concentrations for vaginal tissue were unavailable, our selected range of viable concentrations may not adequately represent the levels needed to maintain vaginal cell culture in a microfluidic device.

The project development was also impacted by limited access to laboratory resources. Restrictions posed by COVID-19 limited in-person experimentation and thus impacted the project trajectory. Collaboration with the Pompano laboratory provided the means to prototype and test the designs, but materials were limited to the resources utilized to develop the microfluidics for immune applications that is the focus of their research. Hence, we were unable to test the device with mechanically relevant biomaterials or study such integration with samples of vaginal tissue.

Although Dr. Hakim's laboratory investigates vaginal wound healing, their research is also impacted by limited access to human vaginal tissue samples. Only recently did vaginal epithelial cells become available for commercial purchase, thus the majority of research has relied on the isolation of primary vaginal cells from patient biopsies[3]. Thus, future work must be done to develop protocols for harvesting tissue from patients and maintaining culture for the vaginal fibroblasts and smooth muscle cells that cannot be obtained otherwise. Thus, the limited supply of all three tissue types will present challenges for demonstrating the viability and efficacy of the developed VoC device. To address this issue, the team has applied for additional funding to create a first-in-world Vaginal Tissue Biobank using specimen collected directly from patients at Texas Children's Hospital. Hence, upon completion of VoC development, the team must first acquire the necessary tissue samples to incorporate all three vaginal cell types into the device.

Downstream Applications

In order to facilitate innovation and novel treatment options to improve vaginal and gynecologic health, the ability to model both healthy and diseased vaginal tissue is imperative.[3] The device developed in this study serves as an essential first step towards the creation of such a platform for studying vaginal cell interactions. The further developed system will comprise human vaginal cells in a layered engineered tissue, creating a 3D physiological model of healthy and diseased vaginal tissue that vastly improved upon prior 2D models. This novel platform will allow researchers to elucidate the underlying mechanisms of vaginal cell interactions for the development and interrogation of targeted therapeutics for improved vaginal tissue outcomes. Specifically, this device will be applied to accelerate Dr. Hakim's K08 award for understanding the mechanisms behind vaginal wound healing. Successful completion of the VoC will enable the research team to conduct high-throughput studies and reveal critical insights that will promote the translation of therapeutic options and prevention strategies for vaginal scar formation. Thus, the developed system serves as a crucial first step toward improving patient vaginal scarring outcomes for people with vaginas worldwide.

Beyond immediate wound healing applications, the simplicity of the VoC design allows for the modeling of a wide variety of vaginal tissue conditions and disease states. Hormones, growth factors, and the tuning of mechanical properties of integrated biomaterials can be incorporated to more accurately model specific vaginal physiologies, such as the atrophic effects of menopause, which up to 50% of post-menopausal women experience but still remains

understudied and without sufficient treatments.[3] Additionally, future iterations of the VoC may integrate the complexities of the vaginal microbiome which also undergoes cyclical changes and maintains the acidic vaginal environment that contributes to the progress of conditions such as bacterial vaginosis, urinary tract infection and increased susceptibility to sexual transmitted diseases upon its disruption.[3] Thus, the device developed in this project provides the foundation for future researchers to build specialized platforms for the generation of the vast body of research on vaginal tissue needed to improve female health.

Therefore, the Vagina-on-Chip model established in this study holds the potential to transform the study of vaginal tissue by providing the very first platform designed specifically for the integration of its three major cell types to replicate the organization of native tissue. Furthermore, the incorporation and analysis of microfluidics present a novel implementation of media flow to maintain prolonged cell viability and mimic the dynamic vaginal cell microenvironment. Finally, the open-top design and multiplexed platform of the final VoC design establishes the unique opportunity to conduct real-time observation and data collection to facilitate the high-throughput development of translational treatment strategies.

Materials and Methods

Materials

An acrylate-based resin, BV007a was used for 3D printing. This material is cytotoxic but accessible and effective for printing microstructures. Water was dyed with blue food coloring and used to simulate cell media. 2% agarose was also dyed with food coloring and used for insertion into the device channels to simulate hydrogel loading.

Methods

Mechanical Design Modeling and Printing

All iterations of the device were modeling in CAD software, Autodesk Fusion 360. The iterations were 3D printed in BV007a using the MiiCraft 50 Ultra printer, a stereolithography 3D printer. Basic functionality of the device was tested after printing by loading agarose dyed with food coloring into the cell culture chambers of both designs. Evidence from subsequent simulations drove changes to be made to the CAD model, which was then re-printed and tested with agarose to validate design functionality.

Velocity Profile Generation

Microfluidic geometries of the top-view of the cell culture chamber were modeled using the Computation Fluid Dynamics (CFD) module in COMSOL Multiphysics Software. Fluid flow was simulated from the left channel with a boundary condition of 100 µm/s to generate a contour plot of the velocities across the model. Velocities across the chamber diameter of 5 mm were measured to generate a plot of the parabolic flow profile. This velocity profile was generated to evaluate fluidic delivery across the cell culture chamber area.

Oxygen Diffusion Simulation

Microfluidic geometries of the side-view of the original cell culture chamber (5 mm depth) were modeled using the Transport of Diluted Species module in COMSOL Multiphysics Software. Oxygen was chosen as the limiting factor, and thus the diffusion coefficient was set to 2.4×10^{-9} m^2/s. The top of the gel area was selected and Initial Value 1 was set at 0.2 mM to reflect the oxygen concentration used in OoC literature. The bottom of the chamber was then selected and Initial Value 2 was set to 0 mM. The time range was set from 0 to 1 hour with a step size of 0.1. A contour plot was generated to reflect the oxygen concentrations measured down the center of the chamber. This simulation was then repeated for gel thicknesses of 1, 2, 3, and 4 mm and measured at time points 0.1, 0.5, and 1 hour.

End Matter

Author Contributions and Notes

M.S., S.C., G.C., J.M., and K.M. designed research, M.S., S.C., and K.M., performed research, M.S. and S.C analyzed data; and M.S. wrote the paper.

The authors declare no conflict of interest.

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