AWARD NUMBER:	W81XWH-21-1-0108
TITLE:	Connexins as Potential Biomarkers and Therapeutic Targets for Vascular Malformation
PRINCIPAL INVESTIGATOR:	Jennifer S. Fang, Ph.D.
CONTRACTING ORGANIZATION:	The University of California, Irvine, CA
REPORT DATE:	March 2022
TYPE OF REPORT:	Annual
PREPARED FOR:	U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012
DISTRIBUTION STATEMENT:	Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE		Form Approved OMB No. 0704-0188

1. REPORT DATE March 2022	**2. REPORT TYPE** Annual	**3. DATES COVERED** 01Feb2021-31Jan2022

4. TITLE AND SUBTITLE

Connexins as Potential Biomarkers and Therapeutic Targets for Vascular Malformation

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-21-1-0108

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Jennifer S. Fang, Ph.D.

E-Mail: jennifer.fang@uci.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

UNIVERSITY OF CALIFORNIA, IRVINE
141 INNOVATION DR STE 250
IRVINE CA 92617-3213

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
DOD PRMRP

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Blood vessels form during development through coordinated signaling between vascular endothelial cells (EC) and their adjacent cell neighbors. Inherited mutations that disrupt these signaling processes can lead to vascular malformations with profound consequences on vascular organization and function. In the rare congenital disease Hereditary Hemorrhagic Telangiectasia (HHT), loss-of-function mutations affecting the Alk1 cell surface receptor drives the appearance of disorganized vascular lesions prone to sudden, serious rupture. The goal of this study is to understand how Alk1 mutation may promote vascular malformation by dysregulating connexins (Cx), constituent proteins of vascular gap junctions that support direct cell-cell signaling of small electrochemical signals. In the first year of this study, we have made significant progress towards understanding this question. We have improved our understanding of how Cx expression is altered by Alk1 loss-of-function and have developed (and are continuing to develop) key tools for manipulating connexin expression in primary endothelial cells, alone or in combination with Alk1-deficiency. We have performed studies to better understand the impact of dysregulated Cx expression on EC behavior, gap junctional coupling, and are currently assessing the impact on vascular lesion formation in a microphysiological model of HHT. Lastly, we have received institutional approval for a clinical study in collaboration with the UCLA HHT Center of Excellence to collect HHT patient samples to understand how variations in Cx gene sequence might impact clinical presentation of HHT in patients.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRDC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	14	

TABLE OF CONTENTS

Page

INTRODUCTION

Blood vessels form during development through coordinated signaling between vascular endothelial cells (EC) and their cell neighbors to support organized and controlled cell proliferation and migration to ensure proper organization of the resulting vascular network. Inherited mutations that disrupt these signaling processes can lead to the development of malformed vessels with profound consequences on vascular organization and function. In the rare congenital disease Hereditary Hemorrhagic Telangiectasia (HHT), loss-of-function mutations affecting the Alk1 cell surface receptor drives the appearance of disorganized vascular lesions prone to sudden, serious rupture. HHT affects 1 in 5,000 live births and has variable presentation, making both HHT and associated vascular malformations (VM) difficult to diagnose. Furthermore, although HHT symptoms often begin in childhood and progress in severity, patients typically only receive a definitive HHT diagnosis at >40 years of age. Thus, it is possible that many active and retired military personnel currently harbor undiagnosed HHT that might put them at risk for sudden and severe bleeding, as well as other major health problems. Thus, a major goal of this study is to improve our understanding of how Alk1 drives vascular malformation in HHT, and to understand whether targeted proteins might serve as potentially useful early screening tools for HHT. Specifically, our aim is to understand how Alk1 mutation may promote vascular malformation by dysregulating connexins (Cx), constituent proteins of vascular gap junctions that support direct cell-cell signaling of small electrochemical signals.

KEYWORDS

Hereditary Hemorrhagic Telangiectasia, HHT, Alk1, Vasculature, Vascular Malformation, Blood Vessels, Arteriovenous Malformation, Connexin, Gap Junction, Microphysiological Systems, Organ-on-a-Chip

ACCOMPLISHMENTS

Major Goals and Accomplishments

In the first year of this study, we have made significant progress towards the major goals and milestones of this project. As outlined in more specific detail below, we have improved our understanding of how Cx expression is altered by Alk1 loss-of-function and have developed (and are continuing to develop) key tools for manipulating connexin expression in primary endothelial cells, alone or in combination with Alk1-deficiency. We have performed studies to better understand the impact of dysregulated Cx expression on EC behavior, gap junctional coupling, and are currently assessing the impact on vascular lesion formation in a microphysiological model of HHT. Lastly, we have received institutional approval for a clinical study in collaboration with the UCLA HHT Center of Excellence to collect HHT patient samples to understand how variations in Cx gene sequence might impact clinical presentation of HHT in patients.

Specific Objectives and Outcomes

Specific Aim 1: Determine how abnormal Cx expression in the vessel wall contributes to VM development.

Overall Progress: ~50%

Major Task 1A: Map Cx expression and localization in a developing VM.

The goal of this task is to determine how Cx expression is affected by Alk1-deficiency in EC and perivascular cells in a developing VM. We have now confirmed using real-time PCR (**Figure 1**) and Western blot (**not shown**) that Alk1 signaling strongly promotes expression of vascular connexin Cx43. In addition, Alk1 signaling activation (via BMP9 and BMP10) promotes Cx37, whereas Alk1 deficiency completely downregulates Cx37 and Cx40 (**Figure 2**). We are in the process of generating Cx-fluorescent protein fusion reporter constructs from existing publicly- and commercially-available tools to demonstrate this finding in real-time. Many of those tools are already in-hand (gift from J. Burt, The University of Arizona) while others have been delayed in their shipping to us due to COVID-related supply chain issues. Thus, we have been delayed in our progress towards developing Cx-reporter constructs. We currently anticipate completing this task in the next 6 months. In the meanwhile, we have pursued this research question using an alternative approach involving classic real-time PCR and immunofluorescence strategies, and we are currently using these tools to systematically map changes in Cx expression and localization in our HHT-on-a-chip platform.

Figure 1. Alk1 knockdown (via IPTG) strongly promotes Cx43 expression.

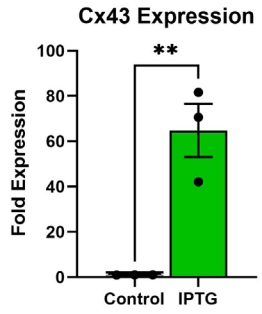
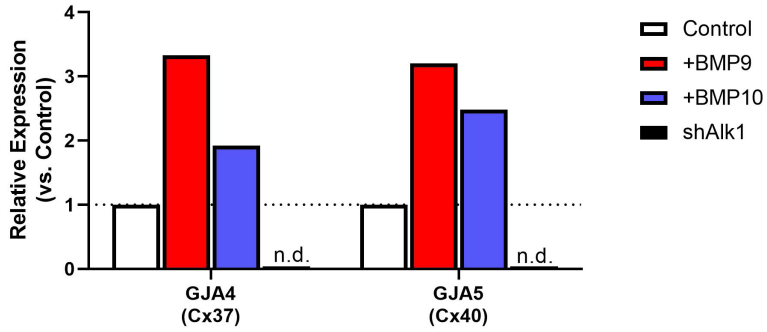


Figure 2. Alk1 signaling activation (via BMP9 or BMP10) promotes Cx37 and Cx40, whereas Alk1 knockdown (via shRNA) eliminates endogenous Cx37 and Cx40 expression. (n.d. = not detected)



Milestone Progress: ~60%

Major Task 1B: Determine effect of "HHT-like" Cx expression profile on VM progression.

The goal of this task is to determine how an "HHT-like" Cx expression profile may promote (or not) the formation of VM. Using publicly- and commercially-available source plasmids, we are currently generating and validating an inducible Cx43 expression constructs using basic molecular biology and cloning approaches. Although Cx43 shRNA constructs have been validated, shRNA constructs targeting Cx37 and Cx40 are only predicted and have not been experimentally validated, we generated and have screened Cx shRNA silencing constructs. Using real-time PCR, we have identified those that significantly knockdown endogenous Cx expression in endothelial cells. In particular, we have identified two shCx37 constructs that almost completely eliminate endogenous Cx37 expression (**Figure 3**). Furthermore, shCx37 #2 promotes expression of Cx43 – evidence that Cx37 regulates Cx43 expression. This finding simplifies our required approach, as we may not need to separately drive Cx43 expression in these cells to produce an HHT-like Cx expression profile. Based on these findings, we are currently cloning shCx37 #2 into an inducible expression construct to enable inducible Cx37 silencing alongside upregulated Cx43 in endothelial cells. Upon completion of this, we will assess the impact of this constructs in our HHT-on-a-chip platform. In the meanwhile, we will continue to screen for an effective shRNA construct that knocks down Cx40.

Figure 3. Screening of predicted shCx37 and shCx40 constructs reveals two shCx37 constructs (#1 and #2) that significantly knockdown endogenous Cx37 expression. shCx37 #2 also promotes Cx43 expression, making it an ideal candidate for further development. Predicted shCx40 constructs were not effective.

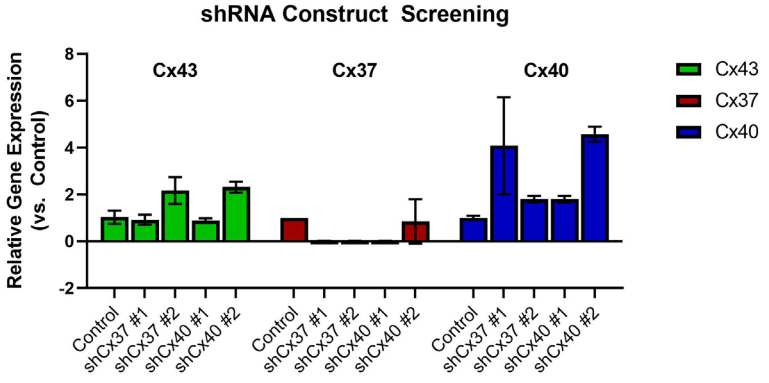
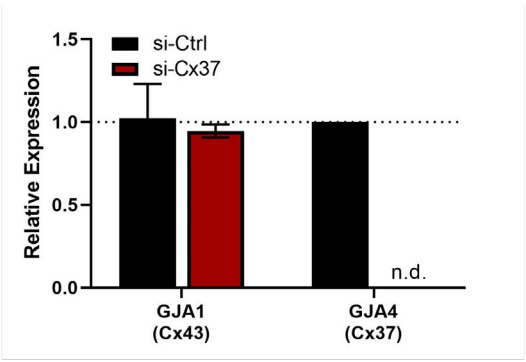
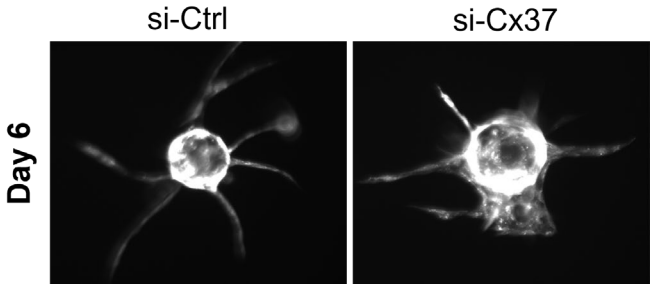


Figure 4. Knockdown of endogenous Cx37 (via siRNA, top) results in malformed vessel sprouts in an *in vitro* Bead Assay model of angiogenesis, wherein fluorescently-labeled endothelial cells sprout from a coated plastic bead. In cells lacking Cx37 (siCx37), sprouts are dilated, and some sprouts develop into highly disorganized and enlarged, sheet-like structures (bottom) suggesting destabilization of the vessel sprout. (n.d. = not detected)





Separately, we also pursued studies to understand how loss of Cx37 or Cx40 expression (as occurs with Alk1 knockdown, see **Figure 2**) may alter endothelial cell function during progression of a VM. For this, we used a standard *in vitro* angiogenesis assay wherein endothelial cells are coated upon the surface of a bead and induced to undergo angiogenesis. This model is useful because vessels form rapidly, and thus effects of transient Cx knockdown (using conventional silencing RNA, siRNA) can be observed. Using this approach, we found that silencing RNA that targets and downregulates Cx37 expression leads to the appearance of enlarged and extremely dilated sprouts,

suggestive of vessel wall destabilization (**Figure 4**). This occurs even in the absence of concomitant changes in Cx43 expression, suggesting that dysregulation of Cx37 may itself be a key driver of vascular malformation. We will examine whether Cx37 knockdown using shCx37 #2 (**Figure 3**) produces similar effects in this *in vitro* angiogenesis model as well as in our HHT-on-a-chip platform.

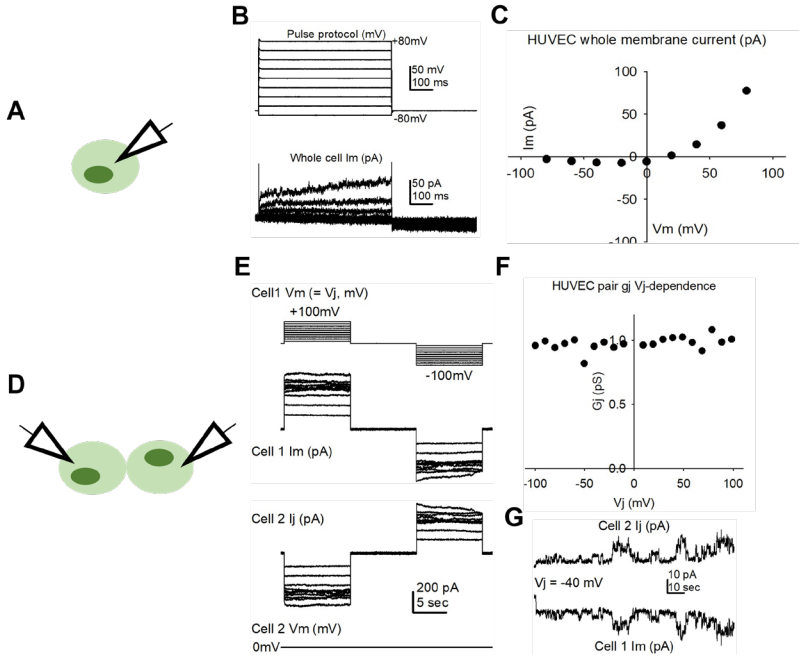
Milestone Progress: ~60%

Major Task 1C: Determine effect of "HHT-like" Cx expression profile on gap junctional communication.

The goal of this task is to use established dual whole-cell patch clamp to understand normal endothelial-endothelial and endothelial-mural gap junctional coupling, and to determine how this may be impacted by enforced expression of an "HHT-like" Cx profile. In the first year of this study, we encountered technical hurdles (related to both adapting existing equipment for this purpose as well as optimizing conditions for recording cell-cell communication from primary endothelial cells) that significantly challenged pursuit of these studies at our current institution. In the last month, we reached out to an existing collaborator – Dr. Jose Ek Vitorin at The University of Arizona, a well-recognized electrophysiologist in the field of gap junction research – about whether he felt these studies were possible from primary endothelial cells. He provided initial proof-of-concept confirmation that dual whole-cell patch clamp is possible from primary endothelial cells, and further determined that under basal conditions, these cells are coupled primarily via Cx43 gap junctions – a relatively surprising finding. Based on this initial work, we are currently planning to request approval from the Department of Defense for Dr. Ek Vitorin to perform additional electrophysiology studies to determine the impact of manipulated Cx expression profile on endothelial and mural cells.

Milestone Progress: ~30%

Figure 5. Primary human endothelial cells were assessed under basal conditions using A) whole cell voltage clamp to B) apply a progressive pulse protocol. C) Successful recording of membrane current from these cells indicate that patch clamp electrophysiology can be performed on this cell type. D) Patch clamp was performed on a pair of primary endothelial cells (dual whole cell voltage clamp), where E) an electrode was used to apply a voltage stimulus to Cell 1, while the other was used to record from Cell 2. This approach measures gap junctional coupling between cells. F) Cells were strongly coupled, and conductance between the cells was high enough that voltage-sensitive gap junctional gating could not be detected. G) Monitoring of cell pair recovery from application of halothane (a pan-gap junction blocker) over time enables detection of individual channel opening events in both voltage-stimulated (top) and voltage-sensing (bottom) cells. Frequency histogram analysis of these data show channel conductance substates consistent with Cx43-predominant gap junction channels.



Specific Aim 2: Determine how Cx expression profile may predict VM risk and severity.

Overall Progress: ~20%

Major Task 2A: Determine lesion-specific changes in EC Cx expression.

The goal of this task is to assess Cx expression from HHT patient vascular lesion donor samples against adjacent healthy tissue. We are in the process of establishing a clinical study in collaboration with Dr. Patricia Loftus, a rhinologist at University of California-San Francisco (UCSF) who will recruit HHT patients for this study and who will perform sample collection. Dr. Loftus has consulted with us on the logistics of recruiting patients for this study, as well as the specific details of how tissue will be collected. Over the past year, we successfully authored the Human Subjects Research master protocol under which this clinical study will be performed, and we obtained initial approval from the University of California-Irvine (UCI) Institutional Review Board for work related to Major Task 2B (see below for more detail). We are currently in the process of identifying a study coordinator for the UCSF study site who will oversee immediate study enrollment and sample delivery. Once this person has been identified at UCSF, we will obtain approval for UCSF to begin recruiting subjects into this portion of our clinical study and begin collecting tissue biopsies for further analysis.

Milestone Progress: ~15%

Major Task 2B: Determine effect of Cx SNP variants in HHT patient outcome.

The goal of this task is to obtain donor blood samples from healthy and HHT patients, and to assess Cx SNP frequencies in these two populations. We will also compare SNP frequencies against patient histories. For this task, we authored a Human Subjects Research master protocol and – after much effort – obtained initial approval for the protocol for all UCI-specific work (Protocol #20216546, approved 11/03/21) from UCI's Institutional Review Board and the Department of Defense. We immediately submitted an amendment to add the University of California-Los Angeles (UCLA) study site, where Dr. Justin McWilliams (Director, UCLA HHT Center of Excellence) will oversee subject recruitment and enrollment into our clinical study for the purposes of donating peripheral whole blood for SNP analysis. Dr. McWilliams will oversee Victoria Rueda, MPH, who will serve as the on-site study coordinator for this study. Again, after much effort, we recently received approval for UCLA to be added to our protocol (approved 2/24/22), and those amended and approved documents were filed with the Department of Defense this week. We have a scheduled UCI-UCLA Study Team meeting planned for 3/10/22 to discuss the launch of this clinical study, and we anticipate starting subject enrollment shortly thereafter. In the meanwhile, our efforts to include UCSF have been delayed by the need to identify an available study coordinator for that site, which we anticipate will be resolved in the next month.

Milestone Progress: ~30%

Specific Aim 3: Determine whether manipulation of Cx expression in vessels can resolve or prevent VM.

Overall Progress: ~40%

Major Task 3A: Determine effect of "Alk1-intact" Cx expression profile on VM progression.

The goal of this task is to determine whether forcible expression of an "Alk1-intact" Cx expression profile in endothelial cells will prevent vascular lesion formation in the presence of loss of Alk1 expression. We have obtained wild-type mouse and human Cx37 and Cx40 expression constructs (gift from J. Burt, The University of Arizona), and are currently in the process of cloning these sequences into Tet-inducible lentivirus backbones. Simultaneously, we have refined our HHT-on-a-chip model to ensure that this model reliably and reproducibly produces vascular lesions to varying degrees of severity, such that modulatory effects of altered Cx expression profile can be detected. We have found that when Alk1-deficient endothelial cells (via application of IPTG) are seeded into our HHT-on-a-chip platform, microvascular structures expand over time to form enlarged and dilated lesions. Importantly, while some vessel networks appear hyperdense but with an otherwise regular appearance, in other devices, Alk1-deficiency leads to profound remodeling and microvascular regression resulting in highly-abnormal dilated structures (**Figure 6**). These data indicate that there is sufficient phenotypic range in the HHT-on-a-chip platform such that disease-modifying effects of enforced Cx expression will be detectible. Those studies will proceed as soon as our Cx37 and Cx40 expression constructs are in-hand and fully validated. Separately, we have assessed the impact of Cx43 knockdown in an *in vitro* model of angiogenesis, and found a dramatic reduction in the number and length of new vessel sprouts (**Figure 7**). This supports the hypothesis that "Alk1-intact" downregulation of Cx43 limits vessel growth to prevent vascular malformation, and this finding will be confirmed in the HHT-on-a-chip platform.

Milestone Progress: ~40%

Major Task 3B: Determine effect of gap junction blockers on VM progression.

The goal of this task is to determine whether pharmacological gap junction inhibition can resolve or prevent vascular lesions in the HHT-on-a-chip platform. In addition to studies showing that there is a range of phenotypic severity in the HHT-on-a-chip platform (**Figure 6** and discussed above), we also adapted the HHT-on-a-chip platform for higher-throughput drug studies. We have now developed a well-plate format version of the HHT-on-a-chip platform which has tripled our experimental capacity and now enables us to perform sophisticated dose response drug screening studies. With this improved platform now in-hand, we have purchased the pan-gap

Figure 6. Alk1-deficient endothelial cells (via IPTG) form hyperdense and disorganized microvascular structures that enlarge over time (yellow arrowheads). B) In some networks, microvasculature remains hyperdense but otherwise regular in appearance whereas in other devices, the microvasculature collapses into highly disorganized, dilated, and abnormal-looking lesions.

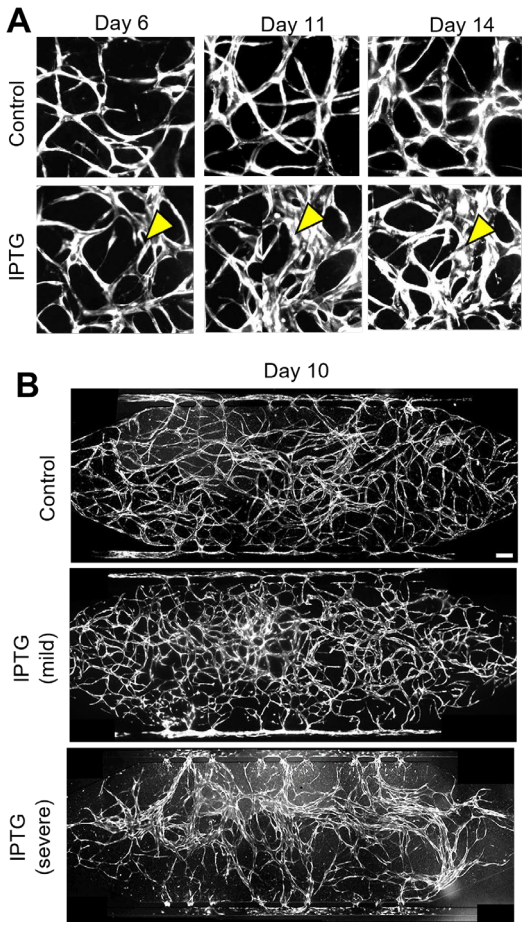
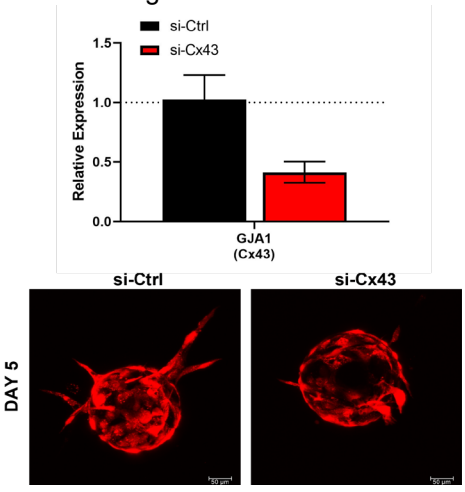


Figure 7. Cx43 knockdown by silencing RNA (top) profoundly limits angiogenic sprout number and length.



junction blocker carbenoxolone, and we have obtained the Cx43-specific mimetic peptide SRPTEKT-Hdc (gift from J. Burt and S. Boitano, The University of Arizona). Studies to assess the impact of these gap junction inhibitors in the well-plate format version of our HHT-on-a-chip platform are now underway, and we expect results shortly.

Milestone Progress: ~75%

Major Task 3C: Use next-generation sequencing to identify Cx-regulated downstream targets.

The goal of this task Is to perform bulk- and single cell RNASeq on “HHT-like” and “Alk1-intact” endothelial cells in monolayer, as well as from the HHT-on-a-chip platform. In preparation for these studies, we have optimized cell sample preparation and submission procedures for bulk and single cell RNASeq from monolayer and from the HHT-on-a-chip device. As described above, we are in the final stages of developing reliable tools for generating “HHT-like” and “Alk1-intact” Cx expression profiles using inducible Cx expression and silencing constructs. Once these tools have been validated, we will immediately generate and submit samples for transcriptomic sequencing and analysis.

Milestone Progress: ~25%

Opportunities for Training and Professional Development

The project was not intended to provide significant training and professional development. Nonetheless, during this period, Dr. Fang supervised a UCI undergraduate student – Vivian Bich Van Vu Dang – who performed a comprehensive literature review of current HHT clinical treatment approaches and their impact, which has provided valuable insight into the impact of the current work.

Dissemination of Results to Communities of Interest

During this reporting period, Dr. Fang presented our current work in a platform presentation at the 2021 Vascular Biology conference, held virtually in October 2021. Vascular Biology is a multi-day conference organized by the North American Vascular Biologists’ Organization (NAVBO) and is a major meeting for vascular biology researchers. Dr. Fang also plans to present this work at the upcoming 2022 World-MPS Summit in New Orleans, LA, which will disseminate findings to researchers focused on developing organ-on-a-chip technology. Lastly, we have maintained close connection with CureHHT, the nation’s foremost HHT patient advocacy group, and we intend to partner with this organization to ensure that work from this study reach HHT patients and clincians, where they might be most impactful.

Goals for Next Reporting Period

Our goal for the next reporting period – which will be the final reporting period for this project – is to complete all studies outlined for this project. This includes completing all Cx reporter, expression, and silencing constructs and analyzing their impact in the HHT-on-a-chip platform as well as by next-generation sequencing. We also intend to complete all gap junction inhibitor drug studies in the HHT-on-a-chip platform. Although we were significantly delayed by the UCI IRB approval process, we are optimistic that we will make significant progress towards our study recruitment, enrollment, and sample collection goals for our clinical study. Lastly, we intend to prepare and submit a manuscript by the end of the next reporting period, as well as use these pilot studies to apply for additional extramural funding to further explore the role of connexins in HHT-related vascular malformation and other diseases involving disorganized blood vessels.

IMPACT

Impact on the Principal Discipline(s):

Findings from this past reporting period have clearly demonstrated a central role for Cx37 in vessel organization. Our ongoing studies should further confirm and expand upon this initial finding, as well as address the relative importance and role(s) of other vascular connexins.

Impact on Other Discipline(s):

Work during this reporting period to adapt the HHT-on-a-chip platform for higher-throughput drug screening studies are likely to have profound impact not just on HHT translational research using this model, but more broadly for several related models in the lab that will also be able to take advantage of this technology to efficiently perform large-scale drug screening studies.

Impact on Society:

Nothing to report for this period.

CHANGES / PROBLEMS

Changes in Experimental Approach

We have not made significant changes to our experimental approach. Thus, we have nothing to report for this section.

Actual or Anticipated Problems or Delay

We encountered three challenges that delayed our work in this reporting period, and we outline steps we have taken to address these obstacles below.

First, COVID19-related supply chain issues resulted in the back-ordering of necessary reagents, including molecular biology cloning reagents, cell culture media, device fabrication supplies, and disposable plastics. While these persistent supply chain issues did not impact our expenses, they significantly delayed our research progress in the past reporting period as we were forced to delay experiments until necessary reagents were delivered and/or to prioritize experiments based on limited supply availability. We have noticed that this supply chain issue is beginning to subside as the nation undergoes economic recovery from the COVI19 pandemic, and we are optimistic that this will mitigate our immediate supply chain issues in the next reporting period.

Second, we were significantly delayed due to an unexpectedly lengthy application and approval process through the UCI IRB office. After much effort, we have now obtained approval for our IRB protocol, as well as approval for our UCLA study site to begin study recruitment and enrollment. Thus, although we are frustrated by the significant delay this has posed on our work, we are optimistic that we can now go ahead with this study in the upcoming reporting period.

Third, we encountered significant challenges when attempting to generate electrophysiological data at UCI. Existing large equipment, although available, was not optimized for single- or dual- whole cell patch clamp – a technique critical for studying gap junction communication. In addition, we found primary endothelial cells to be tricky to patch clamp due to their relatively flat morphology. To address these issues, we contacted Dr. Jose Ek Vitorin at The University of Arizona, who is a well-recognized expert in gap junction electrophysiology. He demonstrated with his own equipment and cells that (with some finesse) gap junctional coupling between primary endothelial cells can be measured by single- and dual- whole cell patch clamp (see above, **Figure 5**). Given that technical challenges have already delayed the collection of these data and that only a year now remains on this project, we believe that it would be most efficient for Dr. Ek Vitorin to collect the remaining electrophysiology data outlined in this project, and we intend to immediately request approval from the Department of Defense for this.

Changes That Had Significant Impact on Expenditures:

We have not made significant changes that have impacted our expenditures outside of what is described in our approved Budget Justification and Statement of Work. Thus, we have nothing to report for this section.

Changes in Human Subjects, Vertebrate Animals, Biohazards, and/or Select Agents

We have not made any changes that have impacted our human subjects work. We do not have any approved work with vertebrate animals, biohazards, or select agents. Thus, we have nothing to report for this section.

PRODUCTS

Publications, Conference Papers, and Presentations

- **Journal Publications:** Nothing to report for this period.

- **Books or other Non-Periodicals:** Nothing to report for this period.

- **Other Publications, Conference Papers, and Presentations:**
    1. “Alk1-Deficient Endothelial Cells Drive Vascular Malformation in a Microphysiological Disease Model of Hereditary Hemorrhagic Telangiectasia.” 2021 Vascular Biology (virtual)

Websites or Other Internet Sites

Nothing to report for this period.

Technologies or Techniques

As part of this project, we modified the HHT-on-a-chip platform for a standardized well-plate format, and we designed a custom widget to support high rates of intravascular flow and fluid shear stress. This technology will be described in the primary peer-reviewed paper associated with this project, and it will be made available to the research community upon that paper’s publication.

As part of this project, we are developing and validating several Cx reporter, expression, and silencing constructs. Completed tools will be described in the primary peer-reviewed paper associated with this project, and they will be made available to the research community upon that paper’s publication.

Inventions, Patent Applications, and/or Licenses

Nothing to report for this period.

Other Products

Nothing to report for this period.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Individuals
The following individuals have worked at least one person month per year on this project during the reporting period.

Name	Jennifer S. Fang
Project Role	Primary Investigator
Research Identifier	ORCID ID: 0000-0001-5703-2239
Nearest Person Month Worked	8
Contribution to Project	Dr. Fang planned experiments, generated tools, collected data, and provided technical and administrative oversight and support for this project, including device fabrication and supply purchases.
Funding Support	• Department of Defense (this award) • NIH/NCATS

Name	Vivian Bich Van Vu Dang
Project Role	Undergraduate Student
Research Identifier	None
Nearest Person Month Worked	3
Contribution to Project	Ms. Dang performed a literature review of HHT clinical studies to identify the impact of current standard-of-care HHT drugs on patient outcomes.
Funding Support	• None (volunteer)

Changes in Active Other Support for Senior/Key Personnel

There have been no changes in active other support for senior or key personnel, thus there is nothing to report for this period.

Other Organizations as Partners

Organization Name	University of California-Los Angeles (UCLA)
Location of Organization	Los Angeles, CA
Partner's Contribution	**In-Kind Support:** Dr. Justin McWilliams will be providing his time and effort to identify prospective subjects for clinical study enrollment. **Facilities:** The facilities at the UCLA HHT Center of Excellence will be used to recruit and enroll HHT patients into the study, and to obtain and process donor samples. **Collaboration:** Staff at the UCLA HHT Center of Excellence will be involved in study coordination and subject enrollment.