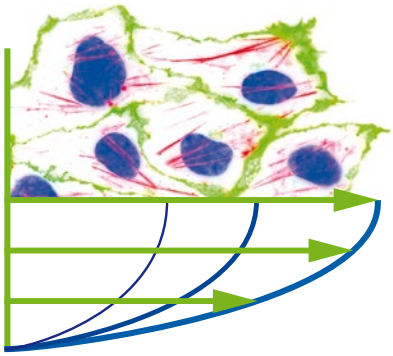
Northwestern

cells in focus ibidi

International **Cell Culture Under Flow** Meeting 2024



July 24–25 · Northwestern University, Chicago, IL, USA

Abstract Book

Welcome

It is a great pleasure to welcome you to the second International Cells Under Flow Meeting 2024. This year's conference was organized in collaboration with Prof. Luisa Iruela-Arispe from Northwestern University, Chicago, USA.

The following abstract book contains the program overview and summaries of all the posters and oral presentations that will be showcased at the conference. We hope it will serve as a useful resource and guide throughout the event.

This is the second "International Cell Culture Under Flow" meeting, and just like our outstanding first meeting, this conference aims to continue fostering discussions & collaborations and bringing together scientists from a variety of fields—including vascular research, immunology, mechanobiology, and barrier function—who cultivate cells under shear stress. As a participant, you will have the opportunity to showcase your recent work and exchange knowledge among both emerging and established scientists. Your contribution is invaluable to the success of this event, and we look forward to the insights and discussions your work will inspire.

Seven sessions will cover the following topics:

- Microvascular Morphogenesis
- Mechanotransduction
- Molecular Mechanisms of Shear Stress Response
- Atherosclerosis
- Renewal and Repair
- Mechanotherapeutics, Cancer, and Inflammation
- Future Technologies and Applications for Cell Culture Under Flow

We hope you have a wonderful time and enjoy the conference!

Venue

Northwestern University, Chicago, IL, USA

Meeting location:
Simpson Querrey Building, Main Atrium, Ground Floor,
303 E Superior St, Chicago, IL 60611, USA

Public parking:
Erie Ontario Self Park, 321 E Erie St, Chicago, IL 60611

Directions from parking:
Exit the parking ramp on E Erie St and turn left (west). Walk to N Fairbanks Ct and turn right (north). In one block, you will reach the Simpson Querrey Building, which is between E Huron St and E Superior St on N Fairbanks Ct. There are entrances to the building on both Huron (south entrance) and Erie (north entrance) and both entrances lead to the main atrium, where the conference will be held.

Additional parking:
Northwestern Garage, 222 East Huron Street, Chicago, IL 60611

We would like to thank our collaboration partner, Prof. Luisa Iruela-Arispe and the administration staff at Northwestern University for their support and assistance. Without their collective efforts, this conference would not be possible.

Contact

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Presenter Guidelines

We kindly request all presenters, on behalf of all participants, to be punctual and adhere to the allocated time slots for their presentations, ensuring a seamless program and maintaining the scheduled break times. Invited speakers are kindly asked to prepare their presentation for 30 minutes and 5 minutes question time, while accepted speakers are scheduled for 10 minutes presentation time followed by 5 minutes for questions.

For the oral presentations, presenters have the option to utilize their personal computer or the provided session computer. Please note that the session computer will run on Windows only. If a MacBook is required, presenters should either utilize their own device or ensure compatibility of their presentation with Windows PowerPoint. If using your own notebook, a USB-C to HDMI adapter will be available to connect your computer to the projector. We also recommend bringing a copy of your presentation on a USB drive and testing your presentation beforehand to avoid any compatibility issues.

For the poster presentations, presenters can set up their posters during registration and during the breaks on the first day. Pins will be available to secure the posters on the display boards. Posters can remain up for the entire two days of the conference. We kindly ask all poster presenters to be present at their posters during the scheduled poster session for engaging discussions and to answer questions.

Hands-On Course

The Cell Culture Under Flow Hands-On Course will take place the day after the conference, Friday, July 26th, which required an additional registration. This guided workshop will allow participants to discuss and share their questions, ideas, and knowledge. If you are interested in attending other Hands-On courses, visit ibidi.com for details on upcoming events.

Registration and Reception

8:30 am		Registration

Welcome and Keynote Lecture

9:30–10:15 am — Chair: Roman Zantl (ibidi GmbH, Germany)

9:30 am	10 min	**Welcome:** *Luisa Iruela-Arispe (Northwestern University, USA) / Roman Zantl (ibidi GmbH, Germany)*
9:40 am	30 + 5 min	**Keynote:** Flow-Induced Endothelial Polarity *Luisa Iruela-Arispe (Northwestern University, USA)*

Session 1: Microvascular Morphogenesis

10:15–11:05 am — Chair: Roman Zantl (ibidi GmbH, Germany)

10:15 am	30 + 5 min	**Keynote:** Coordination of Vascular Morphogenesis at Endothelial Cell-Cell Interfaces *Matthew Kutys (University of California San Francisco, USA)*
10:50 am	10 + 5 min	Oscillatory Shear Stress Modulates Lymphatic Progenitor Cells Maturation Into Lymphatic Vessels With Anti-Cancer Phenotypes *Nancy Lightsey (University of Notre Dame, USA)*
11:05 am	35 min	**Coffee break**

Session 2: Mechanotransduction

11:40 am–1:00 pm — Chair: Paul Evans (Queen Mary University of London, UK)

11:40 am	30 + 5 min	**Keynote:** Flow-Mediated Mechanisms of Endothelial Cell Resilience *Julia Mack (University of California Los Angeles, USA)*
12:15 pm	10 + 5 min	Low Shear in Short-Term Impacts Endothelial Cell Traction and Alignment in Long-Term Under High Shear *Sangyoon Han (Michigan Technological University, USA)*
12:30 pm	10 + 5 min	Mechanotransduction via Endothelial Adhesion Molecule CD31 Initiates Transmigration and Reveals a Role for VEGFR2 in Diapedesis *Bill Muller (Northwestern University, USA)*

12:45 pm	10 + 5 min	CD36-Mediated Lipid Uptake Mediates Endothelial Stiffening Under Disturbed Flow and in Aortic Arch *Victor Aguilar (University of Illinois, USA)*
1:00 pm	60 min	**Lunch break**

Session 3: Molecular Mechanisms of Shear Stress Response

2:00–3:20 pm Chair: Tatiana Petrova (University of Lausanne, Switzerland)

2:00 pm	30 + 5 min	**Keynote:** Understanding Endothelial Cell Responses to Flow Through Study of Notch Target Genes *Jan Kitajewski (University of Illinois, USA)*
2:35 pm	10 + 5 min	Notch Signaling Regulates Unc5B to Suppress Endothelial Proliferation and Branching and to Support Junction Formation and Polarization in Response to Flow *L.A. Naiche (University of Illinois, USA)*
2:50 pm	10 + 5 min	Flow-sensitive Endothelial K^+ Channels are Master Regulators of Endothelial Mechanotransduction and Gene Expression *Sang Joon Ahn (University of Illinois, USA)*
3:05 pm	10 + 5 min	Dissection of Endothelial Junctional Dynamics Following Angiogenic Stimulation Through Application of an Engineered Optogenetic Biotin Ligase *Nicholas Leschinsky (University of Illinois, USA)*

ibidi Industry Talk

3:25–4:00 pm Chair: Roman Zantl (ibidi GmbH, Germany)

Industry Talk: Future Technologies and Applications for Cell Culture Under Flow
Nina Huber (ibidi GmbH, GER)

Poster Session

4:00–8:00 pm Poster Session With Snacks and Drinks

Session 4: Atherosclerosis

9:00–10:40 am Chair: Julia Mack (University of California Los Angeles, USA)

9:00 am	30 + 5 min	**Keynote:** Endothelial Responses to Flow in Atherosclerosis *Paul Evans (Queen Mary University of London, UK)*
9:35 am	30 + 5 min	**Keynote:** Flow-Sensitive HEG1 Regulates Endothelial Function and Protects Against Atherosclerosis *Hanjoong Jo (Georgia Tech and Emory University, USA)*
10:10 am	10 + 5 min	MicroRNA Deregulation as the Mechanism of Cadmium-Induced Atherosclerosis *Abeer Mohamed (University of Illinois, USA)*
10:25 am	10 + 5 min	Mechanosensitive Protein FHL2 Tunes Endothelial Flow Response *Shailaja Seetharaman (The University of Chicago, USA)*
10:40 am	30 min	**Coffee break**

Session 5: Renewal and Repair

11:10 am–1:05 pm Chair: Yun Fang (The University of Chicago, USA)

11:10 am	30 + 5 min	**Keynote:** Mechanisms of Lymphatic Vessel Renewal and Repair *Tatiana Petrova (University of Lausanne, Switzerland)*
11:45 am	30 + 5 min	**Keynote:** Effect of Microfluidic on Endothelial Wound Regeneration During Streptococcal Infection *Simone Bergmann (Technical University Braunschweig, Germany)*
12:20 pm	10 + 5 min	Electro-Osmotic Flow Directs Cell Migration and Growth of Endothelial Sprouts *Mark Messerli (South Dakota State University, USA)*
12:35 pm	10 + 5 min	Enhancing the Targeting Efficacy of Endothelial Colony Forming Cells for Renal Regeneration via Kidney-Targeted Liposomal Nanoparticles *Brenda Cruz-Gonzalez (University of Notre Dame, USA)*
12:50 pm	10 + 5 min	Shear-Sensitive Notch Activation via Extracellular Vesicles to Treat Vascular Injuries *Prerak Gupta (University of Illinois, USA)*
1:05 pm	55 min	**Lunch break**

Session 6: Mechanotherapeutics, Cancer, Inflammation

2:00–3:35 pm — Chair: Luisa Iruela-Arispe (Northwestern University, USA)

2:00 pm	30 + 5 min	**Keynote:** Vascular Mechanobiology and Mechano-therapeutics *Yun Fang (The University of Chicago, USA)*
2:35 pm	10 + 5 min	Disturbed Flow Induces Reprogramming of Endothelial Cells to Immune-Like and Foam Cells Under Hypercholesterolemia During Atherogenesis *Christian Park (Emory University & Georgia Tech, USA)*
2:50 pm	10 + 5 min	Mechano-Sensitive ER Protein TXNDC5 Promotes Neointimal Hyperplasia of Arterio-Venous Fistula Through Endothelial-Mesenchymal Transition and eNOS Suppression *Chih-Fan Yeh (National Taiwan University Hospital, Taiwan)*
3:05 pm	10 + 5 min	The Role of Endothelial Ackr1 in Breast Cancer Metastasis *S. Tanner Roach (University of Illinois, USA)*
3:20	10 + 5 min	Endothelial Caveolin-1 and CXCL10 Promote Transcellular Migration of Autoreactive T Cells Across the Blood-Brain Barrier *Ali A. Almousawi (University of Illinois, USA)*

Closing Remarks and Young Scientist Award

3:35–4:00 pm — Luisa Iruela-Arispe (Northwestern University USA) and Roman Zantl (ibidi GmbH, Germany)

ibidi Hands-On Workshop

Louise Ritter (ibidi GmbH, Germany)

9:00 am–3:00 pm
Simpson Querrey Building, Northwestern University, Chicago

Please find all information about the hands-on workshop in the course book.

Keynote 1

Flow-Induced Endothelial Polarity

Luisa Iruela-Arispe

Department of Cell and Developmental Biology, Northwestern University, Chicago, USA

Shear stress influences the polarity of endothelial cells through various mechanisms, including changes in cell shape, alignment, signaling pathways, junctional complexes, and gene expression. This adaptation allows endothelial cells to respond dynamically to the mechanical forces exerted by blood flow and maintain the integrity and functionality of the vascular system. In this talk, I will particularly focus on the effects of laminar shear stress in polarizing Notch receptors downstream of flow and its impact to signaling events.

Keynote 2

Coordination of Vascular Morphogenesis at Endothelial Cell-Cell Interfaces

Matthew Kutys

Department of Cell & Tissue Biology, University of California San Francisco, San Francisco, USA

Vascular morphogenesis is orchestrated by chemical and mechanical signals at endothelial cell-cell interfaces. By recapitulating vascular morphogenesis in biomimetic human microphysiological systems, our work is revealing new insight into key pathways at cell-cell interfaces regulating underlying changes in endothelial mechanics and fate. In this talk, I will detail previously unappreciated mechanisms controlling Notch receptor activation in the endothelium and describe how Notch activation can directly influence cell-cell adhesion and tissue barrier function, altogether informing a model by which transcriptional and cell adhesive programs might be coordinated by a single receptor.

Keynote 3

Flow Mediated Mechanisms of Endothelial Cell Resilience

Julia Mack

Department of Medicine, University of California Los Angeles, Los Angeles, USA

Laminar blood flow in arteries generally supports endothelial cell body elongation, front-rear polarity, and an anti-inflammatory phenotype. But how this polarity promotes resilience and prevents inflammation is unclear. Here, we will discuss a mechanism by which laminar flow promotes endothelial cell resilience via mechanosensitive signaling domains in the apical surface of arterial endothelial cells.

Keynote 4

Understanding Endothelial Cell Responses to Flow Through Study of Notch Target Genes

Jan Kitajewski

Department of Physiology and Biophysics, University of Illinois, Chicago, USA

tba

Keynote 5

Endothelial Responses to Flow in Atherosclerosis

Paul Evans

Biochemical Pharmacology Centre, Queen Mary University of London, London, United Kingdom

Atherosclerosis is a focal disease of arteries that develops at sites of disturbed flow. Endothelial cells at these sites are activated by the local shear stress conditions. My lab has unveiled the underlying mechanisms by integrating cell culture (ibidi system) and *in vivo* experimental approaches.

Keynote 6

Flow-Sensitive HEG1 Regulates Endothelial Function and Protects Against Atherosclerosis

Hanjoong Jo

Department of Biomedical Engineering, Georgia Tech and Emory University, Atlanta, USA

I will discuss how flow regulates HEG1 expression and function in arterial endothelial cells *in vivo* and *in vitro* in a KLF2/4-dependent manner. Using a mouse model with an endothelial targeted deletion of HEG1, I will discuss the protective role of HEG1 in atherosclerosis.

Keynote 7

Mechanisms of Lymphatic Vessel Renewal and Repair

Tatiana Petrova

Department of Biology and Medicine, University of Lausanne, Lausanne, Switzerland

I will discuss the role of flow sensing by lymphatic endothelial cells in lymphatic vascular renewal and repair.

Keynote 8

Effect of Microfluidic on Endothelial Wound Regeneration During Streptococcal Infection

Simone Bergmann

Institute of Microbiology, Technical University Braunschweig, Braunschweig, Germany

Endothelial wound regeneration was monitored under defined microfluidic conditions after cell cultivation using chambered silicon inlets in combination with sticky coverslips. Wound closure under microfluidic is achieved by endothelial cell proliferation and migration and required significantly more time than under static cultivation. Instead of wound closure, cell destruction by Streptococcal and Pneumococcal infection led to enlargement of the wound surface. This combined microfluidic wound healing system enables the analyses of endothelial response to bacterial blood stream infections.

Keynote 9

Vascular Mechano-Biology and Mechano-Therapeutics

Yun Fang

Department of Medicine, University of Chicago, Chicago, USA

We have recently devised a cohort of new precision nanomedicine platforms to target novel dysregulated mechano-sensing mechanisms, a strategy effectively treating vascular complications *in vivo*. Genetics-informed, dysregulated endothelial mechano-sensitive pathways are targeted by precision nanomedicine strategies, leading to reduced vascular diseases in multiple animal models.

Industry Talk

Future Technologies and Applications for Cell Culture Under Flow

Nina Huber

ibidi GmbH, Gräfelfing, Germany

Exposing conventional 2D cell cultures to flow-induced shear stress was a key milestone in the development of more physiologically relevant in vitro models of the circulatory system. The study of e.g. endothelial barrier formation, angiogenesis, or inflammatory processes requires precise regulation of shear stress while maintaining cellular viability over extended periods. Dynamic culture conditions are also becoming more important for 3D cell cultures, which are gaining ground in pharmaceutical and biomedical research. Precisely controlling the cellular environment of 3D aggregates and bioprinted tissues, including the integration of endothelial cells for vascularization, enables the creation of complex and physiologically highly relevant in vitro organ models. Here too, perfusion offers decisive advantages over static culture conditions, such as continuous supply of nutrients, waste removal, and modeling of the interstitial flow. However, these applications require technological advancements of commercial pump systems to ensure compatibility with existing biological methods and to study the mechanisms of major diseases such as cancer, cardiovascular conditions, and autoimmune diseases.

Talk 1 | Poster 14

Oscillatory Shear Stress Modulates Lymphatic Progenitor Cells Maturation Into Lymphatic Vessels With Anti-Cancer Phenotypes

N. Keilany Lightsey[1], Eva Hall[1], Sanjoy Saha[1], Donghyun Paul Jeong[1], Donny Hanjaya-Putra[1,2]

[1] *Department of Aerospace and Mechanical Engineering, Bioengineering Graduate Program, University of Notre Dame, Notre Dame, USA*

[2] *Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, USA*

Our research group have previously reported that human pluripotent stem cells (hPSCs) can be differentiated into lymphatic endothelial cells (LECs) to form lymphatic capillaries. However, these lymphatic vessels lack lymphatic valves that are required for lymphatic pumping. Since oscillatory shear stress (OSS) has been shown to regulate lymphatic valve morphogenesis *in vivo*, we hypothesize that physiological OSS can induce LEC maturation and morphogenesis into lymphatic valves. LECs were differentiated from hPSCs and had differing OSSs applied to compare to static conditions and patient-derived LECs. hPSC-derived LECs under OSS demonstrated similar mature lymphatic-valve forming alignment, cuboidal morphological shape, and OSS-dependent gene expressions relevant to lymphangiogenesis/lymphatic cell identity. Furthermore, hPSC-derived and patient-derived LECs were found to provide differing cancer-related gene expressions in response to OSS. Thus, OSS promotes lymphatic valve phenotypes in hPSC-derived LECs for lymphatic tissue engineering and OSS-dependent lymphangiogenesis can possibly regulate the lymphatic's system response to cancer in LECs and differentiated LECs.

Talk 2

Low Shear in Short-Term Impacts Endothelial Cell Traction and Alignment in Long-Term Under High Shear

Mohanish K. Chandurkar[1,2], Nikhil Mittal[1,2], Shaina P. Royer-Weeden[1,2], Steven D. Lehmann[1], Yeonwoo Rho[3], Sangyoon J. Han[1,2,4]

[1] *Department of Biomedical Engineering, Michigan Technological University, Houghton, USA*

[2] *Health Research Institute, Michigan Technological University, Houghton, USA*

[3] *Department of Mathematical Sciences, Michigan Technological University, Houghton, USA*

[4] *Department of Mechanical Engineering and Engineering Mechanics, Michigan Technological University, Houghton, USA*

Endothelial cells (ECs) adapt to changing fluid shear stress (FSS), crucial for vascular health. High FSS promotes vasodilation and anti-inflammatory responses; low FSS leads to dysfunction. Using traction force microscopy and a flow chamber, we studied ECs' response to shifts from low to high FSS. Initially, low FSS slightly increases traction, but a transition to high FSS induces a significant, prolonged traction increase over 10 hours. Direct high FSS exposure causes an immediate traction spike, then a rapid decrease. The traction vector orientation—critical for EC alignment—is influenced by initial FSS. Granger Causality analysis revealed that traction alignment under direct high FSS causes cell alignment along the flow, while traction perpendicular to flow starting with low FSS leads to perpendicular cell orientation. This highlights the profound impact of initial low FSS on EC traction adjustments and orientation, underscoring the adaptability of ECs to FSS changes.

Talk 3

Mechanotransduction Via Endothelial Adhesion Molecule CD31 Initiates Transmigration and Reveals a Role for VEGFR2 in Diapedesis

Tao Fu*, David P. Sullivan*, Annette M. Gonzalez, Maureen E. Haynes, Prarthana J. Dalal, Nakisha S. Rutledge, Abigail L. Tierney, Julia A. Yescas, Evan W. Weber, William A. Muller

*These authors contributed equally.

Feinberg School of Medicine, Northwestern University, Chicago, USA

Engagement of platelet endothelial cell adhesion molecule 1 (PECAM, PECAM-1, CD31) on the leukocyte pseudopod with PECAM at the endothelial cell border initiates transendothelial migration (TEM, diapedesis). We show, using fluorescence lifetime imaging microscopy (FLIM), that physical traction on endothelial PECAM during TEM initiated the endothelial signaling pathway. In this role, endothelial PECAM acted as part of a mechanotransduction complex with VE-cadherin and vascular endothelial growth factor receptor 2 (VEGFR2); this predicted that VEGFR2 was required for efficient TEM. We show that TEM required both VEGFR2 and the ability of its Y1175 to be phosphorylated, but not VEGF or VEGFR2 endogenous kinase activity. Using inducible endothelial-specific VEGFR2-deficient mice, we show in three mouse models of inflammation that absence of endothelial VEGFR2 significantly (by ≥ 75%) reduced neutrophil extravasation by selectively blocking diapedesis. These findings provide a more complete understanding of the process of transmigration and identify several potential anti-inflammatory targets.

Talk 4

CD36-Mediated Lipid Uptake Mediates Endothelial Stiffening Under Disturbed Flow and in Aortic Arch

Victor Aguilar[1,2], Elizabeth Le Mastera[1], Irena Levitan[1,2]

[1] *Department of Medicine, University of Illinois, Chicago, USA*

[2] *Richard and Loan Hill Department of Biomedical Engineering, University of Illinois, Chicago, USA*

It is known that plasma dyslipidemia and disturbed flow (DF) have synergistic negative effects on endothelial function. Our studies focus on the impact of dyslipidemia and flow on endothelial stiffness, assessed by Atomic Force Microscopy in modified parallel plate flow chambers, with the upper plate removed prior to the measurements. Our earlier studies demonstrated that exposure of HAECs to oxLDL significantly increases endothelial elastic modulus (stiffening), an effect aggravated by DF as compared to laminar flow (LF). Notably, no difference was observed in elastic moduli between cells exposed to DF and LF alone. Furthermore, oxLDL-induced endothelial stiffening is mediated by a scavenger receptor CD36, upregulated by DF. Most recently, we extended these studies to examine the effects of long chain fatty acids (LCFAs), particularly palmitic acid (PA), a prominent LCFA in obesity, and found that PA also induces endothelial stiffening in a CD36-dependent way. Furthermore, we found that endothelial monolayer in aortic arch is significantly stiffer that in the descending aorta and that this effect is fully abrogated by endothelial-specific downregulation of CD36.

Talk 5

Notch Signaling Regulates Unc5B to Suppress Endothelial Proliferation and Branching and to Support Junction Formation and Polarization in Response to Flow

Qanber Raza[1], Taliha Nadeem[1], Seock-Won Youn[1], Bhairavi Swaminathan[1], Ahana Gupta[1], Timothy Sargis[1], Jing Du[1], Henar Cuervo[2], Anne Eichmann[3], Susan L. Ackerman[4], L.A. Naiche[1], Jan Kitajewski[1,5]

[1] *Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, USA*
[2] *Centro Nacional de Investigaciones Cardiovasculares Carlos III- CNIC- (F.S.P), Madrid, Spain*
[3] *Yale School of Medicine, New Haven, USA*
[4] *University of San Diego, San Diego, USA*
[5] *University of Illinois Cancer Center, Chicago, USA*

Notch signaling guides vascular development and function by regulating diverse endothelial cell behaviors, including migration, proliferation, vascular density, endothelial junctions, and polarization in response to flow. Notch proteins form transcriptional activation complexes that regulate endothelial gene expression, but few of the downstream effectors that enable these phenotypic changes have been characterized in endothelial cells, limiting our understanding of vascular Notch activities.

Using an unbiased screen of translated mRNA rapidly regulated by Notch signaling, we identified novel *in vivo* targets of Notch signaling in neonatal mouse brain endothelium, including Unc5B, a member of the netrin family of angiogenic-regulatory receptors. Endothelial Notch signaling rapidly upregulates Unc5B in multiple endothelial cell types. Loss or gain of Unc5B recapitulated specific Notch-regulated phenotypes. Unc5B expression inhibited endothelial migration and proliferation but was required for stabilization of endothelial junctions in response to shear stress. Loss of Unc5B partially or wholly blocked the ability of Notch activation to regulate these endothelial cell behaviors. In the developing mouse retina, endothelial-specific loss of Unc5B led to excessive vascularization, including increased vascular outgrowth, density, and branchpoint count. These data indicate that Notch signaling upregulates Unc5B as an effector protein to control specific endothelial cell behaviors and inhibit angiogenic growth.

Talk 6

Flow-Sensitive Endothelial K^+ Channels are Master Regulators of Endothelial Mechanotransduction and Gene Expression

Sang Joon Ahn[1], Ibra S Fancher[2], Irena Levitan[1]

[1] *Division of Pulmonary, Critical Care, Sleep and Allergy, University of Illinois at Chicago, Chicago, USA*

[2] *Department of Kinesiology and Applied Physiology, University of Delaware, Newark, USA*

Our studies identified flow-sensitive K^+ channel, Kir2.1 as a central factor in resistance arterial biology and vascular tone regulation. Specifically, we found that deletion of endothelial Kir2.1 channels abrogates flow-induced activation of Akt1/eNOS signaling pathway in microvascular endothelium leading to the loss of NO-dependent flow-induced vasodilation (FIV). Here, we explore the role of Kir2.1 channels in endothelial gene expression under static conditions vs. laminar flow. Briefly, downregulation of Kir2.1 using siRNA had only minor effect on gene expression under static conditions but resulted in significant changes in multiple genes' expression under laminar flow. Specifically, the expression of over 7000 genes was affected by the loss of Kir2.1 with predominant effect of increasing their flow sensitivity upon downregulation of channels. Notably, large number of genes affected by the loss of Kir2.1 overlapped with the genes related to atherosclerosis. Pathway analysis suggests major flow-induced pathways through Akt/ERK1/2/MAPK/NFkB networks were regulated by Kir2.1. Overall, these results show that Kir2.1 plays a major role in endothelial mechanotransduction.

Talk 7

Dissection of Endothelial Junctional Dynamics Following Angiogenic Stimulation Through Application of an Engineered Optogenetic Biotin Ligase

Nicholas Leschinsky, Jacob Matsche, Andrei Karginov

Department of Pharmacology and Regenerative Medicine, University of Illinois at Chicago, Chicago, USA

VE-Cadherin is an essential junctional protein involved in the maintenance of the endothelial barrier. Many angiogenic factors induce rapid and dynamic changes in this barrier. While many of the components involved in these processes have been characterized, the dynamic changes in VE cadherin interactome induced by VEGF have not been fully established. Immunoprecipitation approaches are limited in temporal resolution and have very poor sensitivity as they fail to capture weak protein interactions. Proximity labelling techniques enable improved sensitivity but do not provide sufficient temporal resolution. To address this, we generated an optogenetic biotin proximity ligase termed LightR-TurboID. Fusion of LightR-TurboID to VE-Cadherin resulted in a construct capable of labelling known interacting partners with sub-minute resolution. Furthermore, the stability of biotinylation also enables the capture of weak or transient binders that may dissociate from the VE-Cadherin complex. We aim to utilize these characteristics to identify novel components of the junctional machinery and further develop the mechanism of barrier alteration following angiogenic stimulation.

Talk 8

MicroRNA Deregulation as the Mechanism of Cadmium-Induced Atherosclerosis

Imaduddin Mirza[1], Antu Antony[1], Andre Kajdacsy-Balla[2], Abeer M. Mahmoud[1]

[1] *Department of Medicine, University of Illinois, Chicago, USA*

[2] *Department of Pathology, University of Illinois, Chicago, USA*

Epidemiological evidence demonstrates an elevated risk of cardiovascular mortality in those exposed to high cadmium (Cd). Our recent findings in cultured endothelial cells revealed that Cd downregulates mechanosensitive signaling molecules such as eNOS and KLF2/4. These molecules are post-transcriptionally regulated via shear-sensitive miRNAs known as mechano-miRs. We aimed to test if Cd-induced vascular dysfunction is mediated through modulating mechano-miRs involved in inflammation and atherogenesis. We treated endothelial cells cultured under laminar shear stress (ibidi slides) with Cd (5-100 nM). Samples were sequenced for miRNA and RNA, followed by expression and pathway analysis and integration of RNA and miRNA data to identify the functional miRNA that significantly impacted gene expression in response to Cd. Cd altered the expression levels of 329 miRNAs and 2172 genes. On top of the miRNAs are miR10a, miR92a, miR23a, and miR205; the top genes include thrombomodulin, PAI-1, KLK10, and cortactin, all known to be implicated in atherogenic pathways. Discordant miRNA regulation of target genes revealed an enrichment in the atherosclerosis signaling and surface adhesion pathway.

Talk 9

Mechanosensitive Protein FHL2 Tunes Endothelial Flow Response

Shailaja Seetharaman, John Devany, Ha Ram Kim, Emma van Bodegraven, Tracy Chmiel, Shentu Tzu-Pin, Wen-Hung Chou, Yun Fang, Margaret Gardel

Department of Physics, University of Chicago, Chicago, USA

Endothelial cells are essential mechanosensors in the vasculature and play a crucial role in the regulation of angiogenesis, sprouting and barrier function. Defects in mechano-signaling can drive diseases including atherosclerosis and hypertension. However, the precise mechanisms of endothelial force adaptation in normal and disease states remain unclear. Here, using bulk RNA sequencing, we identify differentially expressed genes in response to healthy and atherosclerotic flow profiles. We demonstrate that the transcription as well as protein expression of Four-and-a-half LIM protein 2 (FHL2) are enriched in atherosclerotic flow conditions both *in vitro* and *in vivo*. Atherosclerotic flow phenotypes are characterized by changes in cell and tissue physiology including hypercontractility, discontinuous cell junction morphology, increased vascular permeability. We find that exogenous FHL2 expression is necessary and sufficient to drive the atherosclerotic phenotypes. Overall, our findings demonstrate that FHL2 tunes the contractile set point of adherent cells through actomyosin-microtubule cytoskeletal crosstalk and sustained RhoGTPase signaling.

Talk 10 | Poster 15

Electro-Osmotic Flow Directs Cell Migration and Growth of Endothelial Sprouts

Kushagra Singh and Mark A. Messerli

Department of Biology and Microbiology South Dakota State University Brookings, USA

Applied electric fields (EFs) are used in the clinic to promote healing of chronic epidermal wounds and promote skin transplantation. We are elucidating the mechanisms by which nonexcitable cells in the dermis and epidermis sense and respond to EFs. We hypothesize that EFs promote electro-osmotic flow (EOF) toward the cathode, i.e. electrically-driven water flow that polarizes single cells and directs their migration, most commonly to the cathode. We show that EOF is sufficient to polarize cell surface receptors and therefore may direct cell migration in a manner similar to chemotaxis. Reducing EOF with small molecular weight, neutral viscous polymers, reverses the direction of EF-induced cell migration. Recently we found that EFs also direct the initiation, turning, and growth of endothelial sprouts toward the anode. Sprout growth antiparallel to the direction of EOF is consistent with sprout growth antiparallel to the direction of pressure-driven flow, indicating that applied EFs may promote healing by inducing interstitial flow. Applied EFs possess superior characteristics for inducing interstitial flow in deep regions of dense tissues, compared to pressure-driven flow.

Talk 11

Enhancing the Targeting Efficacy of Endothelial Colony Forming Cells for Renal Regeneration via Kidney-Targeted Liposomal Nanoparticles

B. Cruz Gonzalez[1], F. Fan[2], E. Hall[3], S. Saha[4], D. Hanjaya-Putra[1,3,4]

[1] *Aerospace and Mechanical Engineering, University of Notre Dame, South Bend, USA*

[2] *Chemistry, Michigan State University, East Lansing, USA*

[3] *Aerospace and Mechanical Engineering, University of Notre Dame, South Bend, USA*

[4] *Aerospace and Mechanical Engineering, University of Notre Dame, South Bend, USA*

Endothelial Colony Forming Cells (ECFCs) have demonstrated to exhibit a high proliferative potential since they have the unique ability to create de novo blood vessels *in-vivo*, indicating a higher angiogenic potential. Acute renal failure (AKI) is characterized by a sudden decline in renal function primarily caused by ischemia/reperfusion injury. This type of injury results in significant vascular damage, leading to capillary loss. Researchers have explored the administration of endothelial cells as a potential strategy for treating AKI. After 1 hour, the presence of ECFCs on tissues became scarce. This highlights the need to engineer a solution that can enhance the retention of ECFCs in the kidney, thereby improving their performance in promoting kidney regeneration. In this project, our goal is to utilize Multilamellar Vesicles (MLVs) as vehicles to facilitate cell homing and create what it is refereed as the "backpack molecule" by using a kidney targeting peptide (KTP) which has demonstrated an enhance binding on the kidney. This project aims to design a backpack molecule able to enhance the targeting efficacy of the ECFCs leading to vascular renal regeneration.

Talk 12 | Poster 16

Shear-Sensitive Notch Activation via Extracellular Vesicles to Treat Vascular Injuries

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Extracellular vesicles (EVs) carry ligands that activate distant cell receptors. We engineered EVs with recombinant notch ligands (NL; Jagged1 or Dll4) to treat vascular injuries via canonical Notch activation in endothelial cells (ECs). NL-EVs outperform NL-liposomes in binding to ECs, indicating the presence of additional surface binders. Dll4-EVs rescue adherens junctions in ECs post-lipopolysaccharide (LPS) treatment under shear. Remarkably, incorporating ~6 NL per EV enabled EVs to alleviate edema and vascular hyperpermeability in lung tissue following LPS-induced systemic inflammation in mice, while unmodified EVs did not show the therapeutic effect at the same dose. Mechanistic analysis revealed partial upregulation of canonical Notch targets in CD31+ mouse lung ECs post NL-EVs treatment. Moreover, NL-EVs lost efficacy upon inhibition of Notch by a gamma-secretase inhibitor (GSI), suggesting active involvement of canonical Notch activation. NL-EVs offer potential for vascular injury treatment by shear-mediated Notch activation and VE-Cadherin junction recovery. Future research aims to understand notch ligand presentation by EVs and replicate this process synthetically for therapy.

Talk 13

Disturbed Flow Induces Reprogramming of Endothelial Cells to Immune-Like and Foam Cells Under Hypercholesterolemia During Atherogenesis

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Our previous single cell RNA-seq (scRNA-seq) study revealed disturbed flow (d-flow)-induced reprogramming of endothelial cells (ECs) (FIRE), including inflammation, endothelial-to-mesenchymal transition (EndMT) and endothelial-to-immune-cell transition (EndIT). However, atherosclerotic plaque development occurs only with d-flow and a systemic risk factor such as hypercholesterolemia. To compare the effects of d-flow and/or hypercholesterolemia on endothelial cells, atherosclerosis was induced in C57BL/6 mice by an AAV-PCSK9 and high-fat diet and/or partial carotid ligation (PCL) surgery. Furthermore, to validate our findings at the protein level, we performed EC lineage tracing study using EC-specific confetti mouse exposed to d-flow and hypercholesterolemia. We identified a novel endothelial reprogramming into foam cells (EndFT) in response to d-flow under hypercholesterolemia during atherogenesis. From our validation study, we detected an abundant number of ECs that co-express confetti fluorescence and markers of FIRE at the lumen of LCAs with varying levels of plaque. D-flow under hypercholesterolemia induces FIRE, including inflammation, EndMT, EndIT, and the novel EndFT.

Talk 14

Mechano-Sensitive ER Protein TXNDC5 Promotes Neointimal Hyperplasia of Arterio-venous Fistula Through Endothelial-Mesenchymal Transition and eNOS Suppression

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Background: Arteriovenous fistula (AVF) is the preferred access for hemodialysis in end-stage renal disease. The underlying molecular mechanisms of neointimal hyperplasia (NH) and AVF dysfunction remain poorly understood. This study aimed to explore the role of thioredoxin domain containing 5 (TXNDC5) in NH formation in AVF and assess if targeting TXNDC5 can prevent AVF failure. Results: TXNDC5 was markedly upregulated in the vascular wall of stenotic AVF from hemodialysis patients and in the neointima of mouse aortocaval fistula. Endothelium-specific deletion of Txndc5 in mice significantly reduced AVF neointimal volume, venous wall thickness and collagen deposition 42 days post AVF creation. Lineage-tracing experiments revealed that endothelial cells contribute to neointima formation via endothelial-to-mesenchymal transition (EndoMT). In human umbilical vein endothelial cells, DF decreased eNOS while increasing TXNDC5 and mesenchymal markers, linking TXNDC5 to DF-induced neointimal hyperplasia and dysfunction via EndoMT and eNOS suppression. Conclusion: TXNDC5 is a pivotal mediator of DF-induced NH and AVF dysfunction through regulating EndoMT and eNOS.

Talk 15

The Role of Endothelial Ackr1 in Breast Cancer Metastasis

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Background: Tumor cells can co-opt mechanisms of leukocyte extravasation (LE). Atypical Chemokine Receptor 1 (ACKR1) expression in endothelial cells (EC) has an established role in promoting LE; however, the potential for EC ACKR1 to facilitate the metastatic process through the extravasation of tumor cells has not been studied. Methods: To understand ACKR1 function, we investigated EC ACKR1 expression at the distant metastatic site of the lung, a common site of breast cancer metastasis. We analyzed lung EC ACKR1 expression using immunofluorescent staining of lung vasculature during tumor progression. We developed an EC-specific ACKR1 conditional knockout mouse model (ACKR1 ECKO) to understand the impact of EC ACKR1 on lung metastasis in breast cancer. Results: We observed that tumor implantation in the mammary fat pad leads to a significant upregulation of ACKR1 in the pulmonary venules, a key site of leukocyte and tumor cell extravasation (TCE). We observed spatial clustering of tumor cells near ACKR1-positive vessels, implying a preferential site of TCE. When tumor cells were injected intravenously into mice, we saw reduced TCE in ACKR1 ECKO mice.

Talk 16

Endothelial Caveolin-1 and CXCL10 Promote Transcellular Migration of Autoreactive T Cells Across the Blood-Brain Barrier

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**Equal contributions*

CXCL10 is an interferon-inducible chemokine that can recruit CXCR3+ leukocytes to the central nervous system, leading to neuroinflammation, & demyelination. How CXCL10 promotes leukocyte extravasation and diapedesis across the blood-brain barrier - formed by brain endothelial cells - is poorly understood. Here, we report that CXCL10 mediates CD4+ T cell migration through the brain endothelial cell cytoplasm (transcellular), but not cell- cell junctions (paracellular), via the vesicular trafficking protein Caveolin-1 (Cav-1). Cav-1 promotes CXCL10 aggregation into cytoplasmic stores in BECs in vitro to provide the local, high concentration necessary for recruitment of CXCR3+ leukocytes. This process also requires LFA-1 activity. In the absence of Cav-1, endothelial CXCL10 is secreted, and the local signaling cues are lost. Consistent with our in vitro data, genetic ablation of Cav-1 reduces the severity of active experimental autoimmune encephalomyelitis (EAE), a murine model for multiple sclerosis, by decreasing the infiltration of CXCR3+ T cells into the CNS. Our findings establish a novel mechanism by which BECs utilize Caveolin-1 dependent CXCL10 intracellular stores to license T cells for transcellular migration across BBB.

Poster 1

Integration of Advanced Imaging and MultiOMICs to Elucidate Pro-Atherogenic Effects of Flow-Induced Reprogramming of EC (FIRE)

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Despite the prevalent use of statins and drug-eluting stents, atherosclerosis is a leading cause of death. While atherosclerotic risk factors including hypercholesterolemia are systemic, plaque lesions develop arterial regions exposed to disturbed blood flow (D-flow). Our recent single-cell RNA sequencing and genome-wide expression analysis carried out in the partial carotid ligation model of mouse atherosclerosis uncovered that endothelial cells (EC) are highly heterogenic, while chronic D-flow exposure induces Flow-Induced Reprogramming of EC (FIRE) with characteristic marker expression of inflammation, mesenchymal and immune cell phenotypes. Yet, FIRE and its role in atherogenesis remains to be validated *in situ*. In this context, we demonstrate a novel imaging pipeline by combining a novel tissue clearing method, advanced light-sheet fluorescence microscopy for spatial mapping of multiple multiOMIC targets, and EC-specific lineage tracing model to validate FIRE *in situ*. This novel integration will provide new mechanistic insights into FIRE and atherosclerosis.

Poster 2

The Shear-Dependent S-Nitrosylation of the HEG1 Protein and its Function

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Atherosclerosis tends to develop in areas of disturbed flow (d-flow), while stable flow (s-flow) keeps arteries healthy. Our previous single-cell RNA sequencing database revealed numerous novel endothelial flow-sensitive genes. Our recent study revealed that HEG1 is upregulated in response to s-flow, offering protection against atherosclerosis. However, the underlying mechanisms remain unclear. HEG1 is a highly glycosylated transmembrane protein with EGF-like (EGFL) domains and a cytosolic domain. Predicted protein structure and amino acid sequences show highly conserved and reactive Cys residues in the EGFL domains. Accordingly, we hypothesized that post-translational modification of the EGFL domains would regulate s-flow-dependent HEG1 activation. Results show that s-flow induces S-nitrosylation of HEG1 protein in endothelial cells. When eNOS or NO production was inhibited, both HEG1 S-nitrosylation and overall protein expression induced by s-flow were decreased. My findings indicate that s-flow-dependent S-nitrosylation of HEG1 enhances its roles by activating pathways for HEG1 expression and highlight S-nitrosylation as a potential therapeutic target for atherosclerosis.

Poster 3

Shear Stress-Induced Piezo1 Activation Downregulates Osteoclast Differentiation

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Objectives: Although osteoclasts (OCs) and their precursors express Piezo1 mechanosensory receptor, the role of Piezo1 in RANKL-induced OC-genesis remains elusive. The aims of this study are to establish the role of Piezo1 in sensing the mechanical stress during OC-genesis, and to elucidate the Piezo1-elicited cell signaling. Methods: Mouse bone marrow-derived mononuclear cells were cultured w or w/o M-CSF (25 ng/ml), RANKL (10 ng/ml), Yoda1 (Piezo1 activator, 5 μM) or shear stress (ibidi Pump System, 5 or 20 dyn/cm2). OC-genesis were evaluated by TRAP staining, pit assay and qPCR. To explore Piezo1-elicited cell signaling in RANKL-primed OCs, Phospho-Explorer Antibody Array (PEAA) and W-blot were performed. Results: OC-genesis was downmodulated by shear stress at 20 dyn/cm2 and Yoda1, respectively. According to PEAA, activation of Piezo1 elicited cell signals involving PI3K/Akt axis. Piezo1 activation indeed dephosphorylated Akt which was counteracted by PP2A inhibitor, suggesting an interposition of PP2A in the PI3K/Akt axis. Conclusions: The present study demonstrated that the shear stress-induced activation of Piezo1 can downregulate OC-genesis via PI3/PP2A/Akt axis.

Poster 4

The Role of Endothelial Notch4 in Retinal Angiogenesis

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Leading causes of blindness involve inflammatory angiogenesis. The Notch signaling pathway is established as a critical regulator of angiogenesis, and preliminary data suggests that Notch4 upregulates inflammatory cytokines. However, the specific role that Notch4 plays in mediating angiogenesis in the retina has not been studied. To investigate endothelial Notch4 in the retina, we developed an endothelial cell specific Notch4 conditional knockout mouse (N4ECKO). To understand the physiological function of Notch4, we studied the growth of the superficial and deep plexus at postnatal day 5 (P5) and postnatal day 12 (P12) timepoints respectively. We used immunofluorescent staining to assess the radial outgrowth, density, area, and caliber of vessels. In addition, we evaluated the potential cross-talk between endothelial Notch4 and pericytes as well as mural cells. Endothelial Notch4 may regulate angiogenesis, evidenced by the increased diameter and muscularization of veins as well as reduced formation of the deep plexus in N4ECKO mice. Trends that warrant further exploration include reduced radial outgrowth, spatial clustering of microglia, and reduced vessel density in N4ECKO mice.

Poster 5

Nup93 Prevents Endothelial Stiffening Via Sun1-RhoA Signal Regulation

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Endothelial cells (ECs) are constantly subjected to systemic inflammation and particularly vulnerable to aging. Emerging studies report that as cells age, nuclear pore complexes (NPCs), responsible for nucleocytoplasmic transport, undergo degradation. While an age-associated loss in NPC proteins was recently revealed to also occur in ECs, the role of endothelial NPCs in cellular stiffening is unknown. Here, we show that nucleoporin93 (Nup93), an essential structural NPC protein, regulates the EC cytoskeleton to affect cellular tension. Loss of Nup93 in ECs leads to stress fiber formation, increased stiffness, and enhanced permeability. Mechanistically, Nup93 depletion reduces Sun1 levels, a component of the linker of the nucleoskeleton and cytoskeleton (LINC) complex necessary to physically connect the nucleus with the cytoskeleton. Endothelial knockdown of Nup93 also results in a concomitant increase in RhoA activity, where the re-introduction of Sun1 in Nup93-deficient ECs mitigates RhoA activation. Taken together, we identify Nup93 as a novel regulator of endothelial cytoskeletal remodeling, contributing to the growing concept of nuclear components regulating EC biology.

Poster 6

Targeted mRNA Delivery to the Inflamed Lung for the Treatment of ARDS

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Lung infection by the influenza virus leads to acute lung injury/acute respiratory distress syndrome (ALI/ARDS), however, few pharmacological treatments directly target ARDS. ARDS is characterized by the dysfunction of endothelial cells, epithelial cell injury and the following uncontrolled cytokine storm. Here, we rationally designed an optimized lipid nanoparticle (LNP) that enables efficient delivery of functional mRNA to inflamed mice lungs. Capitalizing on our prior established genetic causality in ARDS, we demonstrated the attractive strategies treating ARDS based on the innovative targeted nanomedicine approaches, that (i) promote endothelium health by endothelium-specific delivery of Krüppel-like Factor 2 (KLF2) mRNA to restore KLF2 which was demonstrated significantly reduced in ARDS mice lungs; (ii) activate epithelial cells anti-viral pathway by epithelium-specific delivery of 2′-5′-oligoadenylate synthetase 1 (OAS1) mRNA to defense against respiratory viral infection. Our data collectively demonstrated that KLF2 mRNA delivery by endothelium-targeting LNP, or OAS1 mRNA delivery by epithelium-targeting LNP, target mice inflamed lung endothelium or infected epithelium, significantly reduce mice ARDS induced by influenza virus H1N1. This project would provide a promising targeting mRNA therapeutic strategy treating ARDS and vascular diseases.

Poster 7

ABCA1-Dependent Cholesterol Efflux Can Regulate Membrane Cholesterol Content in Endothelial Cells

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In the vasculature, H2S elicits vasodilation, which our lab has shown to be dependent on plasma membrane cholesterol content. Moreover, endothelial cell (EC) plasma membrane cholesterol is lower in small mesenteric arteries (Sma) (<150µm; p=0.0056) compared to large mesenteric arteries (Lga) (>300µm). EC membrane cholesterol content is associated with the observed insensitivity to H2S-induced vasodilation in Lga (p<0.0001). Using single-cell RNAseq analysis, we have identified that the cholesterol efflux gene, ATP-binding cassette family a1 (ABCA1), is enriched in ECs from Sma compared to Lga. Although conflicting data exist, shear stress (SS) has been suggested to regulate ABCA1 expression. Therefore, we hypothesize that SS increases ABCA1 expression, decreasing EC membrane cholesterol content. Using pressurized rat mesenteric arteries and cultured human aortic endothelial cells, we show that increasing SS augments ABCA1 expression and decreases EC membrane cholesterol content. In conclusion, SS may be an important regulator of ABCA1-dependent membrane cholesterol efflux in EC, determining the differential sensitivity to vasodilators between Lga and Sma.

Poster 8

Effects of Differential Shear Stress on Nuclear Integrity of Aortic Endothelium

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Vascular cells are constantly subjected to physical forces associated with the rhythmic activities of the heart, which combined with the individual geometry of vessels further imposes oscillatory, turbulent, or laminar shear stresses on endothelial cells. These hemodynamic forces play an important role in regulating the phenotype and gene expression of endothelial cells in different regions of the arterial wall. Here, we find that in areas of non-laminar flow, endothelial nuclei are impacted with some developing abnormal morphology in wild-type mice. These dysmorphic nuclei first become noticeable in 8-week-old mice at the interface of the aortic arch with the descending aorta, and their number and degree of dysmorphia become increasingly severe with age, eventually appearing at non-laminar flow regions of carotid arteries and the abdominal aorta. These nuclei do not exhibit any increased DNA damage compared to non-dysmorphic nuclei, and have heterogenous hetero- and euchromatin levels. Ongoing studies are focused on exploring functional consequences of dysmorphic nuclei on nuclear pore function and chromosomal organization under different types of flow.

Poster 9

Systems Approaches Distinguish Molecular Drivers of Transcriptomic Identity in the Aortic Arch

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The aortic arch arises from the embryonic remodeling of the pharyngeal arches and serves to connect the vasculature of the upper extremities with the aorta and lower body. The combination of developmental origin, local geometry and flow patterns in the arch facilitate the emergence of vascular pathologies including atherosclerosis and aneurysms. Using a combination of lineage tracing and in-vitro exposure to shear stress we sought to elucidate the contribution of developmental origin and oscillatory flow to arch identity. We identified an anatomic sex-invariant, cell type-specific gene signature for the aortic arch and validate these markers immunohistochemistry. Regional divergence of arch-derived endothelial (EC) and vascular smooth muscle cells (vSMC) is driven by the integration of molecular signals from intrinsic factors (developmental origin, vSMC) and extrinsic factors (oscillatory and laminar flow, EC) to drive phenotypic specification of arch-derived cells relative to the aorta. Integration of these regional, cell-specific signature with genomic variants associated with cardiovascular disease risk yields novel insights into site-specific vascular pathologies.

Poster 10

Acetylation of Microtubules is Required for Endothelial Cell Elongation in Response to Shear Stress

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Endothelial cells (ECs) are constantly exposed to mechanical forces, especially shear stress from their direct contact with blood flow. Adaptation to these hemodynamic forces requires significant cytoskeleton remodeling. While much is known about the effect of shear stress on the actin cytoskeleton; the impact of hemodynamic forces on microtubules (MTs) has not been investigated in depth in ECs. Here we employ imaging, chemical, and genetic perturbations to characterize alterations of MTs in response to flow. Our findings revealed that pharmacological suppression of MT dynamics blocks EC elongation and alignment demonstrating their essential contribution in endothelial mechanotransduction. There is a flow-dependent increase in MT acetylation that when manipulated shows a direct relationship between acetylation levels and cell shape. Finally, loss of aTAT1 led to loss of acetylation and a reduction in elongation in response to flow. In contrast, loss of HDAC6 led to a significant increase in acetylation and faster elongation and alignment kinetics. Our findings uncover an essential role for HDAC6 and aTAT1 as key mediators of EC mechanotransduction through MT acetylation regulation.

Poster 11

Piezo-1 Alters Force Transmission in Endothelial Cells Under Fluid Shear Stress (FSS)

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Piezo-1, one of the major surface receptors involved in early EC FSS sensing and integrin activation is greatly explored in biochemical aspect as a mechanosensitive channel to shear stress. However, its role in force transmission is still unexplored. Here, by combining traction force microscopy with a custom flow chamber, we examined Piezo-1 role in human umbilical vein endothelial cells (HUVECs) adapting their traction during long-term direct high and transitions from short-term low shear to long-term high shear stress. We discovered that piezo1 inhibited HUVECs under direct high shear resulted in 4-fold traction reduction as compared to control. The reduced traction magnitude under direct high FSS showed a direct correlation with reduced cell and traction alignment. On the contrary, initial short-term low shear resulted in no significant traction change as compared to control but resulted in reduced traction and cell alignment. Thus, suggesting an independent pathway involved in force transmission and cell alignment under low FSS condition. Taken together, our findings elucidate the significant influence of piezo1 in force transmission and cell alignment.

Poster 12

Pediatric Hematopoietic Stem Cell Transplantation Monitoring. An Angiogenesis *In Vitro* Assay

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Angiogenesis is a relevant process in hematopoietic stem cell transplantation (HSCT) for hematologic malignancies. However, the role of angiogenesis and temporal behavior requires more investigation. We conducted an observational prospective study to evaluate angiogenesis through tube formation assay during the initial period after HSCT. Fifteen pediatric patients who underwent HSCT at Children's Hospital were enrolled in this study. Plasma was collected from the venous blood on Days 0, +7, +14, +21, +28, and +35 after transplantation. The angiogenesis capability of the patient´s plasma was evaluated in HUVEC cells by tube formation assay using the µ-Slide15 Well 3D. After 8 hours of culture, visual fields were captured using an inverted phase microscope; images were analyzed with ImageJ. One-way ANOVA and Dunnett post hoc tests were performed (SPSS version 25). For each patient sample, significant differences among angiogenesis of each day evaluated was considered $p \leq 0.05$.

Poster 13

Hemodynamic Flow Separation Occurs in the Cephalic Arch of Hemodialysis Patients

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There are currently over 800,000 individuals in the United States who receive routine hemodialysis treatment for end-stage renal disease, which requires an arteriovenous access. This is most commonly a brachiocephalic fistula; however, repeated use is complicated by patency loss, frequently due to stenosis in the post-bend region of the cephalic arch (CA) (Bennet et al., 2015). While the CA is known to be a common site for stenosis in hemodialysis patients, it remains unclear what contributes to stenosis at this specific location. We investigated the hemodynamics in the CA using patient-specific millifluidic models constructed from venogram and IVUS data. The models were perfused with fluorescent tracer beads to visualize flow behaviors. We perfused our models at both physiologic (20-40 mL/min) and pathologic (≥350 mL/min) flow rates to observe the hemodynamic profiles that emerge during hemodialysis. Our findings show that the curvature of the CA creates a zone of flow separation (i.e., turbulence). These occurrences of flow separation only emerge at pathologic, high flow rates and are repeated across models. Our results show that areas of flow separation occur most commonly in the post-bend of the CA, where stenosis is most common. These results suggest that flow separation may drive the vascular remodeling that leads to stenosis and patency loss in hemodialysis patients.

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