BIOGRAPHICAL SKETCH

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NAME: Scott Robert Johnstone

eRA COMMONS USER NAME (credential, e.g., agency login): SRJ6NNIH

POSITION TITLE: Assistant Professor, Fralin Biomedical Research Institute at Virginia Tech Carilion

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Glasgow Caledonian University, Glasgow, UK	BSc	06/2004	Biomedical Sciences
Glasgow Caledonian University, Glasgow, UK	PhD	06/2008	Cell and Molecular Biology
University of Virginia, Charlottesville, VA	Post-doctoral	06/2012	Cellular communication and Cardiovascular
University of Glasgow, Glasgow, UK	Research Fellow	10/2016	Cellular communication and cardiovascular

A. Personal Statement

In June 2020, I initiated my laboratory as a New Investigator at the Fralin Biomedical Research Institute (FBRI) at Virginia Tech. I have worked to build my lab over the last three years and have assembled a team including postdoctoral researchers and graduate/ medical/ undergraduate students. Throughout, I have managed staffing, budgets, and research progression leading to publication and patents. I have collaborated widely, in academic and clinical settings, to establish the best research and to ensure the success of our projects. Our research is focused on understanding the basic cellular mechanisms, signaling, and functions in vascular physiology and pathology. I have studied cell communication through single membrane channels such as pannexin and gap junction channels (e.g. *J. Immunol 2020, J. Cell Sci 2023*). A major aspect of my research has been understanding how protein modifications (e.g. phosphorylation and nitrosylation) regulate channel signaling and protein interactions (e.g. Cx43/cyclin E, Circ. Res. 2012). We have focused on aspects of mural cell differentiation, investigating the effect of platelet-derived growth factor-BB (PDGF-BB), and studying these in novel in vitro and in vivo applications in mice (*Am. J. Path. 2009, Circ. Res. 2012, Biomolecules 2023*). My research themes center around cardiovascular physiology and disease including: i) Pathways involved in the recruitment of inflammatory cells in the vasculature, ii) understanding the process of vascular cell differentiation during disease progression, and iii) cell-to-cell signaling and its control of the vasculature. I have employed a wide range of approaches to help understand these disease processes from the biochemical level in cells to animal models, including multiple transgenic lines targeting smooth muscle cells, macrophages, and endothelial cells, and translation using human saphenous vein models. Translational research is at the core of our research, and our long terms goals are to develop novel therapeutics and techniques as described in our recent review (*Int. J. Mol. Sci. 2021*) and in our patent filing. My research has been funded via fellowships in the UK and USA, and recently through an American Heart Association Career Development Award.

Ongoing and recently completed projects that I would like to highlight:

- VT Proof Of Concept Grant, Virginia Tech 06/24-0625
  - CycliCx: a novel drug for the targeted treatment of vascular disease
  - Johnstone SR (PI)
- Seale Innovation Award, Virginia Tech 01/24-12/24
  - Cell-based therapies: Using macrophages (Φ) to deliver exosome-therapeutics to sites of vascular tissue damage
  - Johnstone SR (PI)
- Johnstone Operations/ Startup Funds, Virginia Tech 06/20-05/26
  - Johnstone SR (PI)
- Seale Innovation Award, Virginia Tech 01/23-12/23
  - Identification of cell-permeable nanobodies to reduce smooth muscle proliferation
  - Johnstone SR (PI)
- NIH-F31 – Predoctoral Award (Sedovy) 08/23-02/26
  - The role of endothelial connexins in vascular wound repair, Award to Ms. Meghan Sedovy, graduate student, Johnstone lab
  - Johnstone, SR (Sponsor)
- American Heart Association – Predoctoral Award (Sedovy) 01/23-08/23 (returned after award of NIH-F31)
  - The role of endothelial connexins in vascular wound repair, Award to Ms. Meghan Sedovy, graduate student, Johnstone lab
  - Johnstone, SR (Sponsor)
- American Heart Association Career Development Award 06/19-05/23
  - Connexin regulation of cell proliferation
  - Johnstone, SR (PI)

Pending

- NIH-R21
  - Development of a novel intracellular targeting nanobody that blocks Cx43-mediated pathological proliferation
  - *Score 1%, awaiting council decision*

Citations:

1. Scott H, Dong L, Stevenson A, MacDonald AI, Srinivasan S, Massimi P, Banks L, Martin PE, **Johnstone SR**, Graham SV. The human Discs large protein (Dlg1) interacts with and maintains Connexin 43 at the plasma membrane in keratinocytes. *J Cell Sci. 2023 May 9. [Epub ahead of print*] PMID: 37158057.
2. King DR, Sedovy MW, Leng X, Xue J, Lamouille S, Koval M, Isakson BE, **Johnstone SR***. Mechanisms of Connexin Regulating Peptides. Int J Mol Sci.2021 Sep 22;22(19). doi: 10.3390/ijms221910186. Review. PubMed PMID: 34638526; PubMed Central PMCID: PMC8507914.
3. Yang Y, Delalio L, Best AK, Maccal E, Begandt D, Lee C, Milstein J, Donnelly J, Miller AM, McBride M, Shu X, Koval M, Isakson BE and **Johnstone SR***. Endothelial pannexin 1 channels control inflammation by regulating intracellular calcium. *J. Immunol.* 2020 Jun 1;204(11):2995-3007 *2020,* PMCID: PMC7336877
4. **Johnstone SR**, Kronke B, Straub AC, Best AK, Dunn CA, Mitchell LA, Peskova Y, Nakomoto RK, Koval M, Lampe PD, Columbus L and Isakson BE. MAPK phosphorylation of connexin 43 promotes binding of cyclin E and smooth muscle cell proliferation. *Circ Res*. 111:201-211; 2012. PMCID: PMC3405546

indicates corresponding author

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2022 – Present	Assistant Professor, Department of Surgery, Virginia Tech Carilion School of Medicine
2021 – Present	Facility Director of Histology Core, Fralin Biomedical Research Institute.
2020 – Present	Assistant Professor, Fralin Biomedical Research Institute, Virginia Tech Carillion.
2020 – Present	Assistant Professor, Department of Biological Sciences, Virginia Tech.
2017 – 2020	Instructor of Research, Robert M. Berne Cardiovascular Research Center, University of Virginia School of Medicine

2016-2017 Lecturer in Biomedical Sciences, Department of Biological and Biomedical Sciences, Glasgow Caledonian University, Glasgow, UK
2012-2016 Research Fellow, British Heart Foundation Glasgow Cardiovascular Research Centre, Institute of Cardiovascular and Medical Sciences, University of Glasgow, Scotland UK
2008-2012 Post-doctoral Researcher, Robert M. Berne Cardiovascular Research Center, University of Virginia School of Medicine

Scientific Appointments and Professional Memberships

2023-Present Elected Council Member, North American Vascular Biology Organization (NAVBO)
2023 Study Section (Ad Hoc, ECR): NIH IVPP June 2023
2022 Associate Editor: Journal of Vascular Research
2021-Present Member: North American Vascular Biology (NAVBO)
2021-2023 Study Section (Ad Hoc): American Heart Association Career Development Award
2020 Junior Associate Editor: Journal of Vascular Research
2019-Present Member: The American Association of Immunologists
2016 Co-Editor, Gap Junction Protocols Book, Springer Science and Media, Methods Molecular Biology, ***21,000 downloads and >60 citations***
2015 Guest Editor, *Biochemical Society Transactions*
2015-present Member: British Society for Cardiovascular Research
2015-present Fellow of the Higher Education Academy, UK
2012-present Member: British Atherosclerosis Society
2012-present Member: British Cardiovascular Society
2008-present Member: American Heart Association
2004-present Member: Biochemical Society

Honors

2023 Co-Organizer, 2023 Vascular and Heart Research Symposium, Roanoke, VA, USA
2022 Invited Speaker: The Ohio State University
2022 Session Co-Chair: 22nd International Vascular Biology Meeting, Oakland, USA
2022 Session Co-Chair: International Gap Junction Conference, ACoruna, Spain
2022 Co-Organizer, 2022 Vascular and Heart Research Symposium, Roanoke, VA, USA
2017 Co-Organizer, International Gap Junction Conference, Glasgow, UK
2015 Co-Organizer, British Society for Cardiovascular Research, Glasgow, UK
2014 Co-Organizer, UK Gap Junction Conference, Glasgow, UK
2012 Co-Organizer, UK Gap Junction Conference, Glasgow, UK

Patents/ License

- Patent #WO2021021716 - COMPOSITIONS AND METHODS FOR INHIBITING NEOINTIMAL FORMATION
  - U.S. Provisional Patent filed 07/25/2019: "Modification of mitogen activated protein kinase phosphorylated connexins 43 and cyclin E activity to control arterial smooth muscle proliferation"
  - Patent conversion 07/27/2020: PCT/US2020/043710,
  - Published 04.02.21, Inventors: **Johnstone**, Isakson
  - Description: The patent covers the discovery and use of peptide (CycliCx) inhibiting Cx43 and Cyclin E interactions to limit neointima formation

C. Contributions to Science

1) Inflammation and cell signaling in the vasculature. Macrophage accumulation plays an integral role in vascular disease progression. Understanding the tissue environment and cell type within developing plaques can help define disease progression, severity, and plaque stability. My research contributed to the first identification of a novel macrophage phenotype, Mox, that was induced by chronic inflammation, NRF2 activation, and differential oxidized phospholipids in atherosclerotic plaques (Circ Res, 2010). We provided similar evidence that oxidized phospholipids differentially alter the phosphorylation state of connexin 43 (Cx43), in mouse carotid arteries associated with an increase in smooth muscle cell proliferation (Am J Path, 2010). Macrophage recruitment and retention is a significant component of the early development of atherosclerosis, with ATP shown to be a major mechanism for the recruitment of inflammatory cells. Our early studies characterized a novel family of ATP-release channels Pannexins (Panx) in smooth muscles and endothelial cells and showed differential expression throughout the vasculature (J Vasc Res, 2012). We found that acute

exposure to pro-inflammatory signaling molecules, including tumor necrosis factor-alpha (TNF-alpha), alters Panx1 signaling and promotes ATP release and subsequent leukocyte adhesion and emigration (*Nature Communications,* 2015). As atherosclerosis is chronic and progressive, we studied changes in Panx1 in response to long-term exposure to inflammatory stimuli, including TNF-alpha. We were the first to describe that chronic TNF-alpha treatments increase Panx1 channel expression and opening for uptake of Ca^{2+} to the endothelium, which acts as a feedforward signaling pathway, amplifying IL-1beta translation and release (J Immunology, 2020). A commentary article was commissioned to discuss the importance of our findings in advancing knowledge of Panx1 signaling: Panx1 in inflammation heats up: New mechanistic insights with implications for injury and infection (Cell Calcium 2020). We have now published further works demonstrating the importance of Panx1 signaling control in endothelial dysfunction, venous inflammation, and impaired cardiovascular functions (Circ Res 2021, Science Signaling 2021).

a. Kadl, A. K. Meher AK, P. R. Sharma, M. Y. Lee, A. C. Doran, **S. R. Johnstone**, M. R. Elliott, F. Gruber, J. Han, W. Chen, T. Kensler, K. S. Ravichandran, B. E. Isakson, B. R. Wamhoff, N. Leitinger. Identification of a novel macrophage phenotype that develops in response to atherogenic phospholipids via Nrf2. *Circ Res.* **107:737-746; 2010.** PMCID: PMC2941538
   - Circulation Research Most Read 2010-2011 list. Circ. Res. 2012; 111: e338-e378, doi: 10.1161/ CIRCRESAHA.112.281089

b. A. W. Lohman, I. Leskov, J. Butcher, **S. R. Johnstone**, T. A. Stokes, L. J. DeLalio, A. K. Best, S. Penuela, N. Leitinger, K. S. Ravichandran, K. Stokes, and B. E. Isakson. Pannexin 1 channels regulate leukocyte emigration through venous endothelium during acute inflammation. *Nature Communication* 6:7965, 2015*. Nature Communications*, 6:7965, 2015. PMCID: PMC4824045

c. Y. Yang, L. Delalio, A. K. Best, E. Maccal, D. Begandt, C. Lee, J. Milstein, I. Donnelly, A. M. Miller, M. McBride, X. H. Shu, M. Koval, B. E. Isakson and **S. R. Johnstone***. Endothelial pannexin 1 channels control inflammation by regulating intracellular calcium. *J. Immunol.* 2020 Jun 1;204(11):2995-3007 *2020,* PMCID: PMC7336877

d. M. E. Good, A. P. Young, A. G. Wolpe, M. Ma, P. J. Hall, C. K. Duffy, M. J. Aronovitz, G. L. Martin, R. M. Blanton, N. Leitinger, **S. R. Johnstone**, M. J. Wolf, B. E. Isakson. Endothelial pannexin 1 contributes to impaired cardiac function following myocardial infarction. Circ Res.2021;128(8):1211-1213 PMCID: PMC8049979.

indicates corresponding author

2) Mechanisms of cellular proliferation and protein post-translational modifications: We were the first lab to report on direct cellular interactions with the connexin 43, cyclin E, and CDK2 proteins and demonstrate their effect on cellular proliferation. My work on protein interactions began initially in model systems such as HeLa cells, where I studied how cells divide in response to proliferative stimuli and the correlation to the expression of the gap junction protein Cx43 (*J Cell Biochem*, 2009). In these studies, I confirmed that while the proteins could indeed form gap junctions, the regulation of proliferation was controlled through a pathway that was completely independent of intercellular signaling. We have studied Cx43 as a signaling hub for protein-protein interactions, including the critical role of the cellular environment in altering Cx43-protein post-translational modifications. In mouse disease models, we found that phosphorylation of Cx43 following exposure to oxidized phospholipids is associated with the development of atherosclerotic disease (*Am J Path*, 2009). The role of Cx43 post-translational modifications is key to the control of cell-cell signaling, and we have published a number of articles and reviews looking at different mechanisms of regulation of the connexin proteins in physiology and pathology of disease (e.g. *ATVB*, 2011, Int J Mol Sci 2018, Int J Mol Sci 2021, Comprehensive Physiology 2021, J Vasc Res 2022). One of the key discoveries made during this time was that Cx43 interacts with the cell cycle regulator protein cyclin E and CDK2. In order to detail the nature of this interaction and its impact on disease, we used biochemical approaches (e.g. analytical size exclusion), molecular techniques (e.g. immune-transmission electron microscopy and proximity ligation assays) as well as animal models of neointimal formation to demonstrate that this interaction was critical to smooth muscle cell proliferation and the development of neointima (*Circ Res*, 2012). We have worked to elucidate the impact of the Cx43 and cyclin E interaction in humans by incorporating ex vivo saphenous vein tissue preparations and human cell lines to show that the mechanism is preserved across species and plays an important role in disease. One important aspect is the ability to target and disrupt the complex in disease, so I have now used peptide array techniques to start to map the specific regions of interaction between the proteins. Using this information, I have been able to design and developed a panel of stearate-linked peptides as tools to study the disruption of growth factor-associated proliferation in human vascular smooth muscle cells. These studies are ongoing, and we have a patent related

to the peptides and are developing new technologies (nanobodies) to advance their therapeutic effects with translational potential.

a. **S. R. Johnstone**, A. K. Best, C. S. Wright, B. E. Isakson, R. J. Errington and P. E. Martin. Enhanced connexin 43 expression delays intra-mitotic duration and cell cycle traverse independently of gap junction channel function. *J Cell Biochem*. 1;110(3):772-82; 2010. PMCID: PMC3030924
b. **Scott R. Johnstone**, Jeremy Ross, Michael J. Rizzo, Adam C. Straub, Paul D. Lampe, Norbert Leitinger and Brant E. Isakson. Oxidized phospholipid species promote in vivo differential Cx43 phosphorylation and vascular Smooth muscle cell proliferation. *Am J Path*. 175(2):916-24; 2009. PMCID: PMC2716985
c. A. C. Straub, M. Billaud, **S. R. Johnstone**, A. K. Best, S. Yemen, S. T. Dwyer, R. Looft-Wilson, J. J. Lysiak, B. Gaston, L. Palmer and B. E. Isakson. Compartmentalized connexin 43 *S*-nitrosylation/de-nitrosylation regulates heterocellular communication in the vessel wall. *Athero Thromb Vascular Biol.* 2:399-407; 2011. PMCID: PMC3056333
d. **S. R. Johnstone**, B. Kronke, A. C. Straub, A. K. Best, C. A. Dunn, L. A. Mitchell, Y. Peskova, R. K. Nakomoto, M.Koval, P. D. Lampe, L. Columbus and B. E. Isakson. MAPK phosphorylation of connexin 43 promotes binding of cyclin E and smooth muscle cell proliferation. *Circ Res*. 111:201-211; 2012. PMCID: PMC3405546

3) Heterocellular signaling mechanisms in the vasculature: Endothelial and smooth muscle cell communication plays an integral role in the coordination of vessel function and maintenance of vascular health (Comprehensive Physiology, 2021). We have investigated roles for channel-based control of ATP signaling, Ca^{2+} and IP3 control, and protein post-translational modifications through phosphorylation and nitrosylation that alter blood pressure (ATVB, 2011, Nature 2012, Circulation 2020). My research has been integral in the characterization of the three pannexin (Panx1, Panx2, Panx3) channels in the vasculature. Both ATP and Ca^{2+} signaling from these channels play a key role in the control of of blood pressure. We identified Panx1 and Panx3 were expressed in the vasculature, with differential patterns of expression based on blood vessel size and function (*J Vasc Res*, 2012). The role of Panx1 in smooth muscle cells was unknown, and we used electron microscopy techniques, vascular reactivity studies, and cell-based assays to define Panx1 as a regulator of alpha-1 adrenergic signaling in smooth muscle cell contractions in the microcirculation (*Circ Res*, 2011). In the kidney we have also shown that Panx1 ATP- release modulates renin secretion and blood pressure homeostasis. Heterocellular signaling is not restricted to the endothelium and smooth muscle layers, and there is a complex interplay between circulating and perivascular cells that is largely unknown. Our ongoing work aims to define roles for these cells in the maintenance of vessel health and alterations in signaling that occur during disease.

a. D. Ryan King, Meghan W. Sedovy, Xinyan Eaton, Luke S. Dunaway, Miranda E. Good, Brant E. Isakson, and **Scott R. Johnstone***. Cell-to-cell Communication in the Resistance Vasculature*. Comprehensive Physiology. 2022,* 12(4):1-35. PMID: **35959755**
b. M. Ottolini, K. Hong, E.L. Cope, Z. Daneva, L.J. DeLalio, J.D. Sokolowski, C. Marziano, N.Y. Nguyen, J. Altschmied, J. Jaendeler, **S.R. Johnstone**, M.Y. Kalani, M.S. Park, R.P. Patel, W. Liedtke, **B.E. Isakson**, S. Sonkusare. Local Peroxynitrite Impairs Endothelial TRPV4 Channels and Elevates Blood Pressure in Obesity. *Circulation*, 2020 21;141(16):1318-1333 PMCID: PMC7195859
c. L.J DeLalio, E. Masati, S. Mendu, C. A. Ruddiman, Y. Yang, **S. R Johnstone**, J. A. Milstein, T.C. S. Keller IV, R.I B Weaver, N. A. Guagliardo, A. K Best, K. Ravichandran, D. A Bayliss, M. L. S. Sequeira-Lopez, S. N. Sonkusare, B. Desai, P. Q. Barrett, T. H. Le, A. R Gomez, and B. E Isakson. Pannexin 1 channels in renin-expressing cells regulate renin secretion and blood pressure homeostasis. Kindey Int. 2020;S0085-2538(20)30543-3. doi: 10.1016/j.kint.2020.04.041
d. A. C. Straub, A. W. Lohman, M. Billaud, S. R. Johnstone, S. T. Dwyer, M. Y. Lee, P. Schoppee Bortz, K. Best, L. Columbus, B. Gaston, and **B. E. Isakson**. Endothelial cell expression of hemoglobin α regulates nitric oxide signaling. *Nature*, 491: 473-477, 2012. PMCID: PMC3531883

indicates corresponding author

Current list of articles: 44 indexed, citations 2994, H-index 24 (Nov 23) :
https://www.ncbi.nlm.nih.gov/myncbi/scott.johnstone.1/bibliography/public/