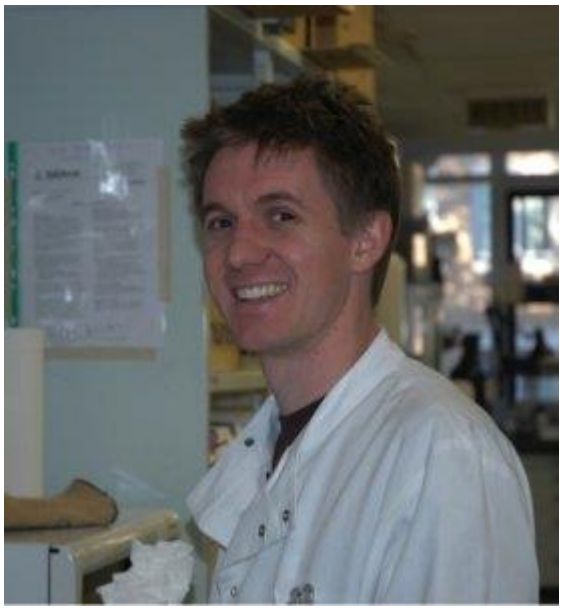
LXXXVIX Wykład JCET, 17.02.2017

Endothelial cell storage and secretion of bioactive components: a crucial role in haemostasis and inflammation



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It is essential that the response to vascular injury or infection is fast; this minimises loss of blood and spread of pathogens. As such, endothelial cells harbour specialised rod shaped storage organelles (WPB) that contain multiple pre-made pro-inflammatory and pro-haemostatic proteins. Within minutes of endothelial cell stimulation WPB are exocytosed and release their stored content into the vasculature thus starting the processes of both haemostasis and leukocyte recruitment. Pro-inflammatory content include P-selectin which on display at the cell surface allows initial rolling and recruitment of leukocytes to sites of tissue injury. The most important haemostatic component of WPB is the glycoprotein von Willebrands factor (VWF) that comprises 90% of stored protein. Upon exocytosis VWF is unfurled by the shear-force present in the blood vasculature to produce millimetre-long protein strings revealing multiple binding sites for platelets. Failure to secrete properly processed VWF result in bleeding disorders (Von Willebrand's disease is the most common inherited bleeding disorder).

My talk will describe a number of novel assays for monitoring WPB trafficking and content secretion including biochemical assays, high-throughput image analysis, high-speed live cell imaging and correlative light and electron microscopy. These approaches allow us to study the mechanics, both of WPB exocytosis and of subsequent, exocytosis related, trafficking events (such as additional compensatory endocytosis).

Our research demonstrates that efficient secretion of VWF requires the formation of an actomyosin ring around the base of WPB that squeezes out the protein content as strings. We also describe some of the upstream signalling molecules required for ring recruitment and demonstrate that this ring is recruited by different physiological stimuli to varying extents both with respect to the timing and the number of exocytic events. Finally we show that following exocytosis an additional round of endocytosis is started. This limits any increase in cell size and helps to control exocytic mode to maintain single granule exocytosis. These processes allow the endothelium to precisely regulate the haemostatic and inflammatory response. Targeting such processes with therapeutics may provide new methods for controlling haemostasis and inflammation.

Short Biography

Thomas Nightingale

Name Thomas Nightingale
Date of birth 20/01/78
Nationality British

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William Harvey Research Institute,
John Vane Science Centre Charterhous Square

Education and Qualifications

Oct 2013-Current: Lecturer-Full time.
The role of intracellular trafficking in controlling inflammation and haemostasis in the blood vascular endothelium.

Sept 2014-Sept 2016: Queen Mary University of London
Postgraduate Certificate in Academic Practice Merit

Nov 2009-Sept 2013: Investigator Scientist University College London-Full time.
Biogenesis and Function of Weibel Palade bodies.

Nov 2005- Nov 2009: Post Doc University College London-Full time. BHF funded.
Characterisation of Novel Proteins involved in the Biogenesis of Weibel Palade bodies in Vascular Endothelium.

Sept 2000-Sept 2005: Brasenose College, University of Oxford.
D.Phil
Regulated Ligand Binding of LYVE1: A Lymphatic Endothelium Specific Hyaluronan Receptor
Prof. D. Jackson

Sept 1996-June 2000: University of Bath, School of Biology and Biochemistry
Undergraduate Degree M.Biochem First

Teaching

Undergraduate and taught postgraduate units

2015/16: I taught Research skills in pharmacology to 1st year BSc pharmacology and innovative therapeutics students. This was the first time this course has been run so together with Prof Nourshargh and Dr Whiteford we generated all course content. I was responsible for designing and delivering tutorials, lectures and seminars as well as marking essays and oral presentations. The group consisted of 21 students and taught lessons took 13h.

2014/15: I supervised and marked the locomotor and FunMED problem based learning session for first year MBBS medical students. Groups were of 8 students and the sessions were 8h and 12h respectively

2013/14 and 2014/15: I was responsible for designing and presenting lectures and demonstrations for the MRes students (BHF scheme and MRC scheme). Groups were typically 10-12 students sessions were 3h.

Publication record

Stevenson NL, White IJ, McCormack JJ, Robinson C, Cutler DF. Nightingale TD. (2017) Compensatory endocytosis in endothelial cells controls Weibel Palade Body exocytic mode. *Under revision for J. Cell Sci.*

Nightingale TD, McCormack JJ, Grimes W, White IJ, Cramer LP, Cutler DF. (2016) Tuning the endothelial response: Differential Release of exocytic cargoes from Weibel Palade Bodies. *Under revision for Blood.*

Nightingale TD, Cutler DF. (2013) The secretion of Von Willebrand Factor from endothelial cells; an increasingly complicated story. *J.Thromb. Haemostasis.* Jun; 11 Suppl 1:192-201

Nightingale TD, Cutler DF, Cramer LP. (2012) Actin coats and rings promote regulated exocytosis. *Trends Cell Biol.* 22(6) p329-37.

Nightingale TD, White IJ, Doyle EL, Turmaine M, Harrison-Lavoie KJ, Webb KF, Cramer LP, Cutler DF. (2011) Actomyosin II contractility expels von Willebrand factor from Weibel-Palade bodies during exocytosis. *J Cell Biol.*194(4) p613-29. *Subject of Biosights JCB podcast highlighting original research*

Rojo Pulido I, Nightingale TD, Darchen F, Seabra MC, Cutler DF, Gerke V. (2011) Myosin Va acts in concert with Rab27a and MyRIP to regulate acute von-Willebrand factor release from endothelial cells. *Traffic* 12 (10) p1371-82

Michaux G, Dyer CE, Nightingale TD, Gallaud E, Nurrish S, Cutler DF. (2011) A role for Rab10 in von Willebrand factor release discovered by an AP-1 interactor screen in C. elegans. *J.Thromb. Haemostasis* 9 (2) p392-401

Nightingale T.D., Pattni K., Hume A.N., Seabra M.C., Cutler D.F. (2009) Rab27a and MyRIP regulate the amount and multimeric state of VWF released from endothelial cells. *Blood* 113(20), p5010-18.

Nightingale T.D., Frayne M.E., Clasper S., Banerji S., Jackson D.G. (2009) A mechanism of sialylation functionally silences the hyaluronan receptor LYVE-1 in lymphatic endothelium. *J. Biol. Chem.* 284(6), p3935-45.

Lui-Roberts W.W., Ferraro F., Nightingale T.D., Cutler D.F. (2008) Aftiphilin and gamma-synergin are required for secretagogue sensitivity of Weibel-Palade bodies in endothelial cells. (2008) *Mol. Biol. Cell.* 19(12): p5072-81.

Metcalf D.J., Nightingale T.D., Zenner H.L., Lui-Roberts W.W., Cutler D.F. Formation and function of Weibel-Palade bodies. (2008) *J. Cell. Sci.* 121(Pt 1): p19-27.

Tharia, H., Nightingale, T.D., Papiz, M., Lawless, A. (1999) Characterisation of hydrophobic peptides by RP-HPLC from different spectral forms of LH2 isolated from *Rps. Palustris. Photosynthesis Research.* 61(2), p157-167.

Managing resources

I co-manage the centre for microvascular research's microscope facility which consists of 3 point scanning confocal microscopes, an Olympus epifluorescent live cell imaging microscope, multiple upright transmitted light microscopes for intravital microscopy as well as a number of tissue culture microsopes. Responsibilities include organising training, upgrades/repairs, administering the microscope budget (from usage costs) and advertising the facility to external users. I helped secure funds for the purchase (Dec 2015) of a Zeiss 800 confocal microscope.

Public Engagement

April-2016: Tour for BHF committee and politicians