ESC First Contact Initiative Grant Report – (Dr Fu Siong Ng, Imperial College London, GB)

Visit details:

Dr Ng visited the laboratory of Prof Igor Efimov at the Department of Biomedical Engineering at Washington University in St. Louis for a total duration of 10 days between 21st April 2011 and 30th April 2011. In addition, during this visit to the United States, Dr Ng also visited Columbia University College of Physicians and Surgeons in New York (3rd May 2011), as well as attending the Heart Rhythm Society (HRS) Scientific Sessions in San Francisco (4th May to 8th May 2011).

Choice of Washington University in St. Louis as the host institution:

Washington University in St. Louis is an internationally renowned private research university, which is home to 12,000 undergraduate and postgraduate students, and has affiliations to 22 Nobel laureates. It recently ranked in the top four private universities receiving funding from the National Institutes of Health (NIH). It is home to the Washington University School of Medicine, and its associated hospitals, Barnes-Jewish Hospital and St. Louis Children's Hospital.

The choice of Washington University as the host institution was influenced in large part by its world-leading reputation in cardiac arrhythmia and electrophysiology research, through its multidisciplinary Cardiac Bioelectricity and Arrhythmia Center (CBAC). Examples of pioneering electrophysiology research at this university include development of the Cox Maze procedure for atrial fibrillation,[1-4] development of non-invasive electrocardiographic imaging (ECGI) [5,6] and leading work on computational modeling of arrhythmias.[7,8]

The visit was to the laboratory headed by Prof Igor Efimov, a professor of Bioengineering. Prof Efimov's laboratory has an international reputation as a leading optical mapping laboratory and has recently published extensively on this technique.[9-13] By developing an excellent collaborative relationship with the transplant surgeons at Barnes-Jewish Hospital and the Mid-America Transplant services, Prof Efimov's laboratory has established the technology and logistics for successful harvesting of human hearts for physiological and functional studies, and as of 2010, has so far studied a total of 113 human hearts.[14] The combination of optical imaging expertise and availability of explanted human hearts for physiological studies makes this the ideal location to carry out optical mapping of the infarct border zones of explanted failing human hearts, and was the main reason why this lab was chosen for this visit.

Research activities during visit:

During the visit, Dr Ng observed and participated in several experiments, including the following:

(1) Optical mapping of explanted human hearts

Experiments using animal disease models have improved our understanding of the electrophysiological remodelling changes that occur in the context of heart failure. However, recent results from optical mapping of human hearts have revealed opposite findings to that from animal models. For example, human optical mapping studies reveal a reduction in transmural dispersion of repolarization,[11] as opposed to an exaggeration in transmural dispersion seen in animal studies.[15]

These results highlight the importance in studying remodelling changes in human myocardium in addition to animal model studies.

Dr Ng participated in the optical mapping of a human left ventricular wedge as described below. In summary, explanted hearts were be immediately perfused through the aorta with a cardioplegic solution and cooled to 4 to 7°C in the operating room. The cardioplegic perfusion washed out blood from the coronary circulation and protected the heart during the subsequent period of wedge isolation. The arrested heart was maintained at 4 to 7°C to preserve tissue during the 15 to 20 minutes delivery from the operating room to the research laboratory. Once at the research laboratory, segments/wedges of human left ventricular wall were isolated for optical imaging. Each wedge contained a section of coronary artery, which was cannulated with a custom-made flexible plastic cannula, and any major arterial leaks were ligated with silk sutures. The wedge was perfused with oxygenated Tyrodes solution, maintaining an arterial pressure of 60-70mmHg. The preparation will also be fully immersed in the perfusion efflux, which assured appropriate superfusion.

Optical mapping of transmembrane voltage (V_m) and calcium transients (CaT) was performed to assess the electrophysiological properties of the heart. The well-established optical mapping protocols of Prof Efimov's laboratory was used.[9-13] After 20 to 30 minutes of washout of cardioplegic solution, gradual warming to 37°C, tissue recovery, and stabilization, the wedge was stained with RH237 (10 to 20µl of a 1mg/ml solution in dimethyl sulfoxide, DMSO), a membrane potential-sensitive fluorescent dye, and Rhod-2-AM, an intracellular calcium indicator (0.2 mg in 0.2 ml DMSO). The left ventricular preparation was immobilized with 10µmol/L blebbistatin (Tocris Bioscience, Ellisville, MO), which is a Myosin II-ATPase inhibitor and thus inhibits cardiac contraction without any effect on action potentials.[16] An optical mapping system with a 100x100 pixels resolution MiCAM Ultima-L CMOS camera (SciMedia) was used to collect fluoresecent signals. Optical action potentials (APs) will be recorded from the optical field of view (20x20 to 30x30 mm^2) with a spatial resolution of 200 to 300 µm/pixel at a rate of 1000 frames/sec.

(2) Testing of new long-wavelength fluorescent dyes

One of the disadvantages of optical mapping as an experimental tool is that the optical signals measured are thought to arise from the superficial layer of cells in the subepicardium.[17] This limits the use of optical mapping as a tool to study the deeper midmyocardial and subendocardial layers in intact hearts. Newer long-wavelength dyes are thought to have deeper tissue penetration and may be used to study properties of the subendocardial myocardium. [18]

Dr Ng participated in experiments assessing the property of new long-wavelength dyes in explanted mouse hearts. The optical mapping protocol described above was used. The properties of a long-wavelength dye (PGH-1) [19] were studied using dual voltage and calcium mapping.

Skills learnt/observed during visit:

1. Dual optical mapping of transmembrane voltage (V_m) and calcium transients
2. Panoramic optical mapping of transmembrane voltage (V_m)
3. Taqman experiments/assays to measure gene and protein expression
4. Optical mapping of explanted human hearts

Outcome of visit (Post-doctoral research at Washington University Oct 2011 - Oct 2012):

As a result of this visit, Dr Ng now plans to spend a year doing post-doctoral research at Washington University between October 2011 and October 2012. A travel fellowship application has been submitted to the British Heart Foundation (BHF) to fund this post-doctoral. The following is a brief abstract of the submitted fellowship proposal:

"Post-infarction ventricular arrhythmias account for a substantial proportion of the 100,000 sudden cardiac deaths annually in the UK. The current strategy of implanting ICDs to abort these arrhythmias does not address the cause and patient selection lacks specificity. The ability to accurately identify patients susceptible to these arrhythmias is poor due to the lack of detailed characterisation of the arrhythmogenic features of human infarcts. To address the hypothesis that there are specific identifiable differences between arrhythmic and non-arrhythmic infarcts, we plan to study infarcted human hearts explanted at the time of transplant surgery, with *in situ* electrophysiological studies and epicardial mapping, *ex vivo* programmed stimulation and optical mapping of transmembrane voltage (Vm) and calcium transients, gene/protein expression studies, and histomorphometry of the infarct border zone. We will compare arrhythmic vs. non-arrhythmic hearts and regions of infarct border zones to characterize and identify infarcts/patients that are prone to ventricular tachyarrhythmias."

Original Hypotheses of planned post-doctoral research

1. There are identifiable differences between arrhythmic and non-arrhythmic **human** hearts, with regards infarct border zone electrophysiology (epicardial electrograms, action potential morphology, conduction), calcium handling, gene and protein expression, and structural morphology.
2. There are identifiable differences between the arrhythmic substrates and infarct border zones of chronic **reperfused** and **non-reperfused** infarcts, with regards myocardial conduction, action potentials, calcium handling, gene and protein expression, and border zone structural morphology.

The research will aim to address the following questions:

1. What are the electrophysiological differences (both at the infarct border zone and in remote viable myocardium) between **arrhythmic vs. non-arrhythmic** chronically infarcted **human** hearts?
2. What are the differences in patterns of gene and protein expression in arrhythmic vs. non-arrhythmic chronically infarcted **human** hearts?
3. What is the functional morphology of the infarct border zone and what are the electrophysiological remodelling changes (ion channels, gap junctions) that underlie the substrate for ventricular arrhythmias in **reperfused** hearts?
4. What is the relationship between VT circuits and infarct substrate in **reperfused** hearts?

Summary:

Dr Ng spent a productive 10-day period at Washington University of St. Louis, funded by the ESC First Contact Initiative Grant. This allowed Dr Ng to observe and participate in research activities in the laboratory, and more importantly, to discuss and evolve research ideas for the future. As a result of this visit, Dr Ng now plans to conduct post-doctoral research at Washington University between Oct 2011 and Oct 2012, and has applied for funding to do so from the British Heart Foundation.

Appendix 1 - References:

1. Cox, J.L., Schuessler, R.B. & Boineau, J.P. The surgical treatment of atrial fibrillation. I. Summary of the current concepts of the mechanisms of atrial flutter and atrial fibrillation. *J Thorac Cardiovasc Surg* **101**, 402-405 (1991).
2. Cox, J.L. et al. The surgical treatment of atrial fibrillation. II. Intraoperative electrophysiologic mapping and description of the electrophysiologic basis of atrial flutter and atrial fibrillation. *J Thorac Cardiovasc Surg* **101**, 406-426 (1991).
3. Cox, J.L. et al. The surgical treatment of atrial fibrillation. III. Development of a definitive surgical procedure. *J Thorac Cardiovasc Surg* **101**, 569-583 (1991).
4. Cox, J.L. The surgical treatment of atrial fibrillation. IV. Surgical technique. *J Thorac Cardiovasc Surg* **101**, 584-592 (1991).
5. Cuculich, P.S. et al. Noninvasive characterization of epicardial activation in humans with diverse atrial fibrillation patterns. *Circulation* **122**, 1364-1372 (2010).
6. Rudy, Y. Noninvasive imaging of cardiac electrophysiology and arrhythmia. *Ann N Y Acad Sci* **1188**, 214-221 (2010).
7. Rudy, Y. Cardiac repolarization: insights from mathematical modeling and electrocardiographic imaging (ECGI). *Heart Rhythm* **6**, S49-55 (2009).
8. Rudy, Y. & Silva, J.R. Computational biology in the study of cardiac ion channels and cell electrophysiology. *Q. Rev. Biophys.* **39**, 57-116 (2006).
9. Fedorov, V.V. et al. Complex interactions between the sinoatrial node and atrium during reentrant arrhythmias in the canine heart. *Circulation* **122**, 782-789 (2010).
10. Fedorov, V.V. et al. Optical mapping of the isolated coronary-perfused human sinus node. *J Am Coll Cardiol* **56**, 1386-1394 (2010).
11. Glukhov, A.V. et al. Transmural dispersion of repolarization in failing and nonfailing human ventricle. *Circ Res* **106**, 981-991 (2010).
12. Li, W., Ripplinger, C.M., Lou, Q. & Efimov, I.R. Multiple monophasic shocks improve electrotherapy of ventricular tachycardia in a rabbit model of chronic infarction. *Heart Rhythm* **6**, 1020-1027 (2009).
13. Ripplinger, C.M., Lou, Q., Li, W., Hadley, J. & Efimov, I.R. Panoramic imaging reveals basic mechanisms of induction and termination of ventricular tachycardia in rabbit heart with chronic infarction: implications for low-voltage cardioversion. *Heart Rhythm* **6**, 87-97 (2009).
14. Efimov, I.R. et al. Multiscale imaging of the human heart: Building the foundation for human systems physiology and translational medicine. *Conf Proc IEEE Eng Med Biol Soc* **1**, 5177-5180 (2010).
15. Akar, F.G. & Rosenbaum, D.S. Transmural electrophysiological heterogeneities underlying arrhythmogenesis in heart failure. *Circ Res* **93**, 638-645 (2003).
16. Fedorov, V. et al. Application of blebbistatin as an excitation-contraction uncoupler for electrophysiologic study of rat and rabbit hearts. *Heart Rhythm* **4**, 619-626 (2007).
17. Efimov, I.R., Nikolski, V.P. & Salama, G. Optical imaging of the heart. *Circ Res* **95**, 21-33 (2004).
18. Walton, R.D. et al. Dual excitation wavelength epifluorescence imaging of transmural electrophysiological properties in intact hearts. *Heart rhythm : the official journal of the Heart Rhythm Society* (2010).doi:10.1016/j.hrthm.2010.08.019
19. Salama, G. et al. Properties of new, long-wavelength, voltage-sensitive dyes in the heart. *J Membr Biol* **208**, 125-140 (2005).