Institution: University of Warwick

Unit of Assessment: A5 - Biological Sciences

Title of case study: Microelectrode Biosensors to Monitor Blood Levels of Physiologically Important Molecules

1. Summary of the impact

For stroke patients and any patient undergoing surgery the time period from diagnosis to treatment is a major factor in clinical outcomes. Research carried out at the University of Warwick has led to the development of sensors that can be used to measure, in whole unprocessed blood, diagnostically useful analytes that can be used to select the best therapeutic treatments. Point-of-care diagnosis and prompt referral to an appropriate care pathway, facilitated by the use of biosensors, will result in efficiency savings for healthcare professionals and the NHS in the long-term, and will also improve patient outcomes. To commercialize these biosensors, Sarissa Biomedical Ltd was founded in 2002, as a UK-based spinout from the University of Warwick. Sarissa sells, around the world, microelectrode biosensors fabricated by a unique enzyme deposition technology protected by patents filed in 2004 and 2008 by the University of Warwick. The diagnostic sensors are based on technology that incorporates Ruthenium Purple and use a sol-gel coating to entrap enzymes on a microelectrode. Sarissa is pursuing human trials of its biosensors as diagnostic tools in two main areas: stroke, and trauma with associated sepsis.

2. Underpinning research

The ability to measure in real-time the production and release of purine-based signalling agents has many potential advantages both for researchers and for clinicians as these agents are ubiquitous signalling agents involved in many important physiological processes, and are also rapidly-released indicators of metabolic stress and are thus relevant to stroke, sepsis and trauma. Although Professor Nicholas Dale invented an adenosine biosensor in 1997, this was large and had limited overall applicability in neuroscience and physiology. Subsequent collaborative research with Dr Bruno Frenguelli, then of University of Dundee, and Professor Michael Spyer, UCL, served as a driver for Dale to invent methods to miniaturize the adenosine biosensor. The first *microelectrode* biosensor relied on the electrodeposition of pyrrole derivatives onto the microelectrode to entrap enzymes[1]. Although this was a significant improvement over the initial biosensor, fabrication of these biosensors was unreliable and time consuming. In 2003, Dale and his colleagues invented a completely novel enzyme-deposition method based on electrodeposition of silicate sol gels (patent WO2004048603) – this was intrinsically reliable, very rapid, used off-the-shelf components, enabled production of the most sensitive microelectrode biosensors to date, and was compatible with a wide range of enzymes, enabling production of the first microelectrode biosensor for adenosine triphosphate (ATP)[2], a key energy/signalling molecule used throughout the body. These methods made it commercially feasible to set up a spin-out company, Sarissa Biomedical Ltd, and to market microelectrodes around the world. Microelectrode biosensors for other clinically important molecules that reflect brain function and metabolic state (glutamate (2009), lactate (2010), glucose (2010), acetylcholine (2006) and D-serine (2011)) soon followed.

Both the adenosine and ATP biosensors proved very useful in elucidating fundamental mechanisms; in neuroscience, for example, they were used to demonstrate the key role of ATP release in CO_2 chemosensing in the brain (a vital life-preserving function)[4], and to measure the release of purines and ATP during cerebral ischaemia[5]. The biosensors also have wider research applications beyond neuroscience, including measuring ATP release in developing systems such as the retina, where it triggers proliferation of retinal stem cells[6]. These highly cited, and important studies, demonstrated the key analytical value of real time biosensing *in vivo* and *in vitro* and thus contributed to the wider adoption of these biosensors by other researchers (creating sales for Sarissa) and an underpinning research base that has led to adoption of these technologies by clinicians. Since 2008, 42 scientific papers have been published, which report data obtained by customers with Sarissa's biosensors.

A number of clinical diagnostic applications were apparent, especially in the field of rapid diagnosis of stroke, transient ischaemic attacks, traumatic brain injury, and sepsis. However the

microelectrode biosensors that we had developed by 2005 were not sufficiently selective to allow the detection of analytes in blood. This is because the specific analytes are present at concentrations some 100-1000 fold less than those of potential electrochemical interferences that could confound any specific biosensor measurements. The next big breakthrough was to produce a mediated biosensor that would allow the specific and selective detection of H_2O_2 (the final product of the enzyme cascades required for analyte detection). Inspiration was taken from the superb selectivity that the mediator Prussian Blue (PB) could impart to electrochemical oxidase-based sensors. PB has a major flaw for clinical diagnostic applications, as it cannot tolerate the presence of Na^+ ions, which are present at high concentrations under physiological conditions. These Na^+ ions destroy PB's catalytic activity towards H_2O_2. The team therefore investigated the possibility of using Ruthenium Purple (RP), which offers similar electrocatalytic properties towards H_2O_2 but tolerates the presence of Na^+ ions. In 2005-2007 Dale and colleagues developed methods for depositing RP on microelectrodes, stabilizing the RP film, and using the same silicate sol-gel methods to add enzymatic layers on top[3,8]. This work resulted in biosensors that had superb selectivity and could measure purines in whole blood, at sub-micromolar levels sufficient for diagnostic applications in stroke, trauma and sepsis. Collaborations in 2007 with Dr Alex Doney (Ninewells Hospital, Dundee) and in 2012-2013 with Prof Chris Imray (University Hospitals of Coventry & Warwickshire) showed that these biosensors could detect purines in unprocessed blood and have detected purines in arterial blood that are rapidly released from the human brain during operations that cause mild brain hypoxia.

Key People:
Prof. Nicholas Dale, Ted Pridgeon Professor of Neuroscience, University of Warwick, 2000 - present: Founder of Sarissa, inventor of sol-gel methods
Dr E. Llaudet, Research Assistant and co-Founder of Sarissa, co-inventor of sol-gel methods, University of Warwick, 2000-2006
Dr F. Tian, Postdoctoral Research Assistant, Senior Scientist in Sarissa and principle inventor of RP sensors, University of Warwick 2004 - present

3. References to the research

Development of microelectrode biosensors for purines – key peer-reviewed publications: Research carried out from 2001 to present day.

1. Llaudet *et al.* (2003). A three-enzyme microelectrode sensor for detecting purine release from central nervous system. Biosens. Bioelectron. 18, 43-52. *DOI: 10.1016/S0956-5663(02)00106-9*
2. Llaudet *et al.* (2005) Microelectrode biosensor for real-time measurement of ATP in biological tissue. Anal Chem 77, 3267-3273. *DOI 10.1021/ac048106q*
3. Tian *et al.* (2007) Ruthenium Purple-mediated microelectrode biosensors based on sol-gel film. Anal Chem 79, 6760-6766. *DOI: 10.1021/ac070822f*

Key initial peer-reviewed publications describing applications of purine biosensors:

4. Gourine *et al.* (2005) ATP is mediator of chemosensory transduction in the central nervous system. Nature 436, 108-111. *DOI: 10.1038/nature03690*
5. Frenguelli *et al.* (2007). Temporal and mechanistic dissociation of ATP and adenosine release during ischemia in the mammalian hippocampus. J Neurochem 101, 1400-1413. *DOI: 10.1111/j.1471-4159.2006.04425.x*
6. Pearson et al. (2005) ATP released via gap junction hemichannels from the pigment epithelium regulates neural retina progenitor proliferation. Neuron 46, 731-744. *DOI: 10.1016/j.neuron.2005.04.024*

The high citations of these papers reflects the significance of the research and its impact in the field.

Patents granted or applied for

7. EP1565565 B1: Method of Producing Sol-gels and Sol-gel Biosensors, granted 2009. (Arising from ref 2; Inventors - Dale, Llaudet, Droniou)
http://www.google.com/patents/EP1565565A2?cl=en
8. EP2126107 B1: Ruthenium Purple Biosensor, granted 2010. (Arising from ref 3; Inventors - Tian, Dale)
9. US Patent 8417314: Ruthenium Purple Biosensor, granted 2013. (Arising from ref 3; Inventors

- Tian, Dale) http://www.google.com/patents/US8417314
10. UK application GB1222074.5: Biosensor devices, filed 7th December 2012, owned by Sarissa Biomedical Ltd. http://www.ipo.gov.uk/p-ipsum/Case/ApplicationNumber/GB1222074.5

Original peer-reviewed funding:

- The Cunningham Trust (2000-2001) Creation of an ultraminiature adenosine sensor. £34k, PI: Dale
- The Wellcome Trust Technology Development Grant (2001-2004) Development and use of a microdagger array for measurement of purines and glutamate in real time during neural activity; Grant reference 065507/Z/01/Z; Amount awarded £285,995. PI: Dale
- The Wellcome Trust Project Grant (2003-2006) Commercialization of purine biosensors: essential tools for the scientific and clinical communities; Grant reference 073027/Z/03/Z; Amount awarded £249,200. PI: Dale

4. Details of the impact

Impacts on commerce:

Sarissa was founded in 2002 as a spinout from the University of Warwick, and began commercial operations in 2004. The underpinning research has led to 2 patent families being awarded to the University of Warwick[7-9] and licensed to Sarissa. In 2012, Sarissa applied for its first patent to cover biosensor arrays (SMARTCap) for diagnostic use[10]. Sarissa's biosensors have international commercial impact as evidenced by US Investigators who have used Sarissa's biosensors to provide underpinning evidence in three US patent applications: US20100284984, US20100165634, WO2013049725. In addition to investment income from two venture capital sources[A], Sarissa has raised grants from Advantage West Midlands 2010 to develop a prototype clinical device for measuring analytes in whole blood – the SMARTCap; and was an SME partner in an FP6 consortium called SANTS (2006-2009, http://www.sants-nanosilicates.com/) providing its expertise, exemplified in refs 2,3,7-9, in silicate fabrication methods to the consortium. Further successful grant funding to develop and evaluate, in substantial clinical trials, new clinical devices based on the IP generated from the underlying research has followed. In 2013, Dale and Sarissa were the lead applicants in a successful bid to the National Institute of Health Research (NIHR) for an Invention for Innovation (i4i) Product Development Award for £575k entitled "SMARTChip: a field-deployable blood test for stroke, capable of detecting brain ischaemia from the earliest stages of pathology".

Employment: Sarissa has continually employed at least one person since 2004 and more during externally funded special projects. In addition, it employs external UK-based consultants for accountancy and business development, creating a total of 13.3 FTE since 2004.

Economy: The company had a turnover of £83k in 2012, up from £62k in 2008, generated mainly from sales into the preclinical scientific communities[B].

Business sector: Sarissa is currently working with scientists and clinicians to investigate and promote the adoption of their biosensor technology for diagnostic purposes. They provide workshops with hands-on demonstrations, external demonstrators (from Europe and USA) and speakers (from UK and USA) to inform customers and potential users about the technology. The fourth workshop was held April 15th 2013. All workshops have attracted delegates from the UK and overseas (Europe, Canada, US), including researchers and representatives from the pharma industry, as well as other biotech SMEs interested in the technology. The workshops provide excellent networking, marketing opportunities and have resulted in new customers adopting the biosensors, including customers from the Pharma sector.

The current product range is the most complete in the world and provides microelectrode biosensors for ten different analytes. Sarissa sells to researchers in the USA, Canada, Japan, Europe, and the UK. These buyers include research groups in universities and increasing sales (10% of total sales in 2012) into non-academic sectors: the pharma industry and US government agencies (FDA)[B].

Impacts on healthcare, practitioners and patient services: Arising from the characterization of the RP biosensors[3,8], and the use of biosensors to measure release of purines during ischaemia[5], Dale and Sarissa are the lead investigators, in collaboration with two NHS Trusts (University Hospitals of Coventry & Warwickshire (UHCW), and University Hospital of North Staffs (UHNS)), in the first clinical trials of the RP-coated purine biosensors for the diagnosis of stroke (funded by

NIHR). This area of innovation is seen as a key area for the NHS, as stroke costs the NHS some £7bn annually, and there are roughly 150,000 stroke incidents per year. The technology invented by Sarissa provides an extremely rapid measurement (minutes) of an analyte (the purines) that is produced within minutes of the onset of stroke symptoms. This test has the prospect of greatly speeding diagnosis, enabling more patients to receive thrombolysis and reducing time from diagnosis to onset of treatment. Every minute saved by Sarissa's technology will improve patient outcomes and reduce the annual cost of stroke treatment to the NHS; even modest improvements resulting from application of Sarissa's technology would give very substantial absolute savings. A clinician from UHNS verified that "*This technology has the potential to improve treatment rates and outcome for stroke patients nationally and worldwide. This has been recognised by the Acute Clinical Studies Group of the Stroke Research Network, who encouraged and supported Professor Dale to develop this into the now successful application for an i4i grant".*[C]

The development and characterization of the RP biosensors[3,7-10] has led to adoption of this technology by clinicians in patient research. Prof Chris Imray, a Consultant Vascular Surgeon at UHCW has been using Sarissa's biosensors during surgery to monitor brain ischaemia (2012-2013). Very exciting results suggest that the preoperative level of blood purines may allow better prioritization of patients at risk of stroke and transient ischaemic attack. Dr Gareth Ackland, a Consultant Anaesthetist and Research Fellow from University College Hospital, London[D], has used Sarissa's biosensors with blood samples from patients suffering from sepsis and surgical trauma (2013). His collaboration with Sarissa is developing proof-of-principle evidence that underpins new IP jointly created with UCL to develop bedside diagnostics of sepsis and trauma, to be exclusively licenced to Sarissa. *"Dale's work has enabled the development of novel modes of detection of inflammation and how this may be managed clinically in real-time"*[D]. The potential market for sepsis/trauma diagnostics is probably an order of magnitude bigger than the stroke market.

Industrial collaborations: A collaboration agreement is in place with Pinnacle Technologies Inc, USA[E], in which Sarissa uses its methods to coat their sensor assemblies. This has allowed Pinnacle to offer a wider range of biosensors, and Sarissa to offer biosensors in a format suitable for chronic *in vivo* recordings. Approximately 10% of Sarissa's product sales now involve biosensors made on the Pinnacle Technologies' assemblies.

Between 2004 and 2010, Sarissa provided business to Sycopel International Ltd by subcontracting them to make microelectrode biosensor assemblies, which Sarissa then coated with a sol-gel biolayer to make a fully functional biosensor. Sycopel also generated further sales through manufacture, to our specification, of a dual potentiostat, an essential instrument required for making the biosensor measurements. This provided to Sarissa's customers suitable instrumentation for use with the microelectrode biosensors and assisted dissemination and uptake of the technology. In 2010, Sarissa bought the rights from Sycopel to take fabrication of biosensor assemblies in-house, using their jigs and methods.

Collaboration is now underway with another SME, Whistonbrook Technologies[F]. Whistonbrook is subcontracted by Sarissa to develop two instruments for clinical use in the NIHR i4i project: one for the SMARTCap device and a second instrument for use with the field-deployable SMARTChip device. In addition Whistonbrook has designed a further dual potentiostat for the non-clinical customers who use Sarissa's sensors. These subcontracts support the development costs of the instruments and will ultimately give Whistonbrook further sales.

5. Sources to corroborate the impact

A. **Investment income into Sarissa:** 2004: £250k from Midven and Mercia and £249k from Wellcome Trust, which is a shareholder in Sarissa; 2010: £170k from Mercia.
B. **Sales figures:** Throughout the world (UK, Europe, Japan, USA, Canada) to Universities, Pharma, the FDA (USA): Available on request.
C. **Letter of Support: Professor of Stroke Medicine**, University Hospital of North Staffordshire NHS Trust (Identifier 1).
D. **Letter of Support: Clinical Scientist**, University College London, (Identifier 2)
E. **Letter of support: President & CEO**, Pinnacle Technology Ltd. (Identifier 3).
F. **Letter of support: Technical Director**, Whistonbrook Technology Ltd. (Identifier 4).