Institution: Durham University

Unit of Assessment: UoA5

Title of case study: Enabling Technology to Improve Cell Growth and Function In Vitro

1. Summary of the impact (indicative maximum 100 words)

3D Scaffold materials and synthetic retinoids for application in animal cell biology developed at Durham University have been commercialized by Reinnervate, a Durham spinout company, using a patent/licensing strategy. Reinnervate has raised £8m venture capital investment and has employed up to 25 people since 2008. Polystyrene-based highly porous polymers which act as 3D in vitro cell culture scaffolds were launched as Alvetex® in November 2010 and a retinoid derivative, designed to control cellular development including stem cell differentiation down neural pathways, was launched as ec23. Alvetex® was voted one of 'The Scientist' magazine's Top 10 Life Science Innovations of 2010.

2. Underpinning research (indicative maximum 500 words)

Initial BBSRC-funded research by Professor Stefan Przyborski and his group (2001-2004) led to the creation of stem cell lines (Przyborski, 2001, Stem Cells; patent PCT/GB2003/003396) and their subsequent differentiation into neural derivatives (Stewart et al. 2003, Stem Cells; Horrocks et al. BBRC, 2003; patent PCT/GB2003/003395). Methodology for the induction of stem cell differentiation and subsequent neural development was subsequently improved through the development of synthetic retinoids developed in collaboration with Professors Whiting and Marder (Dept. of Chemistry, Durham University). These retinoid agonists showed enhanced chemical stability and potency, and decreased variation in the biological response as well enhancing neural development by human stem cells (Christie et al. 2008, OBC; Christie et al. 2010, JNM). Research into mechanisms controlling stem cell differentiation *in vitro* and the formation of 3D neuroprogenitor aggregates (Horrocks et al. BBRC, 2003) and 3D teratomas *in vivo* (Cooke et al. 2006, Stem Cells Dev; Przyborski, 2005, Stem Cells) led to the concept of developing technology to enable the study of cultured cells in 3D models.

There is strong scientific evidence demonstrating that cell growth, differentiation and function in 3D models are significantly enhanced over existing 2D culture systems, providing more accurate information on cell behaviour (Schutte et al. 2011, ADDT). Professor Przyborski established a collaboration with materials scientist Professor Cameron (Department of Chemistry, Durham University) that capitalised on a longstanding existing technology that was applied to the needs of cell biology. Initial proof of concept work demonstrated enhanced neural differentiation on basic 3D scaffolds (Hayman et al. 2004, BBRC). Under an EPSRC grant awarded to Professors Przyborski and Cameron (GR/T24043), polystyrene-based scaffold materials were optimised and tailored to provide 3D supports (Bokhari et al. 2007, JMC). Further optimisation was required to enable 3D cell culture that resulted in engineering of these materials into thin membranes (Bokhari et al. 2007, BBRC).

3. References to the research (indicative maximum of six references)

Stewart R., Christie V., Przyborski S.A. (2003). Manipulation of human pluripotent embryonal carcinoma stem cells and the development of neural subtypes. *Stem Cells*, 21, 248-256. (30 cites)

Bokhari, M., Carnachan, R., Przyborski, S.A., Cameron, N.R. (2007). Effect of synthesis parameters on emulsion-templated porous polymer formation and evaluation for 3D cell culture scaffolds. *Journal of Materials Chemistry,* 17, 4088-4094. (28 cites)

Bokhari, M., Carnachan, R., Cameron, N.R., Przyborski, S.A. (2007). Novel cell culture device enabling three-dimensional cell growth and improved cell function. *Biochemical and Biophysical Research Communications*, 354, 1095-1100. (39 cites)

Christie V.B., Barnard, J.H., Bridgens, C.E., Batsanov, A.S., Cartmell, E.B., Collings, J.C., Maltman, D.J., Marder, T.B., Redfern, C.P.F., Przyborski, S.A., Whiting, A.P. (2008). Synthesis and evaluation of synthetic retinoid derivatives as inducers of stem cell differentiation. *Organic and Biomolecular Chemistry*, 6, 3497-3507. (14 cites)

Christie VB, Maltman DJ, Henderson AP, Whiting A, Marder TB, Lako M, Przyborski SA. (2010). Retinoid supplementation of differentiating human neural progenitors and embryonic stem cells leads to enhanced neurogenesis in vitro. *Journal Neuroscience Methods*. 193(2), 239-45. (8 cites)

Schutte, M., Fox, B., Baradez, M., Devonshire, A., Minguez, J., Bokhari, M., Przyborski, S., Marshall, D. (2011). Rat primary hepatocytes show enhanced performance and sensitivity to acetaminophen during three dimensional culture on a polystyrene scaffold designed for routine use. *Assay and Drug Development Technologies*. 9, 475-486. (9 cites)

4. Details of the impact (indicative maximum 750 words)

The impact of this case study is in a) the provision of valuable cell culture technology products and b) the creation and retention of jobs by the company in an area of the UK that suffers from relative economic deprivation. The 2008-13 Economic Strategy for the NE region gives a high priority to addressing the low levels of R&D investment by the private sector, the low skills base and the lack of employment opportunities (see: http://content.durham.gov.uk/PDFRepository/CountyDurhamEconomicStrategy20082013.pdf).

The global Research Market for cell culture is estimated to be worth $600m annually and is growing at 10% a year. It is estimated that 35 million cell culture plates are used each year for 2D cell growth for many applications in basic research, drug discovery and life science sectors wherever scientists need to better understand the growth, function and disease mechanisms of cells *in vivo*.

Reinnervate Limited (Registered 04468747) was founded in 2002 by Professor Przyborski as a vehicle to commercialise research emanating from his laboratory (Stewart et al. 2003, Stem Cells; Bokhari et al. 2007, JMC, BBRC; Christie et al. 2008 OBC, 2010 JNM), to address the need for improved animal cell culture systems. The Company was very successful in raising seedcorn funding (approx. £60k) from a variety of different initiatives including awards from the Regional Development Agency and Department of Trade and Industry, and such funds were used to support R&D and to perform corporate duties. In 2005, Professor Przyborski negotiated a large commercial loan (£0.5M) to expedite the development and translation of the basic research into marketable products, and further funding (ca. £8M) was raised from investors (VCs, high net-worth individuals, Angel funds) to support R&D, IP and marketing.

Patents have been filed on the technologies developed. Durham University originally filed the patent on the small molecules (WO2008025965) that was subsequently assigned to Reinnervate. The molecule ec23® was then developed commercially by Reinnervate Limited and is currently marketed to stem cell scientists, developmental biologists and the like as a research tool to improve yield and reproducibility of neural cell differentiation in vitro and the study of basic developmental mechanisms. Research that resulted in the engineering of the polystyrene-based scaffold materials into thin membranes was subsequently patented (WO200712588) and assigned to Reinnervate. The concept then went through an extensive development and scale-up phase within the Company, resulting in the development of an optimised and proprietary technology for routine 3D cell culture. The patents were assigned to the Company, and in 2009, the Company established its own independent premises outside the University and completed this process in 2010 by acquiring 5000 sq ft of space in the NETPark Incubator, Sedgefield. In 2011, the Company became fully operational and independent of the University. The product has the trade name Alvetex® (Fig. 1) which was launched into the market Nov 2010.

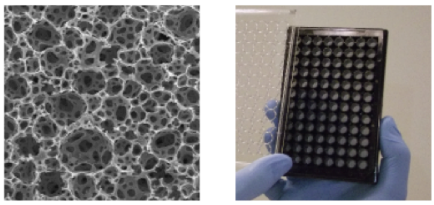

Fig. 1 Scanning electron micrograph of Alvetex® scaffold (left). Example of a 96-well plate product containing Alvetex® 3D culture technology (right).

By 2012 the business employed 27 personnel at NetPark, a further 9 at Durham University and had an experienced management team consisting of a CEO,CSO,CFO, Production Director, Commercial Director, and Marketing and Product Development managers, as well as a Board headed by a Chairman with experience in the life science sector. Many of the support staff are recruited locally from NE England, creating a source of employment and support for the local economy.

Since the launch of the first scaffold, several other formats of the technology such as a 12-well, 24-well and 96-well culture plate, 6-well and 12-well inserts, have been introduced to the market. There are currently 18 distributors including FisherThermo Scientific, Generon (UK), Biozol GmbH (Germany), Chemie Brunschwig AG (Switzerland), THP Medical Products (Austria), In Vitro AS (Denmark),

Euroclone (Italy), and Bio Connect (the Benelux region) are contracted to market the products in different marketing territories around the world. An independent assessment by ThermoFisher Scientific in 2009 concluded that the market for Alvetex® to be in the range of $35-$40 million per annum. Early sales are in line with forecasts and currently amount on average to approximately £15k per month for the first two quarters of 2013.

The ultimate beneficiaries of this impact are the end users who buy and use these technologies i.e. the customers, which are the cell biologists and investigators of the scientific community in academic institutes, government labs, hospitals, biotech and the pharmaceutical industry. Whilst some of the technologies are specialised and will benefit discrete groups of scientists (e.g. stem cell differentiation, cancer cell biology), other products are more generic, and will have far greater penetration and use (e.g. 3D cell culture – applicable to almost any cell biologist currently practising conventional 2D culture techniques). Improving the growth, differentiation and function of cultured cells will have multiple advantages, including decreasing R&D costs, reducing animal usage, improving the predictive accuracy during compound development, and advancing basic research.

Prof. Przyborski was a Finalist in BBSRC's Innovator of the Year competition (2013) for his work with ReInnervate, and a BBSRC Impact study confirms that 'Reinnervate now has more than 1,500 customers around the world, including cell biologists and other researchers in academic institutions, government labs, hospitals and the biotech and pharmaceutical industries, making it a leading company in the 3D cell-culture market. The company's technologies have several applications in life sciences, including stem cell research and tissue engineering in vitro, cancer cell biology, liver toxicology, models of human skin, as well as drug discovery and product development in the academic, biotechnology and pharmaceutical sectors.' In July 2013 Reinnervate announced that a group from Massachusetts General Hospital had chosen Alvetex Scaffold for the first ever 3D osteocyctic cell culture experiments in microgravity. NASA and NIH are funding the study, on the international space station in late 2014, to understand more about bone loss during space flight (see: http://reinnervate.com/alvetex-chosen-for-study-of-bone-loss-during-space-flightmassachusetts-general-hospital-bone-loss-during-space-flight/ and http://www.bbsrc.ac.uk/news/research-technologies/2013/130911-n-technology-set-for-space-experiments.aspx).

The Durham scientists involved in the Reinnervate project were awarded the RSC Rita and John Cornforth award in 2012 recognising the excellence in inter-disciplinary work, at the boundaries of chemistry and biology, to develop Alvetex® Scaffold for three dimensional (3D) cell culture. Furthermore, Alvetex® won an R&D 100 Award in June 2011, identifying it as among the top 100 most technologically significant products introduced into the marketplace over the past year. Alvetex® was named among the winners of The Scientist magazine's Top 10 Life Science Innovations of 2010 with the judging panel commenting that 'Alvetex® Scaffold is an example of innovation to move us closer to better models for mimicking in vivo behaviour of cells with the control offered by in vitro conditions' (Northwestern University Chicago, USA) and 'Alvetex® Scaffold should enable the routine and reproducible creation of 3D cell cultures in the laboratory and extend the concept of 3D culture beyond simple, reconstituted extracellular matrices to complex cellular structures (Environmental Molecular Sciences Laboratory, Richland WA, USA).

5. Sources to corroborate the impact (indicative maximum of 10 references)

www.reinnervate.com - Corporate website displaying information about products, distributors, under-pinning science, technical support, testimonials, publications, news articles, etc.

Publication of intellectual property (Example 1: WO200712588 "Substrate for growth of cultured cell in three dimensions"; international publication date 08/11/2007; Przyborski S; Cameron N; Example 2: WO2008025965 "Retinoid compounds and their use"; international publication data 06/03/2008 Przyborski S; Whiting A; Marder T).

Creation of an independent corporate body Reinnervate Limited - Registered number Companies House 04468747; Registered address: NETPark Incubator, Thomas Wright Way, Sedgefield, TS21 3FD. Corporate headquarters: 5000sq.ft facility, comprising manufacturing suite, clean room, product development laboratory, process development, administration offices, warehousing.

Employment of staff by the company: Chairman: Constantine; CEO: Cooper; CFO: Blain; CSO: Przyborski; Operations Director: Nicklin; Commercial Director: Rowling; Marketing Manager: Lynch;

Product Development scientists: Maltman, Carnachan, Christie, Tholozan, Roger, Mold; Quality Control Manager: Donaghy; Production Manager: Muir; x3 administrators; x5 production operators.

Through a Collaboration Agreement the Company has supported >9 staff within Durham University.
Publication of scientific papers, articles, etc. originating from research associated with the Company
Eg 1) Burkard, A., et al. (2012) *Xenobiotica*, 42, 939-956.
Eg 2) Knight, E., et al. (2011) *Methods in Molecular Biology*, 695, 323-340.
Eg 3) Schutte, M., et al. (2011) *Assay and Drug Development Technologies*, 9, 475-486.

Marketing materials developed by the Company to promote, sell and support products:
general brochures, flyers, webinars on specific applications of technology, videos defining 3D culture and its application, white papers, scientific publications, application notes of specific uses for technology; product protocols for different formats, technical guidance documents and instructional videos; etc. All available via Company website www.reinnervate.com.

International and national awards received in recognition of technology innovation:
R&D Top 100 International Award for the Development of Alvetex® for Enabling 3D Cell Culture as a technologically significant new product (2011); Professor Przyborski received the 2012 Rita and John Cornforth Award for commercialisation of enabling technology by the Royal Society of Chemistry; he was also a Finalist of the 2013 BBSRC Innovator of the Year.

Press releases describing investment in the Company, launch of new products, awards etc.:
on-line news and press release articles; numerous examples of articles in the popular press and magazines; Genetic Engineering News; Screening and Trends in Drug Discovery, etc.

Publication of scientific papers by independent research groups using Alvetex® products:
Eg 1) Pinto, S., et al. (August 2013) *PNAS*, DOI: 10.1073.
Eg 2) Stiles, J.M., et al. (March 2013) *PLoS One*, 8 (3), e60021
Eg 3) Rajan, N., et al. (2011) *Oncogene*, 30, 4243-4260.

Voice of customer quotations directly from beneficiaries and users of Alvetex® products:
a comprehensive list of quotations is available via http://reinnervate.com/alvetex/testimonials/ including collaborators and customers, all of which have used Alvetex® for 3D cell culture.

The Company has a formal exhibition stand which it has displayed at >20 inter/national scientific events and conferences including: American Association for Cancer Research 2013; American Society for Cell Biology 2012, 2013; MipTec Basel 2011; Society for Biomolecular Science 2011; etc. Acting as CSO, Professor Przyborski presents on average 1-2 seminars per month at inter/national locations and events to both the academic and industrial sectors.