Institution: Durham University

Unit of Assessment: 8

Title of case study: Chemical and Materials Technologies for Cell Biology

1. Summary of the impact

3D polyHIPE scaffold materials and synthetic retinoids developed at Durham University for applications in cell biology have been commercialized by Reinnervate, a Durham spin-out company, using a patent/licensing strategy. Reinnervate has raised £8m venture capital investment and has employed an average of 12 FTE staff since 2008, peaking at 27 in 2012. Polystyrene-based highly porous polyHIPE materials which act as 3D *in vitro* cell culture scaffolds were launched under the Alvetex® brand in November 2010 and a retinoid derivative, designed to control cellular development including stem cell differentiation down neural pathways, was launched as ec23®. The products have won several awards and Alvetex® was voted one of "The Scientist" magazine's top 10 Life Science Innovations of 2010.

2. Underpinning research

Interdisciplinary research between Durham University chemists Profs Neil Cameron (DU staff 1997–present), Andy Whiting (DU 2001–present) and Todd Marder (DU 1996–2012) and cell biologist Prof Stefan Przyborski (DU Department of Biological and Biomedical Sciences, returned in UoA 5) began following the creation of the DU Bioactive Chemistry Centre in 2004.

Initial BBSRC-funded research (2001–2004) by Prof. Przyborski led to the creation of stem cell lines and their subsequent differentiation into neural derivatives. This methodology was improved through the application of synthetic retinoids designed in collaboration with Profs Whiting and Marder. Whilst naturally occurring retinoids may be used in a variety of cell differentiation techniques, their inherent instability due to isomerisation in culture media provides opportunities for the use of synthetic retinoids that can control cellular development but also have long term stability. A new class of synthetic retinoids was designed and synthesised at Durham [1] in which an arene carboxylic acid, acetylene and saturated ring replaced the normal retinoic acid system. These retinoids were found to be stable to degradation over extended periods under normal laboratory conditions. The ability of the synthetic retinoids to modulate tissue development, particularly the induction of cell differentiation in a stem cell model of human embryogenesis, was demonstrated [1].

Research into mechanisms controlling stem cell differentiation *in vitro* and the beneficial formation of 3D neuroprogenitor aggregates by the Przyborski group led to the concept of developing technology that would enable the study of cultured cells in 3D models. The shape and function of a cultured cell is significantly affected by the physical environment in which it grows. Cells grown in two dimensions have a large proportion of their surface exposed to the typically flat polystyrene substrate surface and interaction between adjacent cells is therefore localised primarily to their edges. In a 3D scaffold system, however, there is significantly greater scope for cell interaction and signalling which more accurately resembles how cells constitute a tissue, providing far more precise information about cell function. Such *in vitro* assays and models are more effective at modelling the *in vivo* growth of cells. A suitable sterile, inert, solid scaffold would offer several advantages to scientists for 3D cell culture growth including reproducibility, robustness, stability and reduced sample preparation time.

Cameron's research in materials chemistry has a strong focus on the preparation and application of polymers synthesised by high internal phase emulsion (polyHIPE) techniques [2]. Following discussions as part of an internal DU Bioactive Chemistry Centre symposium, Cameron and Przyborski recognised that polyHIPEs offered excellent potential as scaffolds for 3D cell culture since the high porosity of the materials would allow healthy cells to grow within a network of size-controlled pores in the solid polymeric material. Research funded by an EPSRC grant awarded to Professors Przyborski and Cameron (GR/T24043) allowed initial proof of concept research to demonstrate the effectiveness of new 3D polyHIPEs with tailored morphology synthesised by emulsion templating [3,4]. The cells cultured in the pores of the 3D polyHIPE material were not only more functional, but also expressed different proteins which act as markers of neural network development compared to cells grown in conventional 2D culture. Further optimisation enabled polystyrene-based scaffold materials to be tailored to provide improved 3D polyHIPE supports [5] and the engineering of polyHIPE materials into thin membranes [6]. In all cases, cells cultured in 3D were shown to be more functional and more viable than those grown in 2D culture.

3. References to the research

[1] J. H. Barnard, C. E. Bridgens, A. Botsanov, E. B. Cartmell, V. B. Christie, J. C. Collings, T. B. Marder, S. Przyborski, C. P. F. Redfern and A. Whiting, "Synthesis and evaluation of selective stem cell differentiation agents based on synthetic retinoid derivatives", *Org. Biomol. Chem.*, 2008, **6**, 3497-3507. **DOI:** 10.1039/b808574a. **[15 citations]**

[2] A. Barbetta, N.R. Cameron and S.J. Cooper, "High internal phase emulsion (HIPEs) containing divinylbenzene and 4-vinylbenzyl chloride and the morphology of the resulting PolyHIPE materials", *Chem. Commun.*, 2000, 221-222. **DOI:** 10.1039/a909060f. **[74]**

[3] M. W. Hayman, K. H. Smith, N. R. Cameron and S. A. Przyborski, "Enhanced neurite outgrowth by human neurons grown on solid three-dimensional scaffolds", *Biochem. Biophys. Res. Commun.*, 2004, **314**, 483-488. **DOI:** 10.1016/j.bbrc.2003.12.135 **[54]**

[4] R. J. Carnachan, M. Bokhari, S. A. Przyborski and N. R. Cameron, "Tailoring the morphology of emulsion-templated porous polymers", *Soft Matter*, 2006, **2**, 608-616. **DOI:** 10.1039/b603211g. **[68]**

[5] M. Bokhari, R. Carnachan, S.A. Przyborski and N.R. Cameron, "Effect of synthesis parameters on emulsion-templated porous polymer formation and evaluation for 3D cell culture scaffolds", *J. Mater. Chem.,* 2007, **17**, 4088-4094. **DOI:** 10.1039/b707499a. **[28]**

[6] M. Bokhari, R. Carnachan, N.R. Cameron and S.A. Przyborski, "Novel cell culture device enabling three-dimensional cell growth and improved cell function", *Biochem. Biophys. Res. Commun.*, 2007, **354**, 1095-1100. **DOI:** 10.1016/j.bbrc.2007.01.105. **[39]**

This research was funded by various UK research councils including: EPSRC (GR/T24043, 2005-2006); MRC; and 3 × BBSRC and 1 × EPSRC Industrial CASE studentships The quality and international esteem of the research programme is supported by citations of the original papers and a number of invitations to give plenary and keynote lectures at major international conferences (for example, NRC: 32nd Australasian Polymer Symposium, 2011; 23rd European Congress on Biomaterials, 2010; 11th Pacific Polymer Conference, 2009; AW: 1st Annual World Congress on Asymmetric Synthesis, Beijing, 2010; 6th International Conference on Green and Sustainable Chemistry, UK, 2013; SB: Form and Function in Regenerative Medicine, Dublin, 2013; Neurological Disorders, London, 2013).

The Reinnervate team was awarded the 2012 Rita and John Cornforth Award of the Royal Society of Chemistry. This recognises research achievements by scientists working in interdisciplinary collaborative research teams in both the chemistry and life science sectors.

4. Details of the impact

The global research market for cell culture is estimated to be worth $600m annually and is growing at 10% a year. Around 35 million cell culture plates are used each year world-wide for simple 2D cell growth for many applications in basic research, drug discovery and life science sectors; i.e. in all areas where scientists need to better understand growth, function and disease mechanisms of cells *in vivo*.

Durham University spin-out company, Reinnervate Limited [Im1] (UK Company 04468747), was established in 2002 by Pryzborski with the aid of the Durham Business & Innovation Services team as a vehicle to commercialise technology in the bioscience sector and, specifically, to develop enabling technology to improve the growth and function of cultured cells stemming from Durham research in the porous polymer scaffold [3,6] and synthetic retinoid fields [1]. The Company raised seedcorn funding (~£60K) from a variety of different initiatives, including awards from the Regional Development Agency and the Department of Trade and Industry, to support R&D and perform corporate duties. In 2005, a commercial loan (£0.5M) to expedite the development and translation of the basic research into marketable products was raised. A further ~£8M of research funding was raised from investors (Venture Capital funds, high net-worth individuals, Angel funds) to support R&D, IP protection and marketing. In its early stages Reinnvervate was incubated within the Chemistry Department, but by 2009 it had grown to such an extent that it established its own independent premises outside the University. It completed this process in 2010 by acquiring 5000 sq ft of space in the NETPark Incubator, Sedgefield, in which the University has an equity stake. In 2011, the Company became fully operational and independent of the University. 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.

All the products developed and marketed by Reinnervate (ec23® retinoids and the Alvetex® 3D scaffold range) have their origins in DU Chemistry research [1-6].

The stable synthetic retinoids for predictable stem cell differentiation designed, synthesised and described in [1] were patented by DU in 2006 (WO2008025965 “Retinoid compounds and their use”, Przyborski, Whiting and Marder) and the IP rights assigned to Reinnervate. The syntheses of the retinoid compounds were scaled-up and manufactured at High Force Research Ltd (Durham) and are currently marketed through Reinnervate’s commercial partners including Tocris, Enzo Life Sciences, LGC Standards and Amsbio as ec23® [Im2].

The 3D polyHIPE scaffolds derived from porous polystyrene described in references [5] and [6] were patented by DU (WO200712588 “Substrate for growth of cultured cell in three dimensions”, Przyborski & Cameron) and the IP assigned to Reinnervate. An extensive development phase within Reinnervate, involving re-engineering and scale-up of the technology, optimization for 3D cell culture and development of further applications of the 3D scaffolds in bioscience was carried out. This allowed creation of the first Alvetex® products which were launched in November 2010 [Im3]. Since the first scaffold entered the market (see Figure), several other formats of the technology such as a 12-well, 24-well and 96-well culture plate and 6/12-well inserts were introduced. There are currently 18 distributors including ThermoFisher 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) that market the Alvetex® brand in different territories around the world and over 1500 customers world-wide. Growing sales currently amount on average to approximately £15K per month for the first two quarters of 2013.

Figure: porous polystyrene Alvetex® 3D scaffold microstructure (left) and marketed 96-well plate format for cell culture studies (right)

The ultimate beneficiaries of these developments are the life science end users who buy and use the scaffold technology. 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 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 has many advantages, including decreasing R&D costs, reducing animal usage, improving the predictive accuracy during development of drug candidates, and advancing basic research.

A published BBSRC Impact evidence report [Im4] 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.”

Testimonials [Im5] from scientists using the Alvetex® products recorded on the www.reinnervate.com website attest to the effectiveness of the Alvetex® technology for cell biology. These include: "The ability to use Alvetex® Scaffold to create a genuine 3D cell culture enabled us to provide a favourable environment in which our cells could grow, differentiate and proliferate", Professor of Cardiothoracic Surgery, Stanford University, USA; "Oncotest are particularly pleased at the ease of isolation of protein for downstream assays and potential biomarker validation", Head, Throughput Screening Laboratory and Investigator, Oncotest GmbH; "We have been using Alvetex® Scaffold for growing our prostate and colon epithelial cell line [and] have observed some striking differences between 2D and 3D prostate and colon cells surface marker expression", Senior Scientist, Inst. of Biophysics, Czech Republic. In addition, collaborations with various industrial organisations including Tecan, Roslinn Cellab, Medicyte, Kirkstall and Mirus Bio to develop new uses of Alvetex® scaffolds in a variety of healthcare areas are underway.

The Durham scientists involved in the Reinnervate project were awarded the RSC Rita and John Cornforth award in 2012, which recognises excellence in inter-disciplinary research at the boundaries of chemistry and biology, for developing Alvetex®.

Alvetex® has also won numerous technology awards. These include: 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 previous year; and "The Scientist" magazine's Top 10 Life Science Innovations of 2010. The judging panel for the latter award commented 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" (Neil Kelleher, 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" (Steven Wiley, Environmental Molecular Sciences Laboratory, Richland WA, USA) [Im6].

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

[Im1] Company details: Reinnervate Limited (Registered Company No. 04468747), 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. See: http://www.reinnervate.com/.

[Im2] Synthetic retinoids: for additional marketing material see: www.tocris.com/dispprod.php?ItemId=272185#.UYej_oJcSHk, www.enzolifesciences.com/BML-EC23/ec23/ and www.amsbio.co.uk/ec23-synthetic-retinoid-ATRA.aspx.

[Im3] Alvetex®: see http://www.reinnervate.com/ and http://www.fishersci.com/ecomm/servlet/fsproductdetail?storeId=10652&aid=4370711&store=Scientific&segment=lifeScience&WTPromo=%20Spotlight%20Alvetex%20Scaffold.

[Im4] BBSRC Impact evidence reports: http://www.bbsrc.ac.uk/publications/impact/novel-3d-cell-culture-system.aspx.

[Im5] Customer feedback: for quotations directly from beneficiaries and users of Alvetex® products see the comprehensive list at: http://reinnervate.com/alvetex/testimonials/.

[Im6] Technology awards: see information on the web at: http://www.nebusiness.co.uk/business-news/science-and-technology/2011/10/27/bio-tech-firm-reinnervate-wins-major-r-d-award-51140-29669230/, http://classic.the-scientist.com/2010/12/1/47/1/, http://www.biospace.com/news_story.aspx?NewsEntityId=204209&Source=MoreNews and www.reinnervate.com.