Institution: University of Essex

Unit of Assessment: 5 – Biological Sciences

Title of case study: Micro-porous polyethylene: A novel affinity chromatography matrix for the bio-processing industry

1. Summary of the impact

Essex research identified a novel bioprocessing matrix which has since been developed into commercial products and recently launched into external markets by Porvair Filtration Group Ltd. The discovery involved the chemical modification of sintered thermoplastic materials in order to attach biological molecules, so conferring highly specific functionalised properties to an otherwise inert base material. This enabled a new approach for protein immobilisation, having technical and practical advantages over existing processes. As a direct result, Porvair has adopted a new technology and invested £900k in R&D over eight years. Essex research has supported a change in business strategy, enabling entry into new markets, which has in turn both safeguarded and created jobs at Porvair.

2. Underpinning research

In the post-genomic era, the challenge to molecular biology is to understand the function of proteins involved in genome regulation. Studying these proteins in the context of tumourigenesis (the initiation of cancerous tumours) has been an important area of research at Essex for a number of years. Proteins are difficult to study using high-throughput automated processes, due to the wide variation in their properties and interactions with other proteins. Thus protein immobilisation is used as a key enabler and integral part of research, to allow for their analysis and biotechnological exploitation. By the late nineties, there was an extensive range of matrices available for protein immobilisation and purification. However, two key technical challenges remained to be overcome:

(a) Reducing the high non-specific binding properties of commercially available, bead-based matrices for protein immobilisation (such as polysaccharide-based agarose and Sepharose) in order to improve selectivity and specificity, and;

(b) Developing more consistent and convenient experimental configurations, to improve ease of handling and use.

In 2000 Dr Igor Chernukhin, then based at the University of Oxford, was optimising some biochemical protocols when he tried to use solid matrices instead of bead-based matrices. Working alongside Dr Elena Klenova, he found that, after chemical modification, porous discs which were commonly used as filters to protect micropipette tips from aerosol contamination provided a superb matrix for the immobilisation of proteins and peptides. These modified matrices showed improvements in both non-specific background absorption and configuration, and proved to be more efficient and straightforward to use. Subsequently, the filter composition was identified as hydrophobic, porous polyethylene, a material that had not previously been used as a matrix for protein immobilisation. In 2001 Chernukhin (as Senior Research Officer) and Klenova (as Lecturer) relocated to the University of Essex, where they embarked upon an extensive programme of research to advance the initial concept that had been identified. They built upon earlier work, developing the technology into a robust, practical process.

By 2002 Chernukhin and Klenova had advanced their technique to the point where it held commercial potential for application to Chromatin Immunoprecipitation. Dr Paul Thomas (then a

Business Manager at the University of Essex) helped to identify and establish collaborative links with the industrial partner, Porvair Filtration Group Ltd. This proved to be an important step in developing the project, and over the following years commercial prototype development and validation progressed alongside on-going research in an 'open innovation' scenario. In January 2004, the University of Essex provided £10k to support further refinement of the technology, and from March that year Chernukhin adopted supervision of the newly established departmental Proteomics Facility.

Over the course of 2009-10 Chernukhin and Klenova (who was promoted to Professor of Molecular Oncology in 2010) led the testing of prototype column-shaped matrices in chromatin-immunoprecipitation (ChIP) experiments, to examine the study of protein interaction with DNA. This work was published in 2011 (Chernukhin et al., 2011) and underpinned the 2013 patent *Chromatin Immunoprecipitation Assay* (UK Patent No. GB2482209, 2013). It was also drawn upon in later publications from the Essex research group (Gretton and Klenova, 2012; Méndez-Catalá et al., 2013).

3. References to the research [can be supplied by HEI on request]

Chernukhin, I., S.Y. Kang, S. Brown, S. Gretton, C.F. Méndez-Catalá, D. Cowieson and E. Klenova (2011) BioVyon Protein A, an alternative solid-phase affinity matrix for chromatin immunoprecipitation. *Analytical Biochemistry*. 412, 183–188. DOI:10.1016/j.ab.2011.01.036

Gretton, S. and E. Klenova (2012) Breast Tissue Chromatin Enrichment using Chromatrap® Pro-A 1ml Columns. Application note based on the patented Chromatrap® technology. Available at: http://www.chromatrap.com/uploads/documents/TL0010_-Chromatin-Enrichment-App-Note.pdf [Accessed 21 October 2013]

Chernukhin, I., E. Klenova, D. Cowieson and S.J. Brown (2013) *UK Patent No. GB2482209.* Newport, Wales: Intellectual Property Office. Available at: http://north.patent.gov.uk/p-find-publication-getPDF.pdf?PatentNo=GB2482209&DocType=B&JournalNumber=6450 [Accessed 21 October 2013]

Méndez-Catalá, C.F., S. Gretton, A. Vostrov, E. Pugacheva, D. Farrar, Y. Ito, F. Docquier, G.X. Kita, A. Murrell, V. Lobanenkov and E. Klenova (2013) A novel mechanism for CTCF in the epigenetic regulation of *Bax* in breast cancer cells. *Neoplasia*. 15(8), 898–912. DOI:10.1593/neo.121948

4. Details of the impact

Essex research had, for the first time, shown that chemically modified porous polyethylene could be effectively used for protein immobilisation. Furthermore, its use in bead-based matrices could offer both technical and practical advantages over existing processes. Use of this new technique demonstrated the potential to add value in a wide range of contexts, extending beyond the Essex group's original field of research. Through their early and active engagement with the industrial partner Porvair Filtration Group Ltd, Essex researchers maximised this potential, and enabled research insight to be effectively used to underpin commercial impact.

Porvair is a world-leading specialist filtration and environmental technology group involved in designing, developing and manufacturing filtration and separation solutions to a range of industry sectors, including the aviation, molten metal, energy, water treatment and life sciences markets. Historically, the company had principally manufactured industrial grades of porous polyethylene and polypropylene materials, for applications such as silencers for airlines and low cost filters. However, at the time of initial engagement with Essex, Porvair sought to better differentiate itself from competitors through re-direction of R&D into new areas, where porous materials could be used in higher value applications for the biotechnology and pharmaceuticals sector. Porvair's website explains how, alongside collaborative work with a number of other UK universities, partnership with Essex has allowed the company to become more competitive and to enter these previously unobtainable, growing markets [see corroborating source 1].

Porvair's partnership with Essex stimulated significant investment into product development. An email testimony from Porvair's Head of Bioscience New Product Development details how, between 2004 and 2010, the company committed around £450k towards furthering the novel bioprocessing technology provided by Essex [2]. Development activities resulted in the production and launch of two products for commercial exploitation. Firstly, the porous polyethylene material was re-named BioVyon^{TM} and used to develop a column for protein/antibody purification, launched in 2009 [3]. Secondly, a kit for chromatin-immunoprecipitation (ChIP) assays based on functionalised BioVyon^{TM} – Protein A, and named ChromaTrap^{TM} – was launched at the Epigenomics Congress in Boston in April 2011 [4].

Following the initial launch of the BioVyon^{TM} column, the feedback received indicated that further optimisations were needed, and these are currently being conducted. However, the second product – Chromatrap^{TM} – is being adopted and applied effectively in a range of scenarios [5]. One example is its use at the Medical College of Wisconsin. Here, Chromatrap has been used to assist development of state-of-the-art pediatric healthcare within the Children's Research Institute (CRI). A CRI Professor of Pediatrics, Pharmacology and Toxicology comments that "…the most substantial advantage of the Chromatrap system is more efficient and less variable trapping of the immunoprecipitated chromatin such that the signal to noise ratio is larger and influenced less by minor variations in trapping efficiency" [6].

To support uptake and further development, Porvair have continued to devote significant resources to the project. Following Chromatrap's launch, by the end of 2012 the company had invested an additional £450k, and a further £200k spend is anticipated in 2013 [2]. Throughout this time the University has also continued to benefit from product sales, following a licence agreement under which the University receives a 2.5% royalty on the sale of each kit [7]. Porvair has recently applied for patent protection in the US, Australia, Europe, China, India, Brazil and Japan, and the company's continued commitment to this work has also underpinned a number of additional benefits. The technology that came from Essex has supported three jobs at a business unit in Swansea, as well as several Porvair staff throughout the company who continue to devote a significant amount of work to this initiative. Since 2011 the technology has also been responsible, in part, for a Knowledge Transfer Partnership and a Welsh Government A4B (Academic Expertise for Business) project, both with Swansea University [2]. To build upon the initial success and momentum of its first product in this area, Porvair are also active in investigation of expansions to the product range. Planned developments include gravity flow columns and 96-well plate formats, as well as new chemistry matrices using protein G and MeDIP functionalisation.

5. Sources to corroborate the impact [All sources saved on file with HEI, available on request]

[1] Porvair Filtration Group. *Porous Polymers (Vyon® and BioVyon™)* [online] Available at: http://www.porvairfiltration.com/view/AboutUs/NewProductAndMaterialDevelopment/aboutus-newproductandmaterialdevelopment-porouspolymers(vyon%E2%84%A2andbiovyon%E2%84%A2).aspx [Accessed 30 July 2013]

[2] Head of New Product Development: Bioscience, Porvair Filtration Group

[3] Porvair Sciences, 2009. *Excellent column-to-column consistency enhances IgG antibody purification* [online] Available at: http://www.porvair-sciences.com/news/excellent-column-to-column-consistency-enhances-igg-antibody-purification [Accessed 30 July 2013]

[4] Porvair Filtration Group, 2011. *Chromatrap Chromatin Immunoprecipitation (ChIP) Assay Kit* [online] Available at: http://www.porvairfiltration.com/news/highlysensitivespecificchromatinimmunoprecipitation(chip)assaykit.aspx [Accessed 30 July 2013]

[5] Porvair Sciences, 2011. *Researchers Respond Enthusiastically to Chromatrap® Technology* [online] Available at: http://www.chromatrap.com/news/researchers-respond-enthusiastically-to-chromatrap-technology/ [Accessed 15 April 2013]

[6] Porvair Sciences, 2011. *Chromatrap™ assists development of cutting edge pediatric healthcare* [online] Available at: http://www.chromatrap.com/news/chromatrap-technology-assists-development-of-state-of-the-art-pediatric-healthcare/ [Accessed 15 April 2013]

[7] Royalty Agreement Between University of Essex Enterprises Ltd and Porvair Filtration Group Ltd, 22nd May 2012