Title: Culture of HepG2 liver cells on three dimensional polystyrene scaffolds enhances cell structure and function during toxicological challenge
Author: Maria Bokhari, Ross J. Carnachan, Neil R. Cameron, Stefan A. Przyborski
Overview: Growth of HepG2 liver cells in 3D environment enhances their cell structure and function, producing more morphological features of mammalian liver tissue
Highlights:
• Liver cultures in 3D were more heterogeneous in nature compared to 2D counterparts
• Evidence for development of hepatic structures in Alvetex®Scaffold
• Improved viability and albumin secretion, and reduced cell damage in Alvetex®Scaffold vs 2D
• Cells show enhanced resistance to MTX induced cellular toxicity in Alvetex®Scaffold

Title: Novel cell culture device enabling three-dimensional cell growth and improved cell function
Author: Maria Bokhari, Ross J. Carnachan, Neil R. Cameron, Stefan A. Przyborski
Overview: First publication dedicated to description of Alvetex®Scaffold prototypes to support 3D cell culture using model hepatocyte cell line HepG2.
Highlights:
• Cells cultured in Alvetex®Scaffold showed improved HepG2 culture homogeneity
• Evidence for development of hepatic structures in Alvetex®Scaffold
• Increased metabolic activity and albumin secretion by HepG2 grown in Alvetex®Scaffold

Title: Tailoring the morphology of emulsion-templated porous polymers
Author: Ross J. Carnachan, Maria Bokhari, Stefan A. Przyborski, Neil R. Cameron
Overview: Detailed description of the research into the process by which porous polystyrene scaffolds are made, including a review of the different factors that influence the structure and porosity of the material.
Highlights:
• Description of High Internal Phase Emulsions (HIPE) as a method to produce porous material
• Evidence showing how chemical and physical factors influence the formation of polyHIPE
• Development of polyHIPE technology to create tailored materials for specific applications

Title: Enhanced neurite outgrowth by human neurons grown on solid three-dimensional scaffolds
Author: M.W. Hayman, K.H. Smith, N.R. Cameron, S.A. Przyborski
Overview: Original proof of concept study using early stage prototype polystyrene scaffolds to demonstrate their ability to support the growth of cells during culture.
Highlights:
• Growth of human stem cell derived neurons on early stage polystyrene scaffolds
• Use of environmental scanning electron microscopy to visualise cells
• Extraction of total protein from 3D cultures and subsequent immunoblotting to monitor the expression of neural markers.

Title: Growth of human stem cell-derived neurons on solid three-dimensional polymers
Author: M.W. Hayman, K.H. Smith, N.R. Cameron, S.A. Przyborski
Overview: Original proof of concept study using early stage prototype polystyrene scaffolds to demonstrate their ability to support the growth of cells during culture.
Highlights:
• Growth of human stem cell derived neurons on early stage polystyrene scaffolds
• Use of environmental scanning electron microscopy to visualise cells
• Development of methods to coat scaffolds with poly-D-lysine and laminin
• Evidence showing coated scaffolds promote enhanced cellular differentiation in 3D culture
Overview of Key Alvetex®Scaffold Publications

**Title:** Rat Primary Hepatocytes Show Enhanced Performance and Sensitivity to Acetaminophen During Three-Dimensional Culture on a Polystyrene Scaffold Designed for Routine Use  
**Author:** Maaike Schutte, Bridget Fox, Marc-Olivier Baradez, Alison Devonshire, Jesus Minguez, Maria Bokhari, Stefan Przyborski, and Damian Marshall  
**Overview:** Growth and function of primary rat hepatocytes was assessed on 2D and 3D polystyrene substrates. Cells grown on Alvetex®Scaffold exhibit a natural 3D structure, enhanced induction of metabolic enzyme expression, and differential response to expression of genes associated with phase I, II, and III drug metabolism compared with monolayer culture.

**Highlights:**  
- Alvetex®Scaffold provides a solution for routine 3D culture of primary hepatocytes  
- The viability of primary hepatocytes is significantly enhanced when grown on Alvetex®Scaffold  
- Induced expression of cytochrome p450 expression is radically increased in 3D culture  
- Primary hepatocytes show greater sensitivity to cytotoxin when grown on Alvetex®Scaffold  
- Use of Alvetex®Scaffold to support 3D culture of primary cells from cylindroma tumours  
- Early demonstration of technology used for 3D cell culture. Research and prototyping ultimately lead to the development of Alvetex®Scaffold technology.

**Title:** Dysregulated TRK signalling is a therapeutic target in CYLD defective tumours  
**Author:** N Rajan, R Elliott, O Clewes, A Mackay, J S Reis-Filho, J Burn, J Langtry, M Siebel-Bumm, C J Lord and A Ashworth  
**Journal:** Oncogene, 2011, doi: 10.1038/onc.2011.133  
**Overview:** Analysis of the CYLD mutant tumours showed involvement of tropomyosin kinase (TRK). In vitro models including 3D primary cell cultures established from CYLD mutant tumours, demonstrated the function of TRK in inducing cell proliferation in cylindroma tumours.

**Highlights:**  
- Use of Alvetex®Scaffold to support 3D culture of primary cells from cylindroma tumours  
- Long term 3D culture was performed for 28 days followed by drug treatments  
- 3D cultures were analysed using immunoblotting and histological techniques  
- Demonstration of co-culture and interaction of different cell types in 3D Alvetex®Scaffold culture.

**Title:** Adipose tissue-derived stem cells display a proangiogenic phenotype on 3D scaffolds  
**Author:** Evangelos A. Neofytou, Edwin Chang, Bhagat Patil, Lydia Marie Joubert, Jayakumar Rajadas, Sarvij Gambhir, Zhen Cheng, Robert C. Robbins, Ramn E. Baygui  
**Overview:** Co-culturing ASCs with endothelial cells in a 3D matrix environment enabled the generation of pro-vascularized tissue-engineered constructs, potentially surpassing the tissue thickness limitations faced by the tissue engineering community today.

**Highlights:**  
- Demonstration of co-culture and interaction of different cell types in 3D Alvetex®Scaffold model  
- Extensive analysis of 3D culture model using a variety of alternative methods, including fluorescence imaging, confocal, scanning electron microscopy and biochemical assays  
- Clear evidence of enhanced biological responses in 3D Alvetex®Scaffold culture.

Title: Developments in three-dimensional cell culture technology aimed at improving the accuracy of in vitro analyses  
**Author:** Daniel J. Matman, Stefan A. Przyborski  
**Journal:** Biochemical Society Transactions, 2010, volume 38(4), pp1072-1075  
**Overview:** Review contextualising the requirement for routine 3D cell culture technology

**Highlights:**  
- Validation of reference gene stability for APAP hepatotoxicity studies in different in vitro systems and identification of novel potential toxicity biomarkers  
- Introduction of Alvetex®Scaffold and key benefits  
- Illustrative examples of liver and stem cell growth in Alvetex®Scaffold.

**Title:** Emulsion-templated porous polymers as scaffolds for three dimensional cell culture: effect of synthesis parameters on scaffold formation and homogeneity  
**Author:** Maria Bokhari, Ross J. Carnachan, Stefan A. Przyborski, Neil R. Cameron  
**Overview:** Development of emulsion templated porous polymers based on polystyrene for use in 3D cell culture. Research and prototyping ultimately lead to the development of Alvetex®Scaffold technology.

**Highlights:**  
- In-depth evaluation of synthesis parameters on scaffold formation and homogeneity  
- Preparation of scaffolds for 3D cell culture  
- Early demonstration of technology used for 3D cell culture and growth of M063 osteoblasts  
- Evidence of enhanced bone formation in 3D cultures compared to conventional 2D systems