Alvetex® - Revolutionizing Three-Dimensional Cell Culture Technology

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Statement of Purpose: A novel technology called Alvetex® that provides an excellent environment for routine 3D cell growth was developed from a successful collaboration between Durham University and Reinnervate Ltd. We have developed a thin membrane of polystyrene scaffold (200 µm) with a well defined and uniform porous architecture (1) (Fig 1A and 1B).

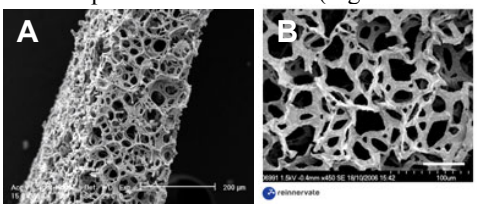

Fig (1):

We have adapted this material specifically for cell culture applications for use in existing culture plates and we have developed this technology from the research laboratory to industrial commercialisation. We have exemplified the application of this technology by growing numerous cell types in 3D including liver tissues (2),(3), bone (4) (osteoblasts) and the development of an artificial skin construct composed of differentiating keratinocytes that mimic the barrier function of the epidermis.

Methods: *Preparation of Alvetex® discs:* This method has been described previously and involves emulsion templating technology (1). *Alvetex® Cell Culture Products:* Alvetex has been developed into a variety of formats standard size 6, 12 (Fig 2A), 24 and 96 well plates and well inserts to fit 6 (Fig 2B), 12 and 24 well plates.

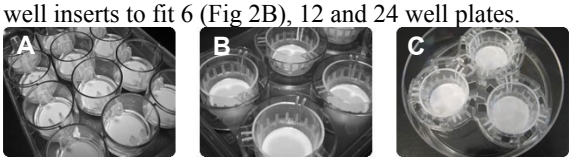

Fig (2):

A cradle housing three 6-well inserts has been developed to fit into a 90mm Petri dish (Fig 2C), in order to accommodate larger media volumes that can support and overcome problems of long term 3D cell culture.

Cell Culture: Populations of human keratinocyte cell line HaCaT cells were initially expanded in 2D plates in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal calf serum (FCS), 100µg/ml streptomycin, 100µg/ml penicillin until they reached 80-100 % confluence. The cells were then detached and seeded on to Alvetex® at a seeding density of $1x10^6$ cells/cm^2, and cultured in keratinocyte specific medium Quantum 153 for 2 days under submerged conditions and then raised to the air/liquid interface and cultured for a further 7-35 days.

Results:

The majority of 3D cell culture products on the market do not demonstrate or overcome the problems of 3D cell culture. Alvetex® demonstrates uniform cell seeding throughout the thickness and diameter of the disc (Fig 3A: Toluidene blue staining of keratinocytes within Alvetex®, Fig 3B: DAPI staining of a 15 mm wide, 200 µm thick section of Alvetex®), which allows high cell loading.

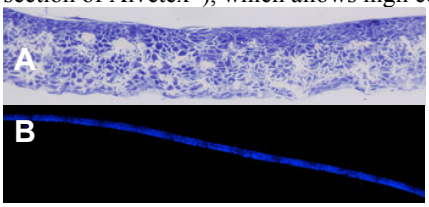

Fig (3):

We have investigated the formation of the epidermal barrier using Alvetex®. There is demand for robust and representative *in vitro* skin constructs that possess barrier function, especially as the use of intact human and animal skin from biopsies or autopsies is limited by availability and regulatory constraints.

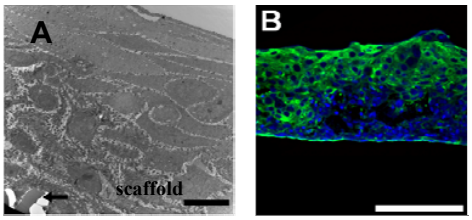

Fig (4):

Keratinocytes cultured on Alvetex® organized themselves and formed structures resembling that of real skin. TEM analysis shows skin cells migrating and differentiating toward the surface of the culture, acquiring keratin, losing their nucleus and undergoing stratification (Fig 4A). This transition from the spinous layer to the larger flattening cells occurs in real skin and ultimately results in the formation of the stratum corneum. Tight junction complexes are differentially regulated during this migration, leading to cells being lost on the surface. Markers of mature keratinocytes such as involucrin are therefore detected in the higher levels of the culture (Fig 4B). These data demonstrate the formation of a robust *in vitro* skin construct that is easy to generate and handle using Alvetex® technology.

Conclusions: Alvetex® has been shown to support routine 3D cell culture. Evidence demonstrates enhanced performance of cells grown in 3D compared to standard 2D cultures. This technology represents an important step to improve *in vitro* assays and *in vitro* tissue formation, and the accuracy and relevance of the data they produce.

References: (1) Carnachan, R. J.; Bokhari, M.; Przyborski, S. A.; Cameron, N. R. *Soft Matter* **2006**, *2*, 608-616; (2) Bokhari, M.; Carnachan, R. J.; Cameron, N. R.; Przyborski, S. A. *Biochemical and Biophysical Research Communications* **2007**, *354*, 1095-1100; (3) Bokhari, M; Carnachan, R. J.; Cameron, N. R.; Przyborski, S. A. *Journal of Anatomy* **2007**, 211, 567-576; (4) Carnachan, R. J.; Bokhari, M; Przyborski, S. A.; Cameron, N. R. *Journal of Materials Chemistry*, **2007**, 17, 4088-4094.