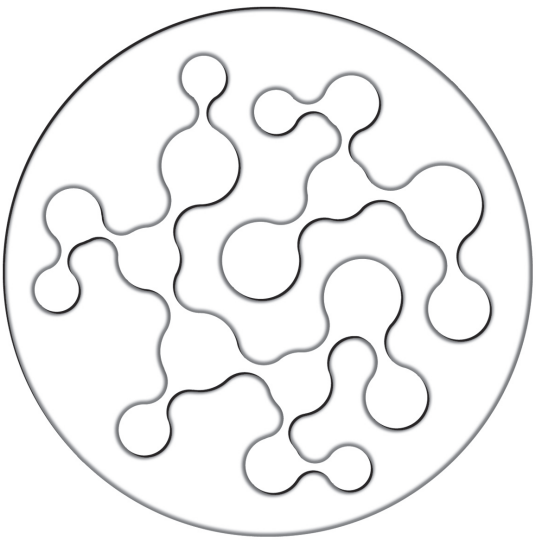


alvetex®

Achieve genuine 3D cell culture
simply and routinely

alvetex®

Achieve genuine three dimensional cell culture simply and routinely

A new dimension to your cell culture research capabilities

- Enables cells to maintain their natural in vivo shape and function
- Accurately mirror in vivo cell environment to improve the accuracy of results
- Understand how cells interact within complex systems
- Enhance cell viability and responsiveness to growth factors and therapeutic agents
- Improve predictive accuracy and enhance measurement of compound safety
- Overcomes the ethical and financial issues by reducing the dependency on animal model testing

Reinnervate's multi award winning technology overcomes the limitations associated with traditional cell culture creating a new dimension for your research. Accurately recreate the natural in vivo cell environment to accelerate your understanding of how cells function and behave.

Recent alvetex® awards

One of the Top 10 Innovations of 2010
The Scientist

2011 R&D 100

Image: Scanning electromicrograph image of alvetex® scaffold

"The ability to use this product in order to create a genuine 3D cell culture enabled us to provide a favourable environment in which our cells could grow, differentiate and proliferate. Using alvetex® to culture adipose stem cells enabled us to rise above the obstacles typically experienced when performing conventional 2D monolayer culture. The 3D polystyrene scaffolds routinely produce a highly porous architecture to study the growth, differentiation and function of cells in new and exciting ways. Reinnervate has created a true working technology that will further advance long-term 3D cell culture research that will ultimately lead to important new discoveries."

Ramin E. Beygui
Department of Cardiothoracic Surgery, Stanford University School of Medicine, California

Image: 3D culture of murine keratinocytes on alvetex®

Contents

One of the Top 10 Innovations of 2010
The Scientist

2011 R&D 100

Introduction to culturing cells in three dimensions (3D)

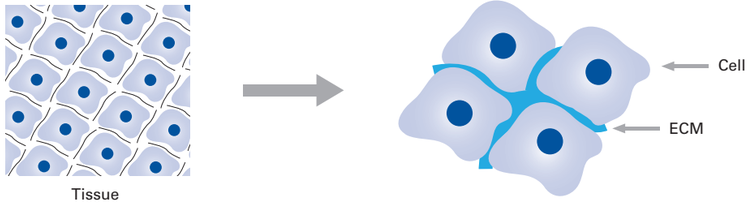
The goal of three-dimensional (3D) cell culture is to eliminate the stress and artificial responses cells experience as a result of cell adaptation to flat, 2D growth surfaces and to create suitable surroundings for optimal cell growth, differentiation and function. Genuine 3D cell culture allows individual cells to maintain their normal 3D shape and structure with minimal exogenous support and interference. Cells are freely able to form complex interactions with adjacent cells and receive and transmit signals, enabling a more natural environment to foster the creation of native architecture found in tissues.

Could the limitations of 2D cell culture be holding you back?

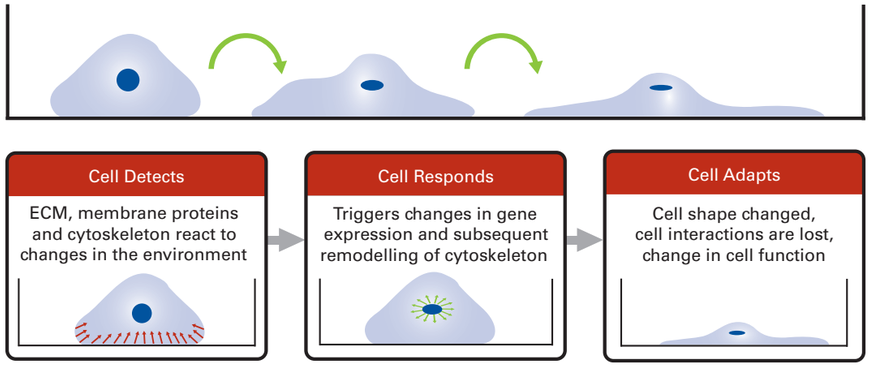
Finding experimental systems that model and provide useful information about in vivo biological processes is one of the most challenging tasks in scientific research. Cell culture enables the growth of cells outside the body in a controlled laboratory environment. Although convenient, culturing mammalian cells results in flat mono-layer cultures. This is dramatically different to the 3D in vivo environment cells experience in the body.

The effects of changing the growth environment on cells

In vivo 3D environment: typically cells maintain a 3D elipsoidal structure and organisation



In vitro 2D environment: cells adopt flattened morphology in a mono-layer



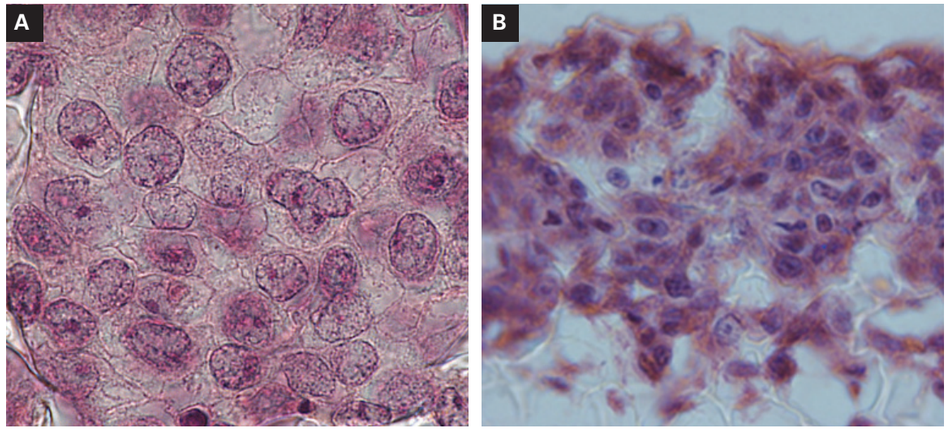
To enable survival in 2D culture, cells are forced to make dramatic changes to their morphology. Gene expression mediated changes to the cytoskeleton result in a flattened cell morphology. These changes can impair cellular functions. Cells grown in the laboratory do not always grow and function in a realistic fashion. This has major implications for research and discovery. For example:

- Inaccuracy of predictive assays in the drug discovery process
- Modification of normal behaviour of cells in response to external stimuli
- Generation of potentially inaccurate / misleading data
- Misunderstanding of complex biological phenomena
- Poor planning and direction of future research programme

Alvetex® - enhances the biological relevance of your cell culture research

By accurately recreating the complex cellular organisation and environment experienced by cells within their native tissues, Alvetex® enables more accurate investigation into the study of cell behaviour and function than ever before possible within conventional 2D model systems.

Cells maintain their natural 3D shape and structure within alvetex®, freely interacting with adjacent cells and laying down extra-cellular matrix which often leads to the formation of "mini slabs" of tissue-like structures. Using alvetex®, the cell biologist can create in vitro models which more accurately mimic the tissue environment, gaining a much deeper insight into the complexities of cell function and behaviour.



Cell Structure: Cells grown within alvetex® maintain their natural shape and 3D organisation:
Image A) 3D cell culture of human pluripotent stem cells within alvetex®. **Image B)** 3D cell culture of liver hepatocytes grown within alvetex®.

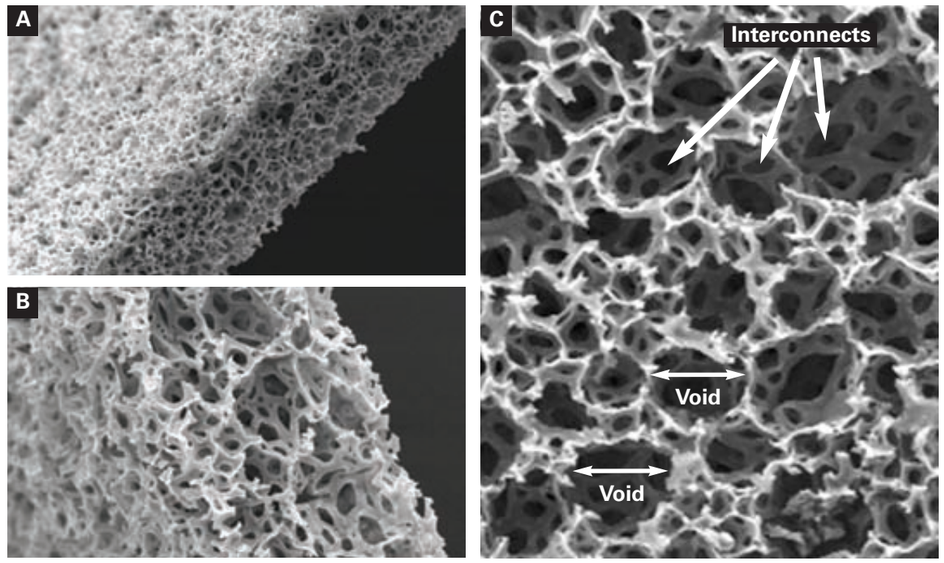
Typical mammalian cells are around 10-25 µm in size and are rarely further than 0-50 µm from another cell or 100-200 µm from a source of nutrients via a blood capillary. Alvetex® is made of the same polystyrene as used in traditional 2D cell culture plasticware. Alvetex® has been designed to enable cells to reproduce natural shape and form to enable the cell biologist to maintain the integrity of the micro-scale in vivo cell environment within simple in vitro models.

Summary: changing cell culture environment impacts on cell behaviour

	Traditional 2D Cell Culture	Alvetex® 3D Cell Culture	Normal In Vivo Environment
General dimensions and physical differences			
Maximum distance of cell from the source of nutrients	0	0-100 µm	0 - 200 µm
Resemblance to in vivo cellular environment	Low	High	n/a
Appearance of cell morphology	Flattened	3D shape	3D shape
Potential for 3 dimensional cell to cell interactions	Very low	High	High
Ability to form complex 3 dimensional cellular structures	Very low	High	High
Upon initial seeding of cells onto plasticware			
Degree of cellular stress placed upon cell structure	High	Low	n/a
Changes to protein and gene expression	High	Low	n/a
Cell surface area in contact with plastic	at least 50%	0-50%	n/a
Post seeding phase			
Ongoing changes to cellular shape	High	Low	n/a
Need for remodelling of cytoskeletal architecture Hig	h	Low	n/a
Deviation from normal in vivo morphology	High	Low	n/a
Cell surface area in contact with plastic	at least 50%	0-50%	n/a
Opportunity for enhanced in vitro cell functionality	Low	High	n/a

A unique micro scale environment to support genuine 3D cell growth

The structure of alvetex® provides cultured cells with an environment and physical space in which to grow in three dimensions. The architecture of alvetex®, as viewed by a scanning electron microscope illustrates voids that are interconnected by pores creating a material with > 90% porosity. Emulsion templating is used to control the size of the voids, optimising the porosity of the material for 3D cell culture. The matrix structure has been designed to enable cells to reproduce an environment that is more consistent with the in vivo cellular environment.

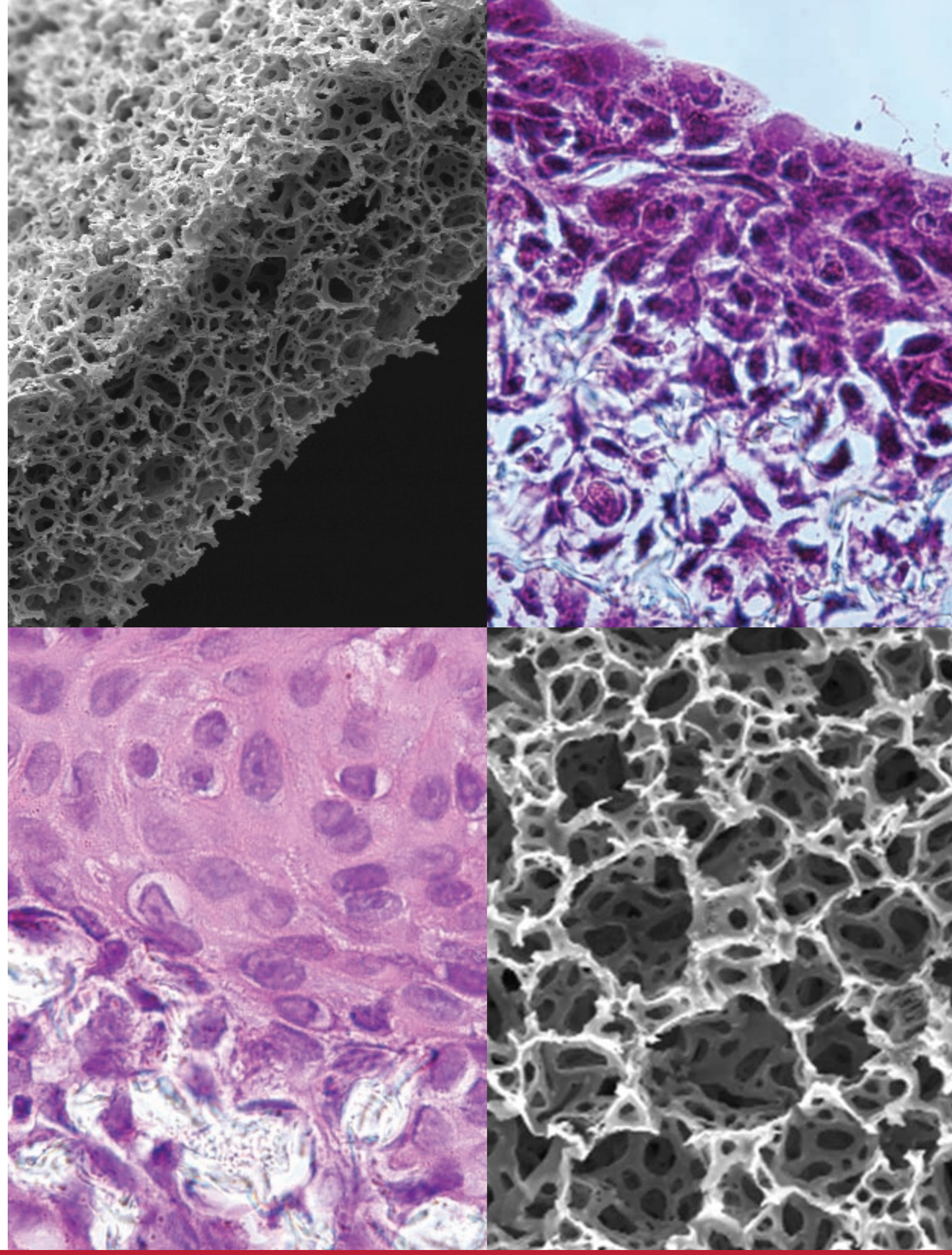


Alvetex® viewed by scanning electron microscope to highlight its porous structure. **Image A)** a 200 µm thick polystyrene scaffold. **Image B)** Alvetex® porosity of >90%). **Image C**) Close up of voids with dimensions of approximately 36-40 µm in diameter and interconnects of approximately 12 -14 µm in diameter.

Alvetex® - Unique scaffold dimensions ideally suited to 3D cell culture

Alvetex® Feature	Benefits of using alvetex® for 3D cell culture
Same polystyrene as existing cell culture plasticware •	• Easily switch between 2D and 3D protocols Inert – no effect on cell growth or function – no new experimental variables • Stable – does not degrade, no change throughout long-term studies • Can be pre-coated with ECM proteins
Consistent scaffold structure – extremely low batch to batch variability (ISO Certified)	• Reproducible, consistent results, low batch to batch variability • Genuine and homogeneous 3D cell growth
Entire scaffold is only 200 µm thick	• No cell is ever more than 100 µm away from nutrients and gasses – mimics in vivo conditions • Cells can feed and excrete via passive diffusion – mimics in vivo conditions
>90% Porosity	• Cells can easily penetrate scaffold and lay down ECM to more closely mimic in vivo conditions • Cells and media move freely through the matrix • Nutrients and waste exchanged by passive diffusion • Partial cell retrieval is possible
Void dimensions 36-40 µm	• Typically up to 75 cells may occupy a single void

Black and White images opposite: Examples of alvetex® scaffold. Note the high (>90%) porosity and consistency of the pore size.
Colour images opposite: Histological images of HaCaT cells grown in alvetex®



There is now an overwhelming body of peer-reviewed data showing stunning differences in cell behaviour when cells are cultured in three dimensions rather than on flat surfaces.

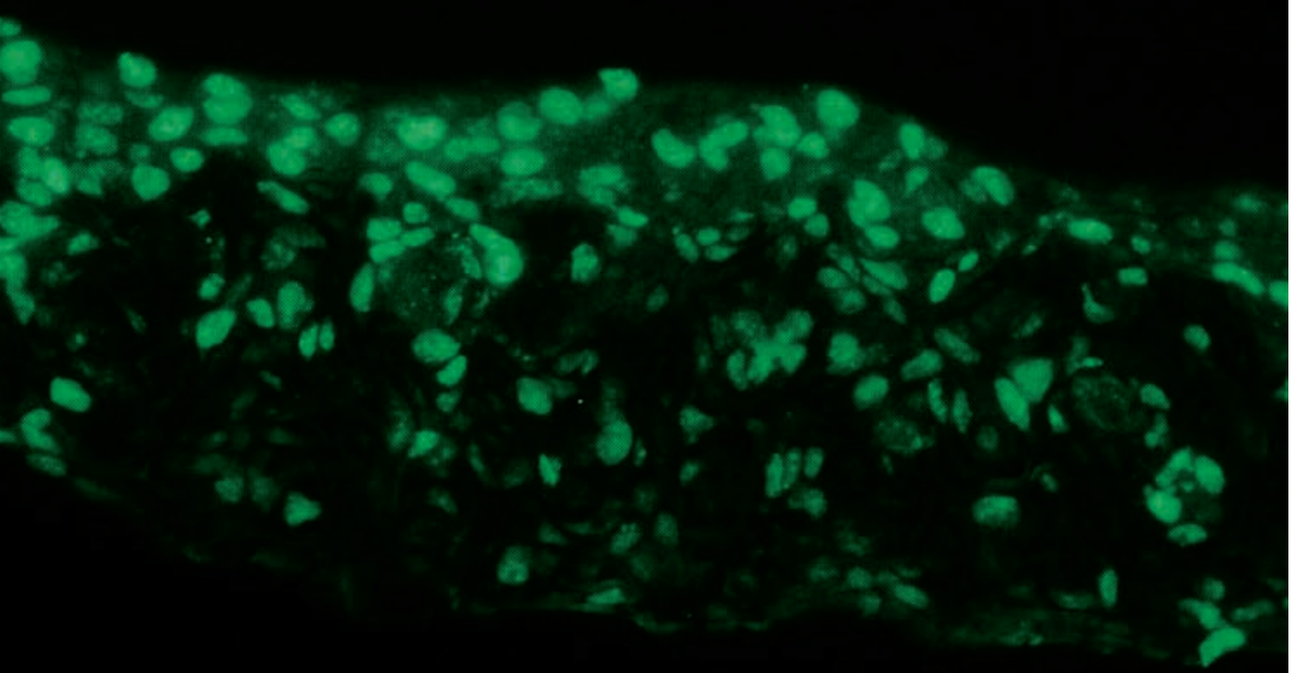
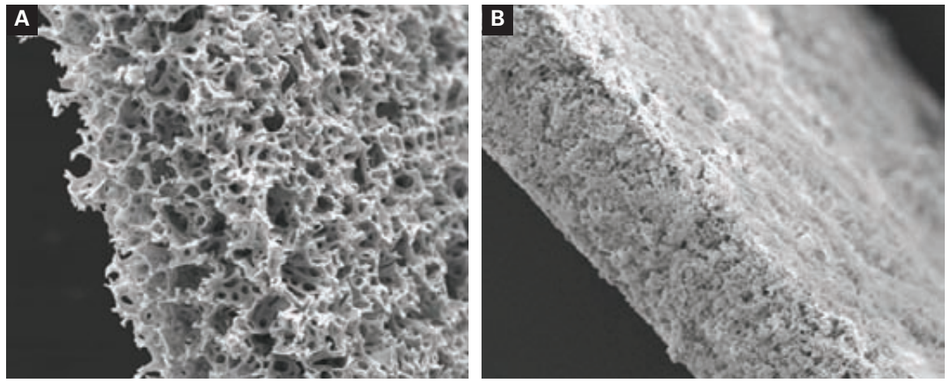


Image: Skin keratinocytes grown in alvetex® with antibody staining for Ki67 showing dividing cells. Ki67 positive cells imaged in green using FITC labelling

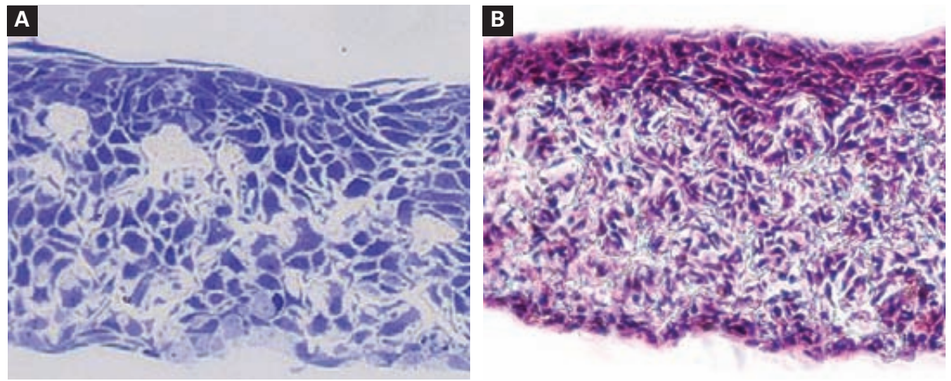
Alvetex® 3D cell culture leads to the creation of mini-slabs of tissue

Cells grow and divide occupying the 3D space within the porous alvetex® scaffold. Cells form complex interactions with one another, behaving in a manner that far more closely mimics normal growth in tissues than is possible using traditional 2D techniques. Cells are free to migrate throughout the matrix, functioning as they would within their natural environment, laying down extracellular matrix and forming organised and complex in vivo-like structures.



3D growth of cultured cells form 200 µm thick 'slabs of tissue'. **Image A)** Naked alvetex® scaffold before cell seeding viewed under scanning electron microsope to show the highly porous scaffold. **Image B)** Cells have grown throughout the scaffold to the point where the alvetex® scaffold is no longer visible.

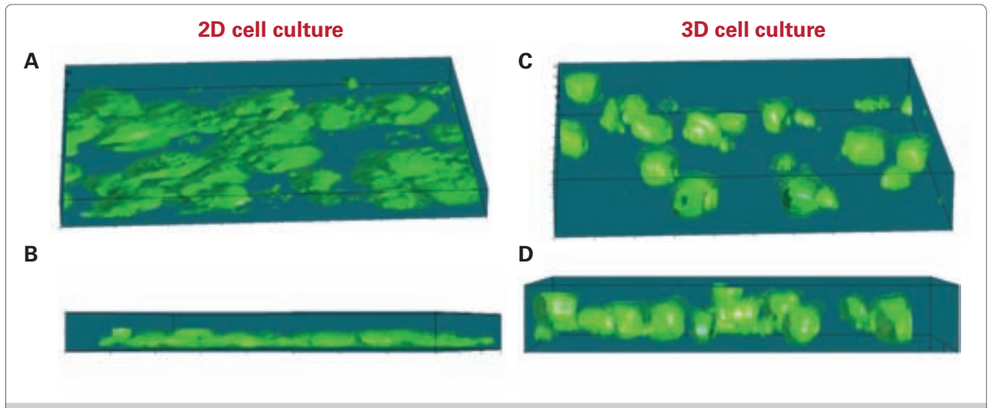
Alvetex® derived cell cultures can be processed just like normal tissue samples and prepared for histology using standard procedures including fixation, embedding, thin sectioning and counter staining.



Tissue processing and staining highlight the complex organisation of cells growing throughout the alvetex® scaffold. **Image A)** Resin sections of alvetex® showing skin keratinocytes stained with Toluidine Blue. **Image B)** Paraffin sectioned skin keratinocytes counter stained with H&E viewed by bright field microscopy.

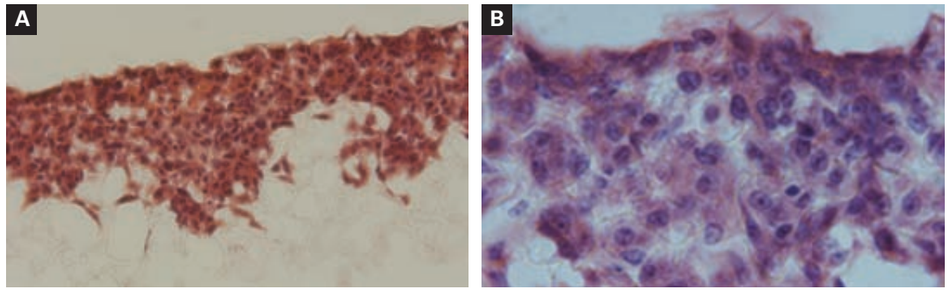
Alvetex® overcomes the limitations of culturing cells on flat plastic surfaces

The geometry and shape of a cultured cell is significantly affected by the physical environment in which it grows. Using alvetex® to culture cells, it is possible to maintain natural 3D cell morphology and replicate the conditions for growth and development that occur within living tissues.

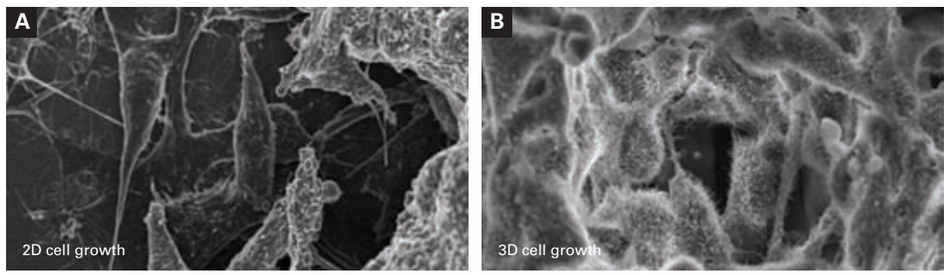


Cells grown on conventional 2D surfaces (A and B) adopt a typical flattened morphology covering a large surface area in horizontal x–y plane (A) and have a reduced height in the vertical z plane (B). In comparison, cells maintained in alvetex® (C and D) retain a more cuboidal morphology and 3D cell structure, particularly in the z-plane.*

Unlike cells grown on conventional 2D substrates where cell morphology is much more varied in appearance, consisting of clumps and individual flattened cells, cells grown in alvetex® exhibit a morphology that is much more consistent with that found within the in vivo environment. The appearance of cells is more homogeneous with a high degree of 3D organisation.



Structure of HepG2 cells cultured in 3D for 2 weeks on alvetex® inserts. **Image A)** Low magnification. **Image B)** High magnification.



Scanning electron microscopy image comparing the cell morphology and organisation of HepG2 liver cells grown in alvetex® versus 2D culture. **Image A)** Structure of cells in 2D is very heterogeneous with poor organisation. **Image B)** Cells in alvetex® scaffold grow homogeneously and develop a 3D form characteristic of liver tissues in the body. (see page 15 for higher magnification image).

Maintaining in vivo cell structure in alvetex® results in improved function

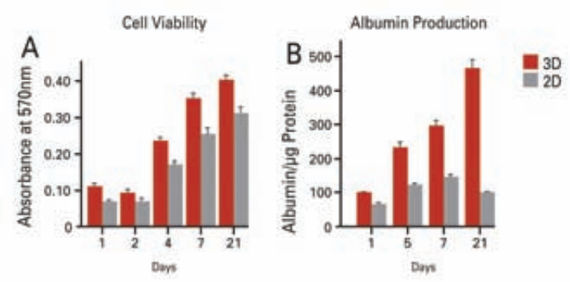
By maintaining the shape and structure of cells and enabling a high level of cell-to-cell interaction, alvetex® enables a much deeper understanding of how cells function in vivo.

Example: Improved cell function and responsiveness

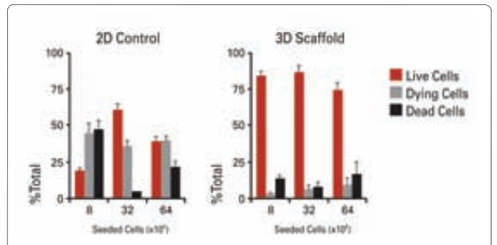
Enabling cells to maintain their natural morphology and 3D organisation leads to improved cell function and responsiveness which is much more representative of the natural in vivo environment. Alvetex® delivers data of unmatched biological relevance. Factors such as cell viability and responsiveness have been demonstrated to be enhanced when growing cells in alvetex® in comparison to 2D mono-layer cultures.

Function and Responsiveness:

Assessment of HepG2 cells grown on 2D and 3D substrates. Cell viability was determined using a MTT assay and showed greater numbers of viable HepG2 cells in alvetex® than on 2D substrates. Similarly, the secretion of albumin from 3D HepG2 cells was elevated compared to standard 2D cultures. In both cases, these data have been normalised to total protein per well to take into account differences in cell numbers. Overall, these results indicate the superior performance of HepG2 cells in 3D culture compared with their 2D counterparts.

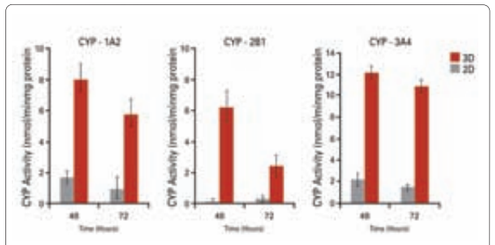


Increased cell viability



Cell viability of rat primary hepatocytes determined by quantification of live/dead cell staining of hepatocytes maintained for 24 hours on 2D plasticware or alvetex® scaffolds. Cells showed greater than 74% viability when grown on alvetex® compared to 2D monolayer culture.*

Increased metabolic responses

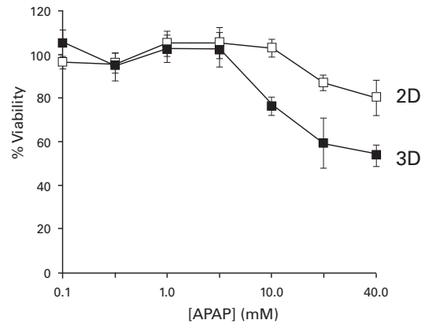


Metabolic responses to model toxicants were significantly enhanced using alvetex®. Primary rat hepatocytes were cultured for 3 days in either 2D or 3D culture, Cytochrome p450 expression was induced in cells using a cocktail of model toxicants.*

Rat primary hepatocytes cultured in 3D showed greater sensitivity to APAP

Cell sensitivity:

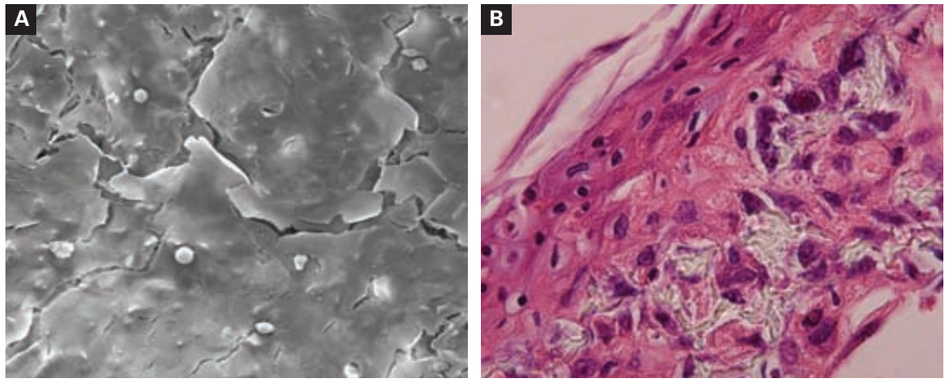
Primary hepatocytes cultured on 2D plasticware versus alvetex® were exposed to a range of acetaminophen (APAP) concentrations for a period of 20 hours and their viability was determined by a standard MTT assay. In general, these data demonstrate that rat primary hepatocytes cultured in 3D using alvetex® show increased sensitivity to the model toxicant, acetaminophen*



*Data generated during a collaborative project between Reinnervate Ltd and LGC Standards – data now published in the following journal:
Title: Rat primary hepatocytes show enhanced performance and sensitivity to acetaminophen during three dimensional culture on a novel polystyrene scaffold designed for routine use. Authors: Maaike Schutte, Bridget Fox, Marc Baradez, Alison Devonshire, Jesus Minguez, Maria Bokhari, Stefan Przyborski, Damian Marshall. Assay and Drug Development Technologies DOI: 10.1089/adt.2011.0371

Imaging reveals the integrity of in vivo structure and organisation

Changes in cellular morphology and function limit the value of cells grown in 2D cell culture. 3D culture systems enable cells to form more complex structures. Various models have been developed to create 3D skin constructs in vitro, including raft cultures. These methods are often technically challenging, involve multiple steps, show poor reproducibility and are difficult to practise routinely. Alvetex® provides an alternative method for 3D growth of keratinocytes, enabling reproduction of natural in vivo structures including the development of the stratum corneum, an essential component of the epidermal barrier. Skin constructs generated on alvetex® can then be used for drug and allergen penetration studies as well as assessment of barrier function.



3D culture of skin keratinocytes on alvetex® resulting in formation of 3D epidermis and maturation of the stratum corneum. **Image A)** Scanning electron microscope image illustrating the formation of the stratum corneum of a 21 day culture. **Image B)** Alvetex® scaffold sectioned and stained with H&E and viewed by light microscopy after 35 days.

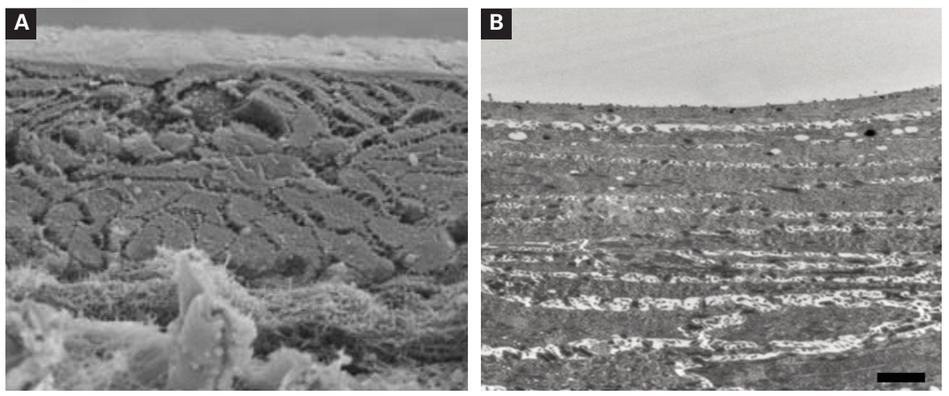


Image A) Scanning electron micrograph image showing full thickness skin construct grown on a layer of collagen on top of alvetex® which includes an upper cornified layer. **Image B)** Transmission electron microscopy demonstrating stratification of keratinocytes in upper layers of the culture.

Image: Scanning electron microscopy image revealing structure of HepG2 cells grown in alvetex®

Alvetex® can easily be coated with ECM proteins

Alvetex® can be coated with extracellular matrix (ECM) proteins and other reagents commonly used to treat cell culture substrates, notably: Collagen I; Collagen IV; Fibronectin; Laminin; Poly-D-lysine; Poly-L-lysine; Poly-D-lysine and Laminin; Poly-L-orthinine and Laminin; Matrigel™; PuraMatrix™.

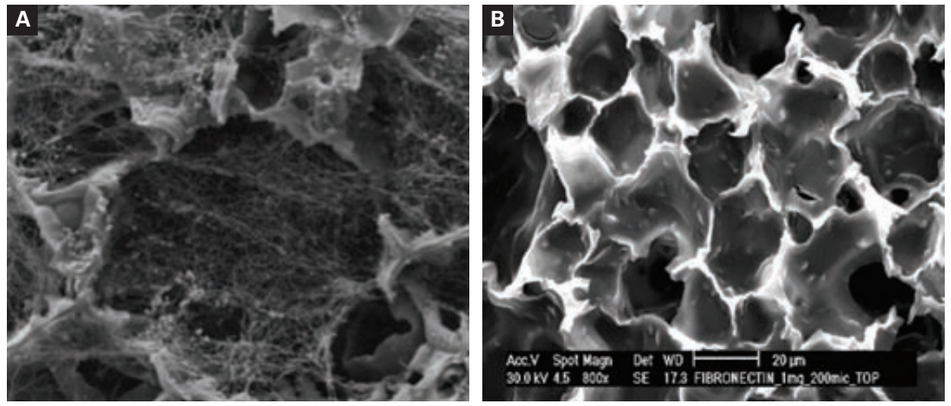
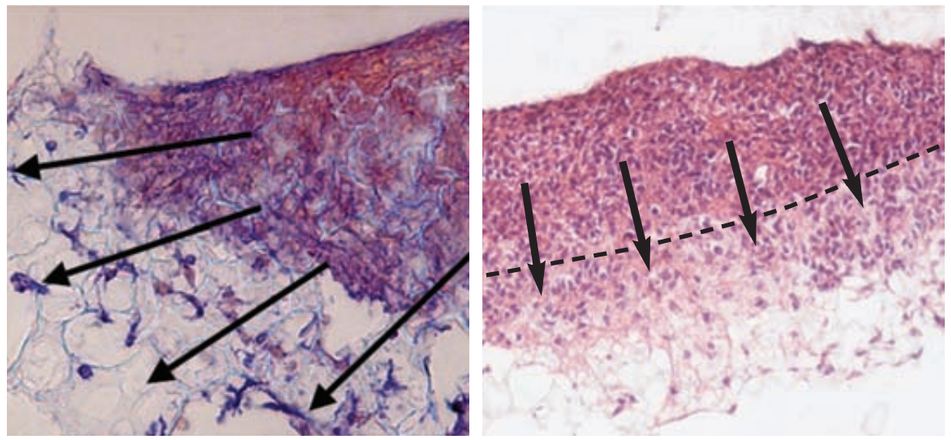


Image A) Scaffold pre-loaded with Collagen IV. **Image B)** Coating alvetex® with fibronectin. The ECM proteins form a web of fibres spanning voids into which cells can grow and migrate in 3D.

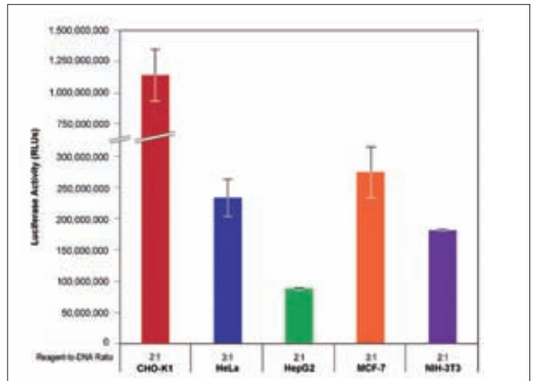
Cells can be explanted directly into alvetex®

Cells can move into alvetex® directly from pieces of primary tissue or cell aggregates, migrating freely into the alvetex® and spreading throughout its structure. Depending on cell type and characteristics, cells may proliferate as well as migrate. By enabling cells to be explanted in this way, alvetex® creates the opportunity for many different applications including tumour cell biology, separation of alternative cell types and establishing and maintaining 3D cultures de novo directly from primary sources, etc.



Examples of cells from tissue pieces placed on top of alvetex® migrating into the structure of the scaffold.

Transfection of various cell types using alvetex® 3D culture plates



3D Transfection of Multiple Cell Types

In collaboration with Mirus Bio, methods have been developed that enable the transfection of cells grown in 3D culture.

Common cell types (HeLa, HepG2, MCF-7, NIH-3T3 and CHO-K1) were seeded at optimised cell densities in 12-well alvetex® 3D plates and adapted to 3D culture conditions for 48 hours. After adaptation, cells were transfected with a novel Mirus Bio formulation combined with a plasmid encoding firefly luciferase at the reagent-to-DNA ratios indicated beneath the bars. Luciferase activity was measured 24 hours post-transfection using a conventional assay. High expression was detected in all cell types demonstrating the efficiency of the new Mirus Bio Transfection Reagent when used with alvetex® 3D culture plates.

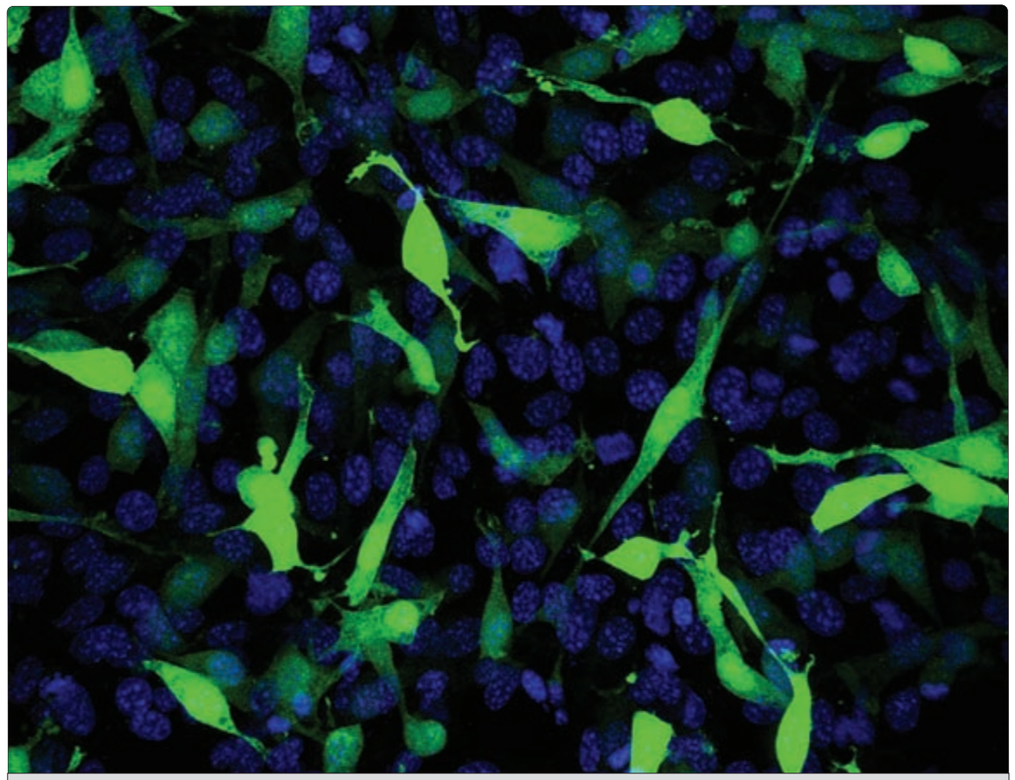


Image: Fibroblasts grown in 3D using alvetex® scaffold technology were successfully transfected with a GFP construct and imaged using confocal microscopy. In brief, cells were transfected with the new Mirus Bio Transfection Reagent for 3D transfection, at a reagent-to-DNA ratio of 3:1, using a GFP-expressing plasmid. Cells were seeded at 48 hours prior to transfection, and the cultures were fixed 24 hours post-transfection. Cells were imaged using a confocal microscope (Zeiss LSM510). The data shows a 40 micron integrated stack of multiple images as viewed from above the intact 3D culture. The position of all the cell nuclei are visualised with Hoechst 33342 (blue) and the positively transfected cells express GFP (green).

Image: Reinnervate founder and CSO Professor Stefan Przyborski

Spend time generating genuine 3D cell culture - rather than optimising

Despite previous attempts to enable genuine 3D cell culture, the majority of researchers today continue to express the need for a universal platform that enables genuine 3D cell culture, without the need for investment in new equipment, additional training or highly labour intensive protocols. Alvetex® technology meets this need and is complimentary with existing plasticware, media and reagents and provides easy access to all the benefits of 3D cell culture.

Limitations of existing technologies available today

Technology	Drawbacks and limitations
Biodegradable polymers	Unstable and degrade over time
Hydrogels	Issues with preparation, storage, variability and expense
Electrospun fibre mats	Fibre mats create a 3D nano surface, not a 3D cell culture
Fibre polystyrene scaffold	Large fibre diameter, little 3D cell growth, mixture of 2D and 3D
Alginate 3D scaffold	Issues with stability, size, not compatible with existing plastic substrates

Alvetex® defines the gold standard for 3D cell culture

- Creating suitable surroundings for optimal cell growth, differentiation and function ☑
- Allowing cells to adopt a natural 3D shape and structure ☑
- Encouraging cells to form complex interactions with adjacent cells ☑
- Reducing stress and aberrant responses as a result of the growth substrate ☑
- Enabling a more natural environment to mimic native tissue structures ☑
- Consistent 3D cell culture growth within the matrix ☑
- High batch-to-batch reproducibility ☑
- Assay compatibility ☑
- Consumable, off-the-shelf product ☑
- Developed for routine use ☑

> “Alvetex® is an example of innovation to move us closer to better models for mimicking in vivo behaviour of cells with the control of in vitro conditions.”

Neil Kelleher (Award Judge of The Scientist Magazine Top Ten Life Science Innovations 2010)
Northwestern University (Chicago, IL, USA).

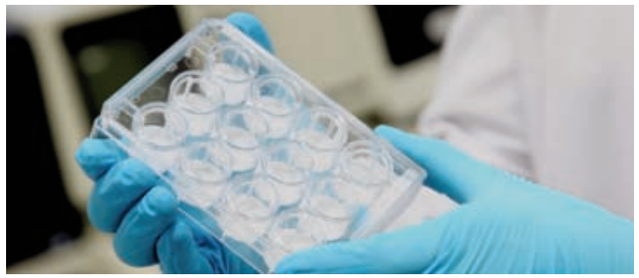
A range of product formats for all your applications

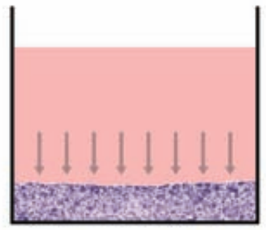
Alvetex® is supplied as 200 µm thick discs that each provide a highly porous polystyrene scaffold in which cells can grow and interact in 3D. Available as inserts or plates, experiments can be designed to meet the needs of the specific investigation or application, whilst accurately reproducing the in vivo 3D cellular environment of your specific cell type. Suddenly a whole range of exciting new investigations become possible:

- Optimise the growth of specific cell types
- Control the degree of cell penetration required within the 3D structure of alvetex®
- Select the appropriate format to the duration of the assay
- Enable long term 3D cell culture

Alvetex® 12 well plate format

Alvetex® in a 12 well format (Product No: AVP002), comprises twelve 22 mm discs of alvetex® inserted into a 12 well plate and held in place by a removable clip. Ideal for short term cultures where the medium is replaced every 1-2 days.



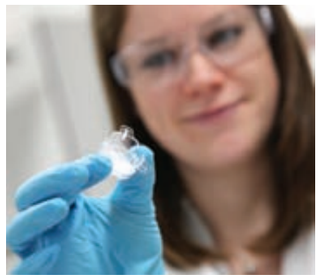


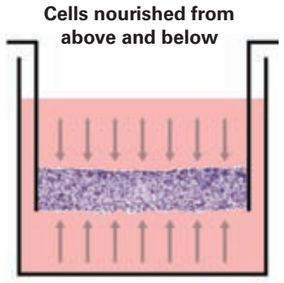
Key benefits:

- Ideal for 3D culture in the top half of alvetex®
- Useful for when restricted cell penetration is required (e.g. cell transfection)
- Cells are fed from the top of the alvetex® scaffold only
- An option for use with expensive cells, reducing cell number

Alvetex® well insert formats

Two sizes of inserts are available: the 6 well insert (AVP004-3) and the 12 well insert (AVP005-3). Supplied individually blister packed, the well inserts have been designed for convenience and to facilitate easy handling of cultures. Well inserts can be accommodated into most standard 6 and 12 well tissue culture plates or with the new well insert holder located in a deep Petri dish (see page 28 for disc sizes and further details).

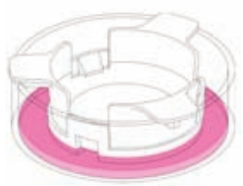




Key benefits of alvetex® inserts:

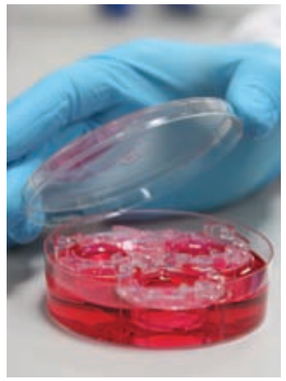
- Enables cells to be fed from above and below simultaneously
- Readily transfer 3D cultures to a fresh plate
- Growth of cells at the air / liquid interface
- Ideal for longer term 3D culture
- Enables co-culture studies (2D and 3D, or 3D and 3D)

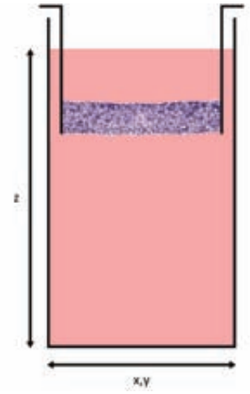
Alvetex® inserts enable three different media fill options

	Media Fill Option 1:	Media Fill Option 2:	Media Fill Option 3:
			
Description:	Media in contact from below only	Media in contact above and below - independent media compartments	Media in contact above and below - connected compartments
Enables:	3D growth at the air/liquid interface	3D growth with two different media constituents	3D growth with two uniform media constituents
Example of Application:	Induction of epithelial stratification (e.g. skin epidermis)	Barrier penetration assay of test compounds Evaluation of compound permeability Growth with different media either side of the scaffold	Create optimal conditions for maximising cell growth and increased viability

Well insert holder in a deep well Petri dish

The innovative well insert holder inside a deep Petri dish (AVP0015) can accommodate up to three 6 well inserts or three 12 well inserts at any one time. Cells can be cultured in 3D for longer term assays reducing the requirement for frequent media changes.



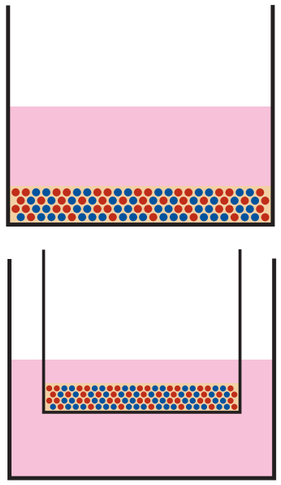


Key benefits of well insert holder in a deep well Petri dish:

- Reduces frequency of media changing as up to 95ml of medium can be used to support a single 3D culture
- Ideal for maintaining long term 3D culture experiments of up to several weeks
- Facilitates the use of a magnetic stirrer to increase media circulation if required

Using alvetex® to create co-culturing experiments

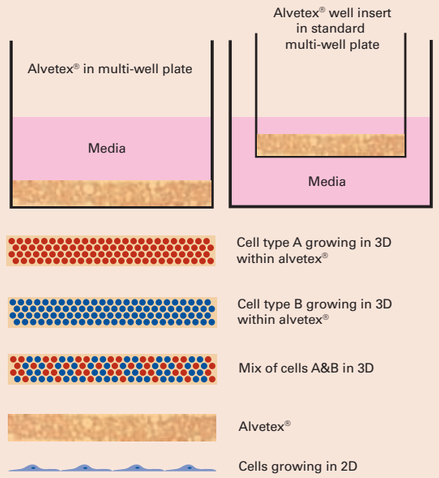
1 Assembly option
3D / 3D co-culture in multi-well plate or well insert



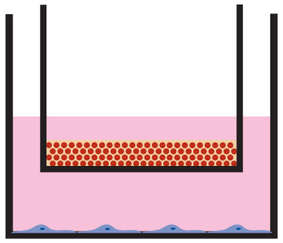
Description
Different cell types cultured together within the same scaffold

Application
Emulate the structure of a tissue comprised of more than one cell type

Key to Image Parts:



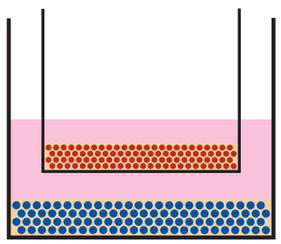
2 Assembly option
3D / 2D co-culture in multi-well plate and well insert combined



Description
Two independent cell cultures one in 2D and one in 3D. Contact is via medium – communication via paracrine factors

Application
Approach used to study the secretion of factors and signalling molecules

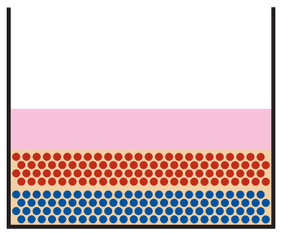
3 Assembly option
3D co-culture in multi-well plate and well insert combined



Description
Two independent 3D cultures. Contact is via medium only and inter-culture communication via paracrine factors

Application
Approach used to study the secretion of factors and signalling molecules

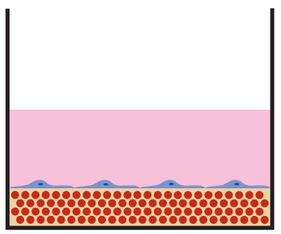
4 Assembly option
3D / 3D co-culture in multi-well plate



Description
Two 3D cultures in direct contact

Application
- Study the direct interaction of cells in contact with one another
- To establish layers of alternative cell types in 3D to mimic tissue structures
- To investigate invasion and migration of different cell types amongst each other

5 Assembly option
2D / 3D co-culture in multi-well plate

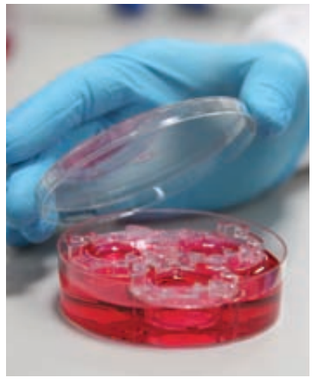


Description
One 2D and one 3D culture layered in direct contact with one another

Application
- Study the direct interaction of cells in contact with one another
- To establish layers of alternative cell types in 3D to mimic tissue structures
- To investigate invasion and migration of different cell types amongst each other

Choosing the right alvetex® format based on assay type

ASSAY TYPE:	Viability assays	Toxicity assays	Proliferation assays	Metabolic Activity assays	Gene Expression assays (qPCR/ microarray)	Protein Expression assays (e.g. western blotting)	Air-liquid Interface assays	Cell Signalling assays	Permeability assays	Transfection assays	Co-culture assays	Invasion assays	Migration assays
Alvetex® 12-well plates	•••	•••	•••	•••	•	•	n/a	•	n/a	•••	•	•	•
Alvetex® 6-well inserts	•••	•••	•••	•••	•••	•••	•••	•••	•••	•	•••	•••	•••
Alvetex® 12-well inserts	•••	•••	•••	•••	••	••	•••	•••	•••	•	•••	•••	•••



"Alvetex® 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."

H. Steven Wiley, Lead Biologist at the Environmental Molecular Sciences Laboratory (EMSL) at Pacific Northwest National Laboratory (Richland, WA, USA) and Award Judge of The Scientist Magazine Top Ten Life Science Innovations 2010

Reinnervate is dedicated to the development of more biologically relevant cell culture technologies and products.

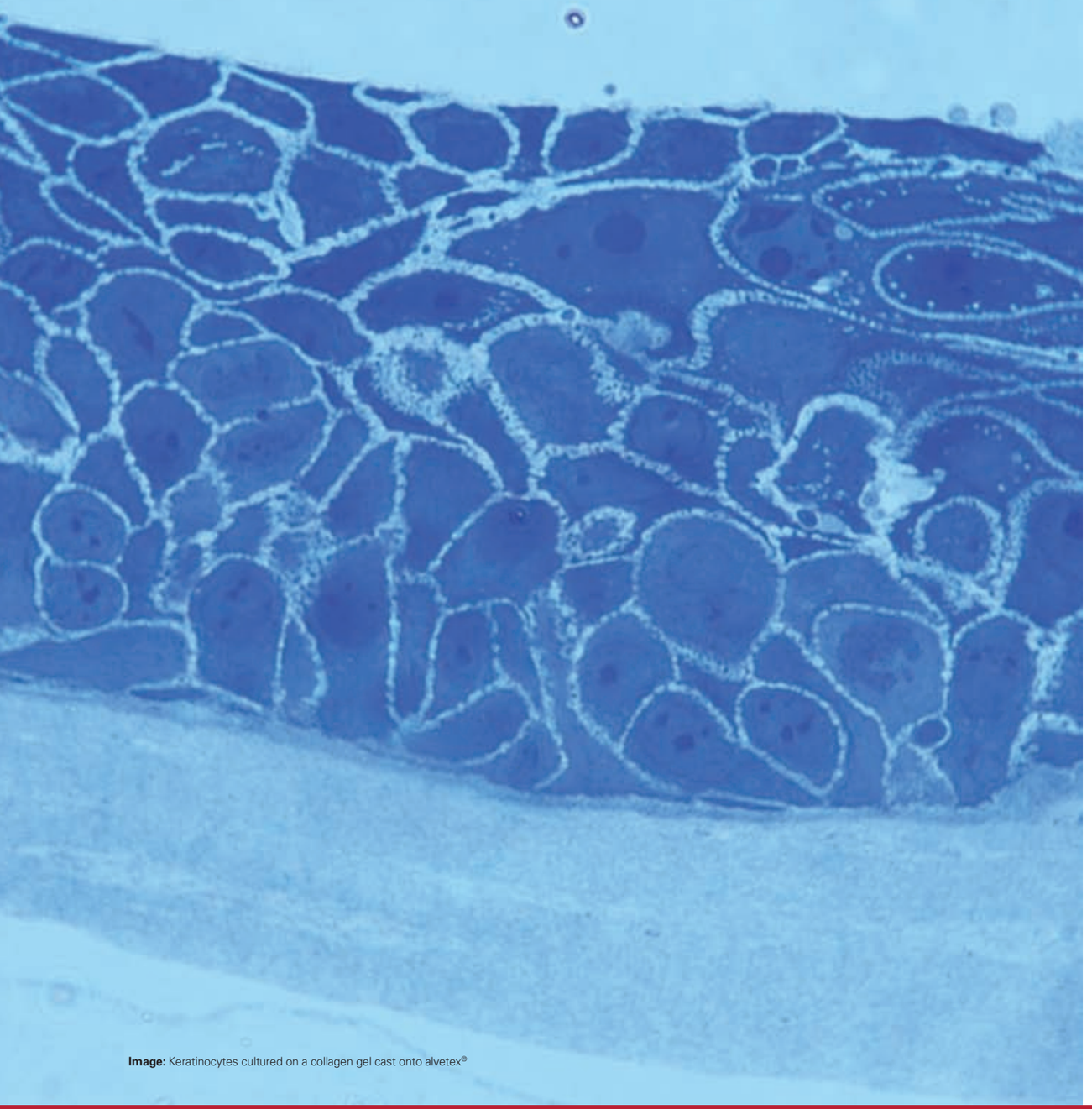


Image: Keratinocytes cultured on a collagen gel cast onto alvetex®

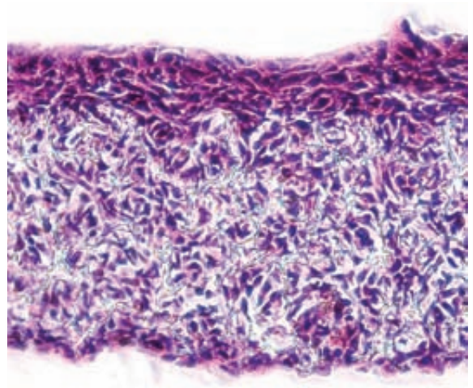
Alvetex® - designed to meet the needs of your research project

Alvetex® is made of the same polystyrene used in standard 2D cell culture plasticware, removing the need to change existing media or adopt new reagents.

Compatible with downstream applications

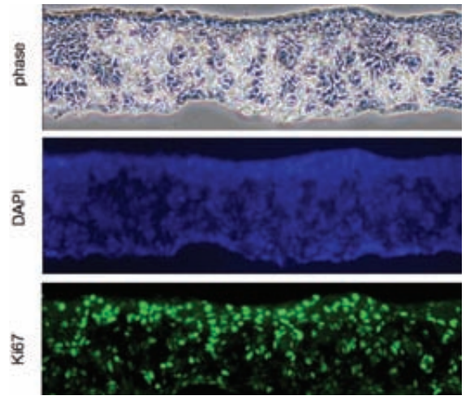
- Tissue processing, fixation, embedding and sectioning
- Bright-field microscopy and photographic imaging
- Cryostat sectioning
- Fluorescence microscopy, confocal, laser capture
- Flow cytometry and cytospinning
- Biochemical assays
- Histological staining, in situ hybridisation
- Electron microscopy – both SEM and TEM
- Immunocytochemistry
- Isolation of viable cells
- Extraction of nucleic acid and total protein

Sectioning and Counterstaining



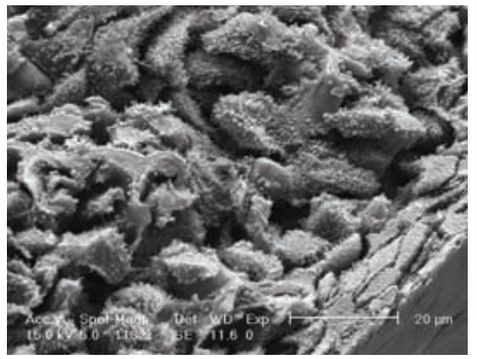
Unlike other 3D cell culture supports, alvetex® can easily be processed like a standard tissue sample. Frozen and paraffin-embedded samples can be sectioned and stained to reveal the native cellular structures inside alvetex®. In this example cells have been fixed in 4% paraformaldehyde, embedded in paraffin wax, sectioned (7 micron) before staining with H&E and cover-slipped.

Immunocytochemistry



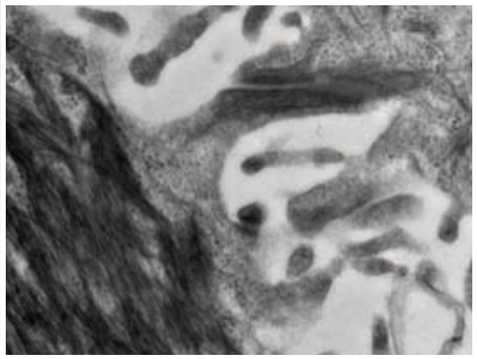
Immunocytochemistry used to visualise the expression of specific protein markers. In this example, cell cultures have been fixed in 4% paraformaldehyde, embedded in paraffin wax and sectioned (10 microns). Antigen retrieval followed by immunocytochemical analysis with the proliferation marker Ki67 (green) and the nuclear stain DAPI (blue) was performed following standard immunocytochemical methods.

Scanning Electron Microscopy



Visualisation of the structure of 3D cultures in alvetex® is made possible using scanning electron microscopy (SEM). Samples are prepared in the same manner as would normally be used for tissues. In this example of skin, cells have penetrated throughout the scaffold and some have stratified on the surface.

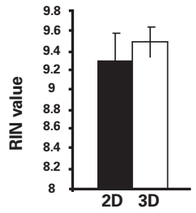
Transmission Electron Microscopy



The ultrastructure of cells grown in alvetex® can be analysed by standard transmission electron microscopy (TEM). At high magnification, cellular structures such as this specialised cell junction linking two cells, are readily visualised.

Gene Expression Analysis

Quality and yield of RNA isolated from primary rat hepatocytes

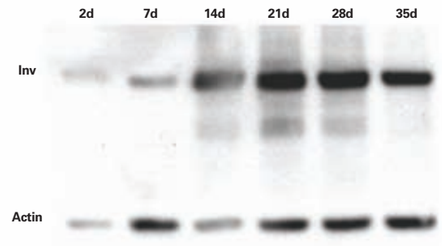


	Sample	ng/ul	RIN
2D	DMSO	82	9.3
3D	DMSO	91	9.5

Isolation of nucleic acid from rat primary hepatocytes grown in alvetex® and conventional 2D cultures. RNA quality was determined by the RIN (RNA Integrity Number) and showed that the quantity and quality of RNA isolated from cells grown on alvetex® was the same if not better than that isolated from standard 2D cultures. Data generated in collaboration with LGC (unpublished).

Protein Expression Analysis

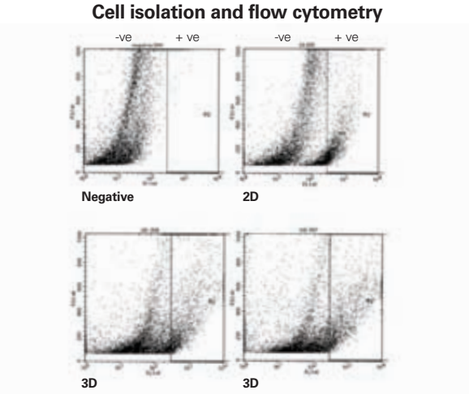
Protein isolation and western blotting



With standard lysis protocols, total protein can be efficiently isolated from cells growing inside alvetex®. This allows for more biologically relevant protein expression analysis experiments to be carried out. Here we showed the increase over time of involucrin (Inv) expression in maturing keratinocytes by western blot analysis.

Isolation of cells from alvetex®

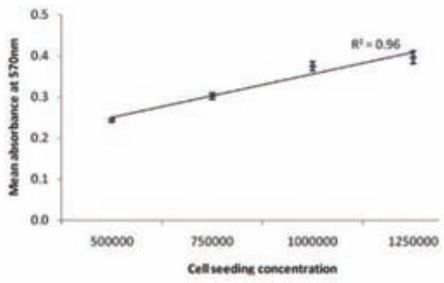
Cell isolation and flow cytometry



It is possible to isolate some viable cells from alvetex® scaffolds for downstream experiments such as flow cytometry, cytospinning and for sub-culture. Here, mesenchymal stem cells induced to form adipocytes were isolated from alvetex® and 2D cultures. Cells were subsequently stained with Nile Red to detect the presence of lipid and analysed by flow cytometry.

Biochemical assays

MTT cell viability assay of cultures grown on alvetex® in 3D



Cells growing in 3D inside alvetex® can be studied using typical biochemical assays such as cell viability assays, apoptosis assays, cell proliferation assays etc. Here we show the measurement of cell viability using a standard MTT assay.

Unlock the potential of your in vitro cell culture with alvetex® and take your research to a whole new dimension

Image: Genuine 3D cell culture; histological images of HaCaTs grown on alvetex®

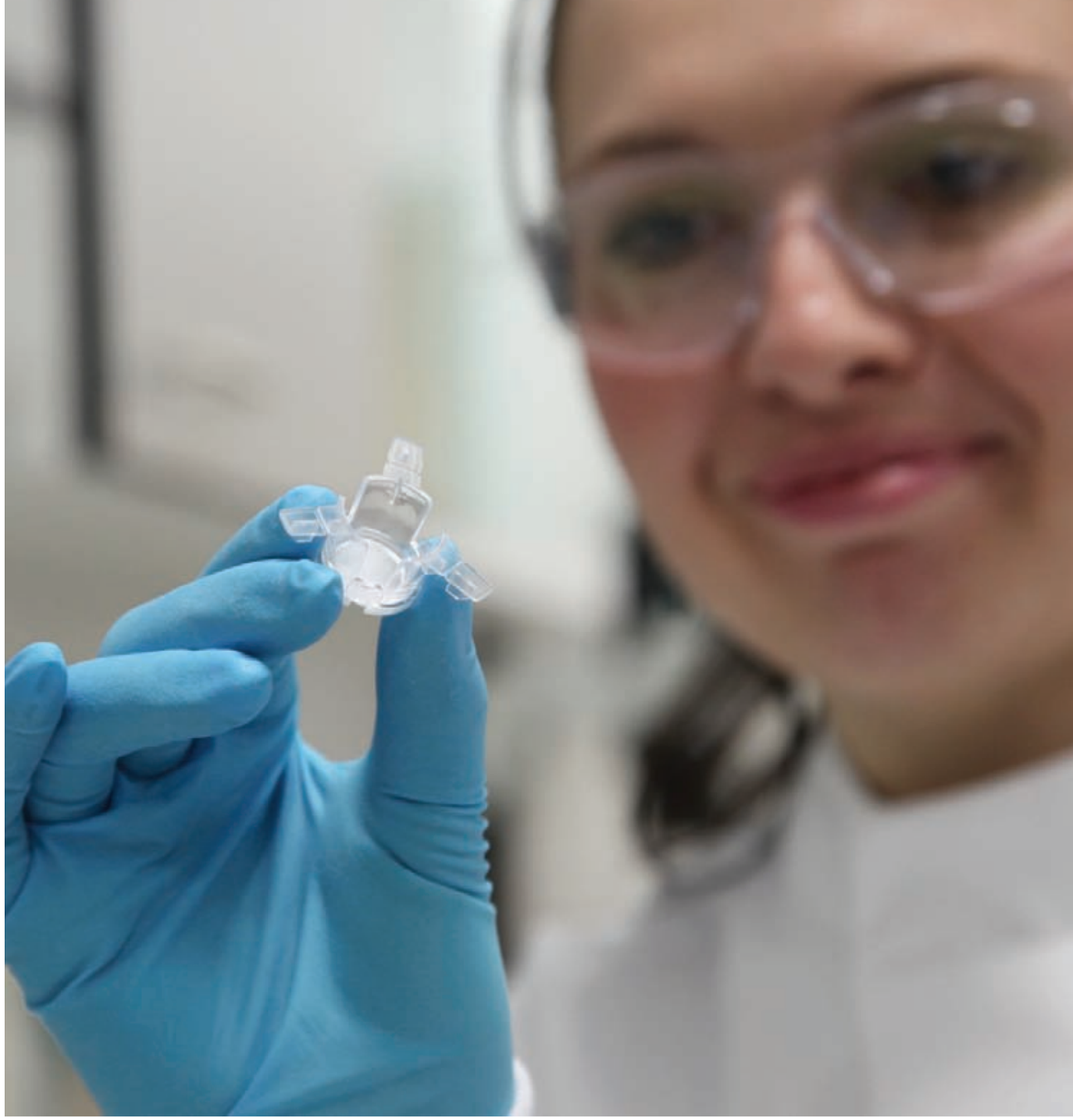
Ordering information

Summary of different alvetex® product formats available

Product Name	Product Format	Size	Duration of Assay	Flexibility	Media Fill options	Typical 3D Growth Pattern	Assembly Options	Can disc be removed?
Alvetex® 12-well plates	12 alvetex® discs within a 12 well plate	22 mm diameter 200 microns thick	Short Term (1-10 days)	Basic	From above only	10-50% penetration of matrix (cell type dependant)	Comes preassembled in 12 well plate	Yes
Alvetex® 6-well inserts	Single alvetex® disc within a well insert	22 mm diameter 200 microns thick	Long Term (1-5 weeks)	High	Multiple options	Throughout matrix	6 well plate or Well Insert Holder (AVP0015)	Yes
Alvetex® 12-well inserts	Single alvetex® disc within a well insert	15 mm diameter 200 microns thick	Long Term (1- 5 weeks)	Highest	Multiple options	Throughout matrix	6 well plate, 12 well plate or Well Insert Holder (AVP0015)	Yes
Well insert holder in a deep well Petri dish	Well insert holder in a deep well Petri dish	N/A	N/A	N/A	N/A	N/A	Can hold 1-3 of any well inserts	N/A

Product Name:	Code:	Description
Alvetex® 12 well plate	AVP002	Single plastic plate with alvetex® inserted
6 well insert	AVP004-3	3 individually sealed 6 well inserts
12 well insert	AVP005-3	3 individually sealed 12 well inserts
Well insert holder in a Petri dish	AVP015	1 holder and Petri dish

Note: all products are gamma irradiated and supplied sterile.



Choose alvetex® today and experience all the benefits of achieving 3D cell culture simply, consistently and reproducibly

For further information, online tools and resources visit: www.reinnervate.com

Useful References

Data on alvetex® have been published extensively in peer reviewed publications exemplifying its use with a large number of cell types (including hepatocytes, fibroblasts, stem cells, tumour cells). These publications also cover the use of alvetex® in a range of applications, particularly those relevant to drug development (e.g. disease modelling and toxicity screening).

1. Schutte et al (2011). Rat primary hepatocytes show enhanced performance and sensitivity to acetaminophen during three-dimensional culture on a polystyrene scaffold designed for routine use. Assay Drug Dev. Technol. Epub ahead of print.
2. Neofytou et al (2011). Adipose tissue-derived stem cells display a proangiogenic phenotype on 3D scaffolds. J. Biomedical Materials Research. Epub ahead of print.
3. Rajan et al (2011). Dysregulated TRK signalling is a therapeutic target in CYLD defective tumours. Oncogene. Epub ahead of print.
4. Fox et al (2010). Validation of reference gene stability for APAP hepatotoxicity studies in different in vitro systems and identification of novel potential toxicity biomarkers. In Vitro Toxicology, 24 (7), 1962-1970.
5. Maltman and Przyborski (2010). Developments in three dimensional cell culture technology aimed at improving the accuracy of in vitro analyses. Biochem. Soc. Trans., 38 (4), 1072-1075.
6. Bokhari et al (2007). Novel cell culture device enabling three-dimensional cell growth and improved cell function. Biochem. Biophys. Res. Comm., 354, 1095-1100.
7. Bokhari et al (2007). Culture of HepG2 liver cells on three-dimensional polystyrene scaffolds enhances cell structure and function during toxicological challenge. J. Anatomy, 211 (4), 567-76.
8. Bokhari et al (2007). Emulsion templated porous polymers as scaffolds for three-dimensional cell culture: effect of synthesis parameters on scaffold formation and homogeneity. J. Materials Chem. 17, 4088-4094.
9. Carnachan et al (2006). Tailoring the morphology of emulsion-templated porous polymers. Soft Matter, 2, 608-616.
10. Barbetta et al (2005). Porous polymers by emulsion templating. Macromolecular Symposia, 226, 203-211.
11. Hayman et al (2005). Growth of human stem cell-derived neurons on solid three-dimensional polymers. J. Biochem. Biophys. Methods, 62, 231-240.
12. Hayman et al (2004). Enhanced neurite outgrowth by human neurons grown on solid three-dimensional scaffolds. Biochem. Biophys. Res. Comm., 314, 483-488.

Pricing Information

Product Name:	Code:
Alvetex® 12 well plate	AVP002
Alvetex® 6 well insert	AVP004-3
Alvetex® 12 well insert	AVP005-3
Well insert holder in deep Petri dish	AVP015

Contact Details for placing orders

www.reinnervate.com

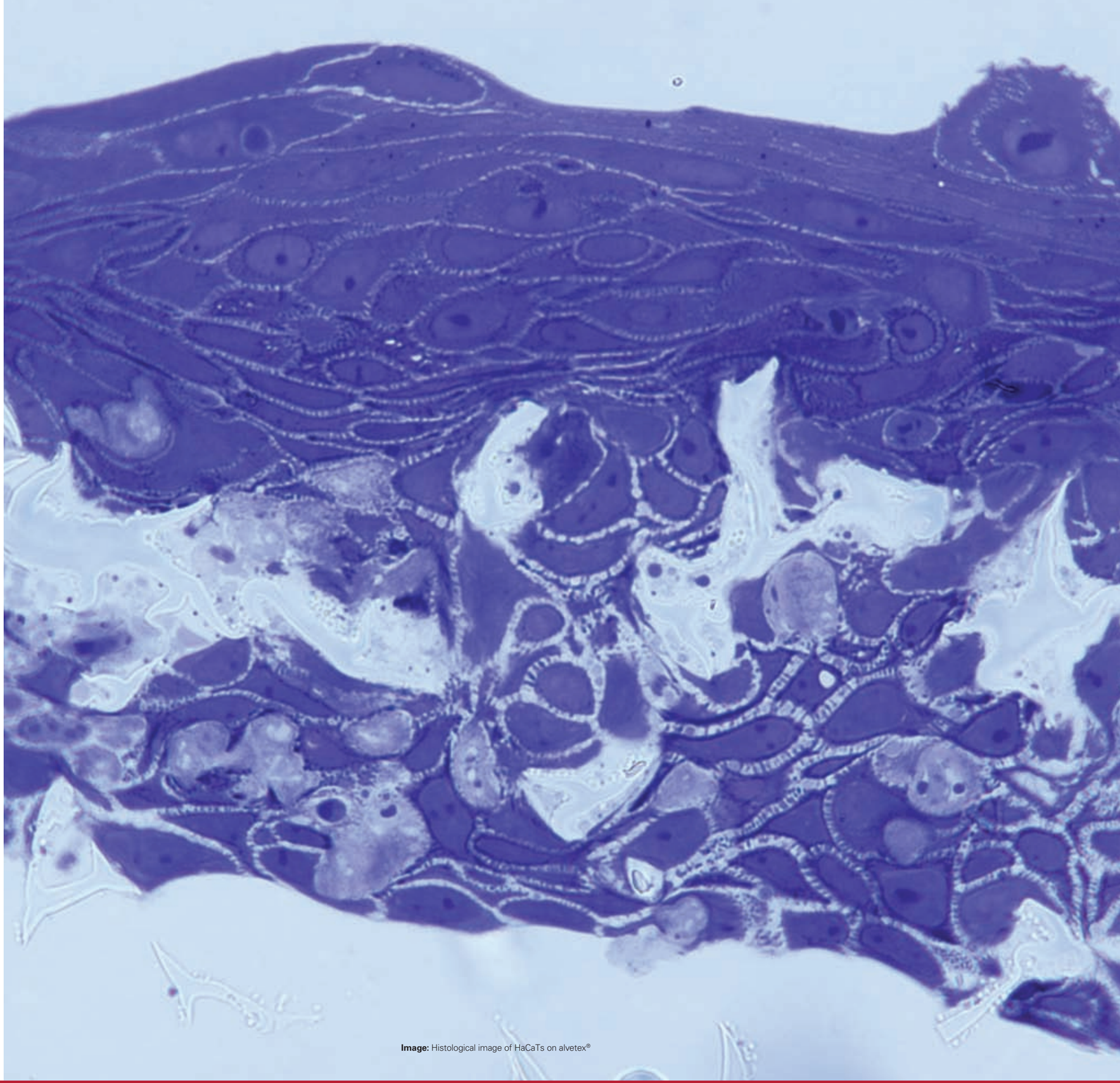


Image: Histological image of HaCaTs on alvetex®

tel: +44 (0)1740 625266
fax: +44 (0)1740 625251

e-mail: info@reinnervate.com
web: www.reinnervate.com

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ENABLING CELL TECHNOLOGIES