alvetex®

the breakthrough in

3D cell culture

Genuine 3D cell culture
Simply and routinely

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AMSBIO is the global source for alvetex®. alvetex® is a registered trade mark of and manufactured by Reinnervate.
Why is 3D Better Than 2D?

**In vivo**

- Real tissue: natural 3D environment

**In vitro**

- ‘The growth and study of cells in the laboratory, outside the body’

Genuine 3D cell culture
Why is 3D Better Than 2D?

Two dimensional cell culture (2D)

- Cells respond to their surrounding environment
- Culturing cells on flat plastic-ware results in artificial 2D monolayers of cells
- Cell interactions are lost, shape changes influence gene and protein expression, hence the function of the cell is different

Genuine 3D cell culture
Why is 3D Better Than 2D?

Three dimensional cell culture (3D)

Recreating the natural 3D environment

Real tissue

How do we define 3D cell culture?

Genuine 3D cell culture
What is Three Dimensional (3D) Cell Culture?

3D cell culture is about creating suitable surroundings for **optimal cell growth, differentiation and function** by:

- Allowing individual cells to maintain their **normal 3D shape and structure** with minimal exogenous support and interference,

- Encouraging cells 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 **tissue structures**,

- Reducing stress and artificial responses as a result of cell adaptation to flat, 2D growth surfaces.

Genuine 3D cell culture
Cultured cells in 3D environments are much more similar to cells in a living organism \((in \ vivo)\) than flat, unnaturally thin, single layer cells grown on 2D plastic:

- **Cell shape:**
  Typical cells in 3D are ellipsoids with dimensions of 10-30\(\mu m\). Cells in 2D are flat with typical thickness of 3\(\mu m\).

- **Environment:**
  Typical cells in 3D have nearly 100% of their surface area exposed to other cells or matrix. Cells in 2D have approximately 50% of their surface area exposed to fluid, approximately 50% exposed to the flat culture surface or intermediate, and a very small percent exposed to other cells.

- **Cell Behaviour:**
  Cells in 3D, as compared to 2D, show differences in Differentiation, Drug Metabolism, Expression (Gene, Protein), General Cell Function, In Vivo Relevance, Morphology, Proliferation, Response to Stimuli, and Viability.

**Genuine 3D cell culture**
<table>
<thead>
<tr>
<th>3D vs 2D Cell Culture Effect:</th>
<th>Examples of Supporting Scientific Literature:</th>
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</thead>
</table>
| Differentiation            | Increased bone differentiation by human stem cells  
Comparison of osteogenesis of human embryonic stem cells within 2D and 3D culture systems  
Tian et al. Scandinavian Journal Clinical Laboratory Investigation, 2008 |
| Drug metabolism           | Enhanced p450 enzyme activity in human hepatocytes  
Enhancing drug metabolism activities of C3A - A human hepatocyte cell line - By tissue engineering within scaffolds  
Elkayam et al. Tissue Engineering, 2006 |
| Gene, protein expression   | Enhanced differentiation of bone by murine marrow cells  
Long-term bone marrow cultures from adult mice show osteogenic capacity in vitro on 3-dimensional collagen sponges  
Shoeters et al. Cell Proliferation, 1992 |
| General cell function      | Increased sensitivity of hepatocytes to model toxicants  
Culture of HepG2 liver cells on 3D polystyrene scaffolds enhances cell structure function during toxicological challenge  
| In vivo relevance          | Increased neuron/astrocyte ratio of rat hippocampal neurons and astrocytes  
Superior survival and durability of neurons and astrocytes on 3-dimensional aragonite biomatrices  
Peretz et al. Tissue Engineering, 2007 |
| Morphology                 | Maintained original spindle-shape morphology of rat olfactory ensheathing cells  
Phenotypical analysis of adult rat olfactory ensheathing cells on 3-D collagen scaffolds  
Wang et al. Neuroscience Letters, 2006 |
| Proliferation              | Decreased proliferation of sheep bone marrow stromal cells  
Engineering of osteoinductive grafts by expansion of ovine bone marrow stromal cells directly on 3D ceramic scaffolds  
Scaglione et al. Biotechnology and Bioengineering, 2005 |
| Response to stimuli        | Upregulation of stem cell survival signaling including beta-catenin, Notch1 and Survivin  
Characterizing cancer cells with cancer stem cell-like features in 293T human embryonic kidney cells  
Debeb et al. Molecular Cancer, 2010 |
| Viability                  | A highly porous 3-dimensional polyphosphazene polymer matrix for skeletal tissue regeneration  
Laurencin et al. Journal Biomedical Materials Research, 1996 |

(Source: 3DCellCulture.com)
alvetex® has particular design attributes to enable routine 3D cell culture:

- **Reproducible and consistent in structure**
- **Inert, does not degrade and remains stable**
- **Adaptable to existing cell culture formats**
- **Material is the same as existing 2D products**
- **Transferable and accessible**
- **Developed as a consumable product**
- **Applications exemplified**

Genuine 3D cell culture
What is Alvetex®

• Highly porous interconnected scaffold made from cross-linked polystyrene

• Discs have a thickness of 200 microns to ensure sufficient diffusion of gases, nutrients and waste products

• Controlled parameters:
  - void and pore size – porosity
  - surface area
  - mechanical /chemical properties

Genuine 3D cell culture
Adapting the Material for Cell Culture

Engineering alvetex® ready for receipt of cultured cells in existing formats of tissue culture plastic-ware

200 µm thick porous polystyrene membrane

Genuine 3D cell culture
The growth of a range of alternative cell types has been demonstrated on alvetex® including...

- fibroblasts
- osteoblasts
- keratinocytes
- neural stem cells
- tissue
- primitive cells
- epithelial cells of the lens
- glioma
- neurons
- pluripotent stem cells
- adipocytes
- placental cells
- astrocytes
- tumour cells
- islet cells
- mesenchymal stem cells

Genuine 3D cell culture
Confocal imaging of hepatocytes (seeded at low density) labelled with a fluorescent dye grown on 3D (alvetex®) and 2D (standard cell culture plastic) polystyrene substrates.

Viable cells (green) imaged using Nikon ECI 3 laser confocal.
In vitro derived 3D tissues grown in alvetex® can be studied using standard molecular and cellular techniques:

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

Genuine 3D cell culture
Paraffin embedded, sectioned (10µm), counter stained

The epidermal cells cultured for 7 days in 3D alvetex®, fixed in PFA, embedded in wax and sectioned (7 micron) before staining with H&E and cover-slipping on standard microscope slides.
Cells grown in 3D have produced a tissue layer that has been fixed and sectioned for immunocytochemistry.

The top panel shows the phase contrast image, the middle panel shows staining with the fluorescent nucleus marker, DAPI, the bottom panel shows staining for Ki67, a marker of proliferating cells.

Note that alvetex® does not auto-fluoresce and is seen as black.
To encourage cell adhesion, differentiation and optimize function, AMSBIO uniquely offers alvetex as a stand alone product or in combination with high quality proteins like Basement Membrane Extract, Collagen I and IV, Fibronectin, Laminin, Poly-lysine, Vitronectin, animal-free MAPTrix recombinant proteins biomimetics etc. can be employed.

Scaffold pre-loaded with Collagen IV.
The ECM protein forms a web of fibers spanning voids into which cells can grown and migrate in 3D.
Optimization of 3D Cell Culture

- The appropriate format of alvetex® should be matched to the **requirements of the assay**
- Careful consideration has been given to the presentation of the scaffold to enable optimal 3D culture over short-term and long-term periods

**Plate**
Ideal for **short-term cultures**

**Insert**
Ideal for **long-term cultures**

5 day culture
**12-well plate vs well insert**
TERA2.cl.SP12 cells
Maintaining 3D Culture Models

Well insert holder inside a deep petri-dish

Key benefits:

• Convenient – reduces frequency of media changing

• 3D culture experiments of up to several weeks

• Petri dish facilitates use of magnetic stirrer to increase media circulation

• 3 Alternative insert heights give users better control over medium volumes

• Raise 3D cultures up to the air liquid interface (e.g. skin application)

• Up to 80ml of medium can be used

Genuine 3D cell culture
Versatile Well Insert Technology

Culture media filled to contact alvetex® from below only

For 3D cell growth at air/liquid interface

Culture media filled to contact alvetex® from above and below independently

For 3D cell growth with different media constituents

Culture media filled to contact alvetex® from above and below connected

For 3D cell growth with uniform media constituents

Genuine 3D cell culture
Single device providing **flexibility** to optimise 3D culture using alvetex®

- **Versatile Well Insert Technology**
  - Compatible with multi-welled plates and Petri dish holder
  - Flexible and maximizes user applications
  - Developed specifically for optimization of 3D cell culture
  - 6 and 12-well formats

**12-well format designed to fit 6- and 12-well plates**

Genuine 3D cell culture
Exemplification of **alvetex**®

**alvetex**® is positioned as a generic solution to 3D cell culture

**Liver toxicity assay**
- Intended as a routine toxicological screen
- Primary application is drug development

**Skin barrier assay**
- Intended as routine skin barrier assay
- Primary applications in cosmetics and pharmaceutical development

**Stem Cell Differentiation assay**
- Creating the 3D environment for cell differentiation
- Multiple applications to enable the formation of more complex tissues

**Adapting cells to 3D culture**
- Derivation of cell lines in 3D
- Adaption of existing cell lines to 3D culture

Genuine 3D cell culture
3D Cell Culture of Liver Hepatocytes

Structure of Liver Cells on alvetex®

HepG2 cells cultured for 2 weeks on alvetex® inserts
HepG2 liver cells grow homogeneously on alvetex® and form structures characteristic of liver tissues in the body.

2D cell growth (by SEM)

3D cell growth (by SEM)

bile canaliculus (by TEM)

HepG2 liver cells grown in 3D on alvetex® outperform their counterparts grown on standard 2D tissue culture plastic.

Primary Hepatocyte Viability Significantly Enhanced Using alvetex®


Genuine 3D cell culture
Cyp p450 expression in primary rat hepatocytes cultured for 3 days in 2D and 3D culture. Cells were induced to express cytochrome P450 using APAP (acetaminophen). Cyp enzyme levels determined by EROD and LC/MS assays.

3D Culture of Keratinocytes

Modeling Skin and Epidermal Barrier Function *in vitro*:

- *In vitro* models show poor development of the stratum corneum, an essential component of the epidermal barrier.
- Models of the stratum corneum will be useful for drug penetration studies, modeling disease, barrier function, etc.
- Demand for *in vitro* skin systems has increased in light of changing legislation concerning the testing of cosmetic products on animals.
Existing 3D Skin: Raft Cultures

Fabrication of organotypic cultures of mammalian skin – current practice

Existing RAFT cultures suffer from several impracticalities:

- Variability
- Preparation and set up time
- Technically challenging
- Not amenable for routine use

Genuine 3D cell culture
Development of the Epidermal Barrier

-alvetex® and dermal equivalent

-alvetex® only

Genuine 3D cell culture
Medium-term 3D Skin Culture with Collagen

Genuine 3D cell culture
Long-term 3D Skin Culture with Collagen

Stratified epidermis grown on alvetex® containing collagen and dermal fibroblasts

Cornified surface of epidermis showing squamous cells lifting at the air / liquid interface

Genuine 3D cell culture
Culture of 3D Epidermis Using **alvetex**® Only

(14 and 21 days, left and right respectively)

Development of the stratum corneum

Genuine 3D cell culture
Culture of 3D Epidermis Using alvetex® Only

(21 and 35 days, left and right respectively)

Maturation of the stratum corneum

Genuine 3D cell culture
Structure of the Epidermis: Characterization of Cell Layers

- Epidermolytic plantopalmar keratoderma (EPPK)
- Epidermolytic hyperkeratosis (EH)
- Epidermolysis bullosa simplex (EBS)

Genuine 3D cell culture
Detection of Keratin 14 – marker of immature keratinocytes

Phase  
DAPI  
Keratin 14
Culture of 3D Epidermis using alvetex®

Detection of Keratin 10 and Involucrin – markers of maturing keratinocytes

Genuine 3D cell culture
Culture of 3D Epidermis using alvetex®

Detection of cytokeratin 1 (ICC) and Involucrin (western blot)
markers of maturing keratinocytes

Genuine 3D cell culture
3D Culture of Stem Cells and their Differentiated Derivatives

Enhancing the Development of Potential Stem Cells using alvetex®

- Cell differentiation is a complex process influenced by the environment in which cells grow and differentiate.

- Current 2D in vitro models limit 3D cell-cell interactions which impacts on their ability to differentiate into complex tissues.

- **Differentiation in 3D creates a more realistic niche environment** enabling more appropriate signaling between cells and the ECM.
Differentiation of bone by rat MSCs

**Osteoblastic differentiation:** DMEM (10% FCS), 100nM dexamethasone, 100 µg/ml ascorbic acid-2-phosphate, 10nm β-glycerophosphate

**Alkaline Phosphatase**
- (early marker)

**Osteocalcin**
- (later marker)

**Genuine 3D cell culture**
Osteoblasts grown on alvetex® remain viable and form complex interactions between adjacent cells.

After 21 days in culture, cells were found to secrete extracellular collagen and form bone nodules.
Differentiation of Mesenchymal Stem Cells on alvetex®

Differentiation of **fat** by rat MSCs

Staining for Oil Red O in cyto-spun fat cells. Note the **presence of lipid droplets** in 3D cells grown using alvetex®

**Elution and measurement of Oil Red O – lipid marker**

**Flow cytometry analysis** of Nile Red (a lipid soluble fluorescent dye) after **cell extraction** from the alvetex scaffold using trypsin

Genuine 3D cell culture
Human Pluripotent Stem Cells

- **Human pluripotent stem cells differentiate** and form complex tissues in teratomas following cell transplantation (Przyborski, 2005)

- 3D cell growth is essential in order to achieve complex differentiation (Blum & Benvenisty, 2007)

3D culture of **Human Pluripotent Stem Cells** on alvetex®

Genuine 3D cell culture
alvetex® loaded with Basement Membrane Extract and seeded with human pluripotent stem cells induced to differentiate for 21 days.

Sample stained with Masson’s Trichome picking out the blue of the collagen present in the Basement Membrane Extract.

Acinus / gland-like structure with central lumen

Scaffold loaded with Basement Membrane Extract (blue)

alvetex® scaffold

Genuine 3D cell culture
Formation of Complex Structures During Stem Cell Differentiation in 3D Cell Culture Using alvetex®

Genuine 3D cell culture
Summary

• **alvetex®** technology provides a versatile platform for routine 3D cell culture that is **adaptable for multiple applications**

• **alvetex®** products are **easy to use, cost effective**, and is widely available to the scientific community

• **alvetex®** technology has been exemplified and its use is becoming widespread

Genuine 3D cell culture
<table>
<thead>
<tr>
<th>Product Description</th>
<th>Catalogue Number</th>
<th>Quantity</th>
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<tr>
<td>alvetex® 12-well plate</td>
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<td>alvetex® 6-well insert</td>
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<td>alvetex® 12-well insert</td>
<td>AMS.AVP005-34</td>
<td>12x 12-well insert</td>
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<tr>
<td>alvetex® well insert holders and deep Petri-dishes</td>
<td>AMS.AVP015-2</td>
<td>2x well insert holders (for 3 inserts each) and deep Petri-dishes</td>
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</tbody>
</table>

*inserts to purchase separately*
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