amsbio

Physiologically Relevant Cell Culture

Scaffolds | Hydrogels | ECM Proteins | Assays

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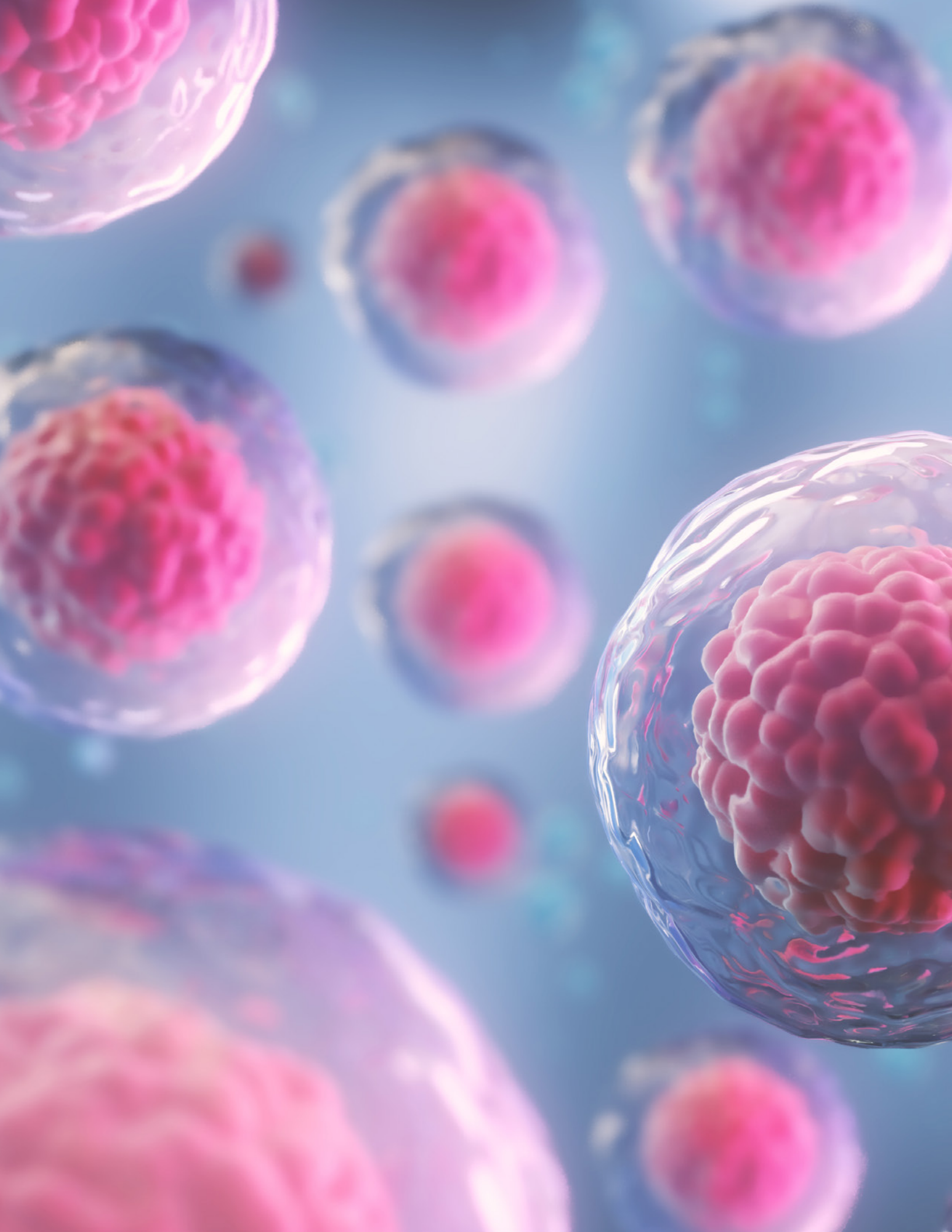


Introduction to Physiologically Relevant Cell Culture

Biological sciences rely heavily on tissue culture techniques. Until recently, the only techniques available for cell or tissue culture were limited to 2D techniques. Reconstituting a physiologically relevant environment in two dimensions is nearly impossible. The cells are exposed to high levels of serum and oxygen, they adapt their morphology to the 2D environment (thus modifying protein expression), and crucial intercellular interactions are reduced to the insignificant levels. While these techniques are successful in supporting many cell lines, there is increasing concern that they provide an inadequate model for *in vivo* physiological contexts. While animal models are the closest *in vivo* experimental system, they have limited applications. Many diseases (such as Down's syndrome) cannot be reliably recreated in murine animal models due to genomic, developmental and functional differences. This handbook contains a collection of tools and methods that are currently used to overcome the challenges that traditional 2D models display.

The biggest drawback with 2D models is the lack of an extracellular matrix. Not only does this lead to altered cell morphology, but crucial interactions between the cellular membrane and the extracellular matrix are lost. We offer artificial scaffolds, hydrogels, and extracellular matrix proteins (recombinant and natural) to address this issue. These products have been used successfully to grow 3D cultures from stem cells, cell lines and tissues.

The use of artificial scaffolds for 3D culture (Artificial Scaffolds section) such as Mimetix® or Alvetex® is particularly prevalent in regenerative medicine. These are known to support generations of various tissue types, such as liver, skin and neurons. This is complemented by the MAPTrix™ range of bio-mimetics, allowing tailoring of tissue culture conditions by addition of signals from specific extracellular proteins and/or growth factors.

2D cell behavior assays provide unrivalled throughput as drug development platforms, but are not accurately predictive of drug activity in the clinical setting. Providing a physiological context in these assays is a way to improve on the high attrition rates frequently observed in clinical trials. This can be achieved by introduction of 3D matrices, bio-mimetic arrays and other approaches covered in Cell Behavior Assays section.

Artificial Scaffolds

Naturally occurring hydrogels are an excellent tool to recapitulate the extracellular environment. However, they are very complex in composition and lack specificity to a particular tissue. As a result, several other solutions for the creation of physiologically relevant environment were developed. One solution involves replacing EHS-based materials with alginate, a naturally occurring hydrogel which is inert. Entirely artificial solutions are Mimetix®, a highly porous electrospun scaffold and Alvetex® a porous slab made of tissue culture plastic.

This enables seamless transition from 2D to 3D environments with several advantages: a very stable scaffold for cells to attach, grow, and differentiate on; and the ability to multiplex several cell types by combining slabs in various co-culture combinations. Alvetex® Strata is a new generation of this technology extending it to tissue slices and embryonic bodies.

MIMETIX® SCAFFOLD

MIMETIX Electrospun Scaffolds

Mimetix® scaffolds mimic the extracellular matrix by providing an ideal architectural environment to support the growth of cells in 3D. They are created by electrospinning the medical-grade polymer poly(L-lactide) (PLLA) into microfibers, which are highly consistent with regard to fibre diameter and pore size, resulting in excellent reproducibility of cell-based assays.

The Mimetix® scaffold is incorporated into standard SBS footprint well plate frames (96 and 384) with bases of superior optical clarity and minimal base distortion. The scaffold depth of 50 µm is thick enough to provide the benefits of 3D cell morphology and behavior, yet thin enough to allow microscopic imaging.

ADVANTAGES

- ✓ True 3D microenvironment
- ✓ Minimal protocol adaption required to switch from 2D
- ✓ Compatible with industry-standard automated handling and imaging equipment
- ✓ Scaffolds are free from animal derived materials and are synthesized using medical-grade polymers
- ✓ Supplied gamma-irradiated in individually-sealed plastic wrapping
- ✓ Excellent well to well and batch to batch consistency
- ✓ Compatible with fluorescent and light microscopy
- ✓ Scaffold can be coated or plasma treated for enhanced wettability

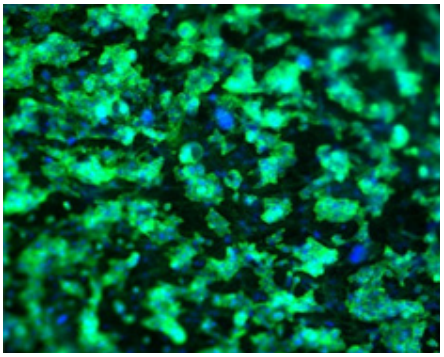


Figure 1. *Co-culture of primary keratinocytes and primary dermal fibroblasts in the Mimetix® scaffold after 24 h (fibroblasts in the background, keratinocytes in the foreground). Courtesy of Dr A.J. Bullock, University of Sheffield.*

SCAFFOLD SPECIFICATION

- ✓ Material: medical-grade poly-L-lactide (PLLA) (FDA-approved)
- ✓ Scaffold thickness: 50 µm
- ✓ Fiber diameters: 4 µm (= pores of 15-30 µm)
- ✓ Overall porosity: app. 80%
- ✓ Non-biodegradable in *in vitro* applications
- ✓ Supplied with low profile lid with condensation rings

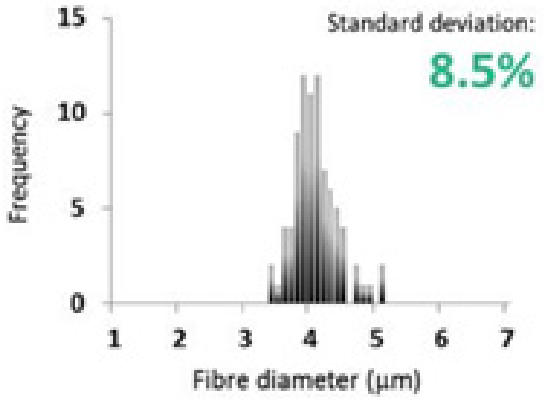


Figure 2. *Standard Deviation of fibre diameter.*

Mimetix® scaffolds come in two formats, as randomly orientated fibres and aligned fibres orientation scaffolds. Mimetix® randomly orientated scaffold is thick enough to provide true benefits of 3D cell morphology and behavior, designed for weight-bearing bone regeneration, corneal repair and neurosciences. It has been evaluated with a range of primary cells, stem cells and immortalized cancer lines. Mimetix® aligned scaffold is ideal for cells needing physical guidance and/or where cellular orientation influences cell behavior and function. It finds applications in cardiomyocytes, Schwann cells and oligodendrocytes studies, it is used for *in vitro* myelination assays as well as nerve conduit and tendon repairs.

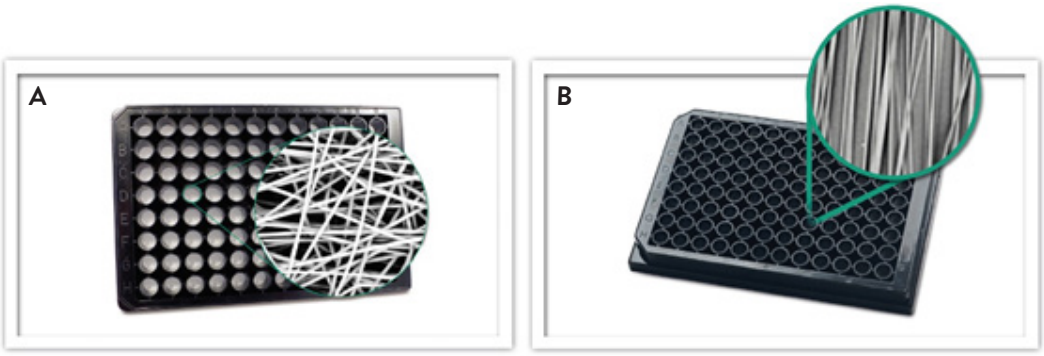


Figure 3. *Different formats of Mimetix® Scaffolds in 96-well plates (**A**) Randomly orientated fibres and (**B**) Aligned fibres orientation*

BENEFITS OF MIMETIX MULTIWELL PLATES IN 3D ASSAYS

- ✓ True 3D environment
- ✓ High consistency for reproducible cell-based assays
- ✓ Ready to use, sterile, standard size plates are compatible with industry-standard automated handling and imaging equipment
- ✓ Minimal protocol adaption to switch from 2D to 3D cell culture
- ✓ Material does not degrade or alter over the course of an experiment
- ✓ This scaffold provides advantages of 3D cell morphology and behavior, yet allows microscopic imaging

Product	Description	Catalogue No.	
		1 pack	8 pack
Mimetix® 384-well plate	384-well plates, fixed scaffold	AMS.TECL-001-1X	AMS.TECL-001-8X
Mimetix® 96-well plate	96-well plates, fixed scaffold	AMS.TECL-002-1X	AMS.TECL-002-8X
Mimetix® 12-well plate	12-well plates, removable discs and retaining rings	AMS.TECL-003-1X	AMS.TECL-003-8X
Mimetix® 6-well plate	6-well hanging inserts	AMS.TECL-004-1X	AMS.TECL-004-8X
Mimetix® 96-well plate aligned scaffold (2 µm fibre)	96-well plate, fixed scaffold	AMS.TECL-005-1X	AMS.TECL-005-8X
Mimetix® 12-well plate aligned scaffold (2 µm fibre)	12-well plate, cell crown inserts	AMS.TECL-006-1X	AMS.TECL-006-8X
Multiwell starter pack 1	1 x 12-well + 1 x 96-well plate	AMS.TECL-007-1X	
Multiwell starter pack 2	1 x 384-well + 1 x 96-well plate	AMS.TECL-008-1X	
Multiwell insert starter pack 3	1 x 12-well removable discs + 1 x 6-well plate with inserts	AMS.TECL-009-1X	

Mimetix® Custom Services

AMSBIO offers a custom service for electrospun fibres. These scaffolds can be precisely engineered based on **customers' choice of material, format, fibre diameter etc**. Mimetix® scaffolds are produced in Class VII cleanroom using state of the art electrospinning equipment from IME Technologies and are ISO 13485 certified. Each scaffold batch is checked under a scanning electron microscope (SEM) before shipment to the customer.

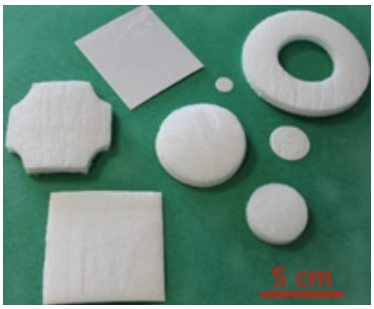


Figure 4. *Custom Mimetix® inserts.*

BENEFITS OF MIMETIX® CUSTOM SERVICES

- ✓ The manufacturing capabilities to develop personalised electrospun scaffolds from a large variety of polymers in custom formats
- ✓ Blend or co-spin various polymers, and can form complex nano-composite material
- ✓ Materials can be manufactured into implantable medical devices, coatings to improve the acceptance of implants, or supports for implantation of autologous or allogeneic cells for reconstructive therapy
- ✓ Electrospun scaffolds can be designed to mimic the extracellular matrix in terms of its architecture, chemical functionality and mechanical properties, therefore providing an ideal substrate for cell growth, differentiation and tissue repair
- ✓ Scaffolds can be made from FDA approved polymers and formulated to degrade over a defined period *in vivo* or be used as carriers for therapeutic compounds
- ✓ Bioactive compounds can be incorporated into the fibre structure for targeted release or modifying surface properties

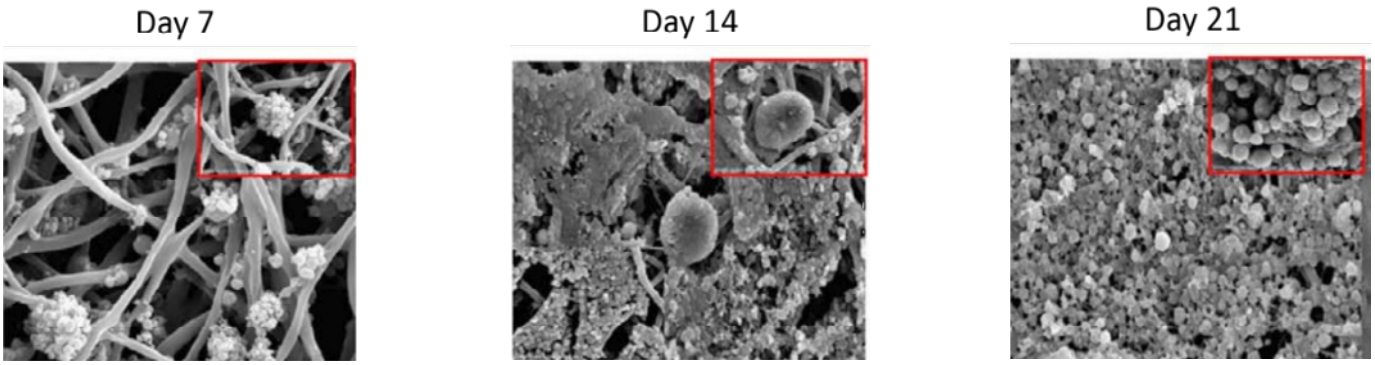


Figure 5*. 21-day culture of hMSCs in the PLLA + Collagen/HA scaffold, showing mineral deposition on the surface of osteoblast-like cells at day 14 and 21. Published by researchers at University of Malaysia.*

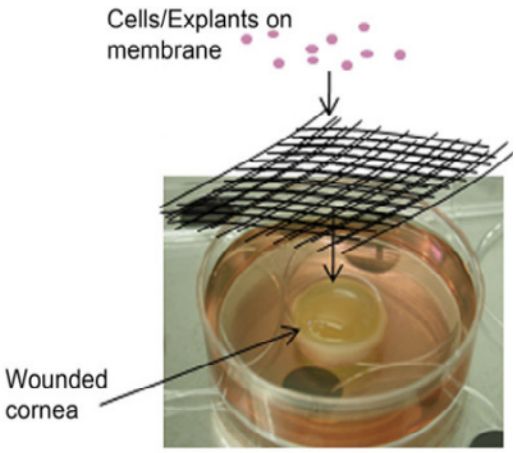


Figure 6*. These electrospun scaffolds are used in a project on corneal surface regeneration led by Professor Sheila MacNeil at the University of Sheffield. The project is supported by the "Welcome Trust Affordable Healthcare for India" programme and was conducting first-in-man studies in 2015.*

Selected Applications of Mimetix® Scaffolds

Mimetix® scaffolds have proven to be versatile and consistent environment for various research fields. Here we highlight a few experimental outcomes achieved using these electrospun scaffolds:

HIGH THROUGHPUT MYELINATION ASSAY

Our aligned electrospun Mimetix® scaffold allows the differentiation of more than 80% of oligodendrocyte precursors into mature oligodendrocytes in 14 days. 90% of the oligodendrocytes produce sheath lengths comparable to *in vivo* within 7 days. Mimetix® also provides physical guidance and support for Schwann cells.

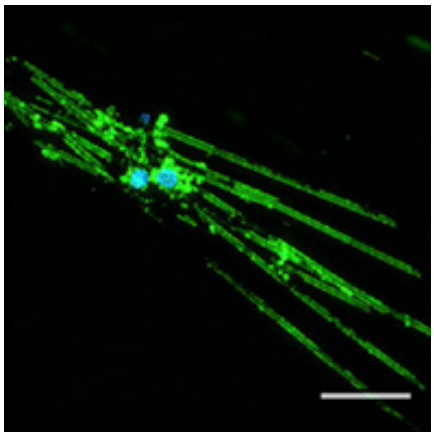


Figure 7. *Cortical oligodendrocyte precursors differentiating into active oligodendrocytes and lay myelin on the Mimetix® scaffold in the absence of neurons. Cells stained after 14 days for myelin basic protein (green) and Hoechst (blue). From Bechler M. E. et al. (2015) Curr. Biol. 25 (18).*

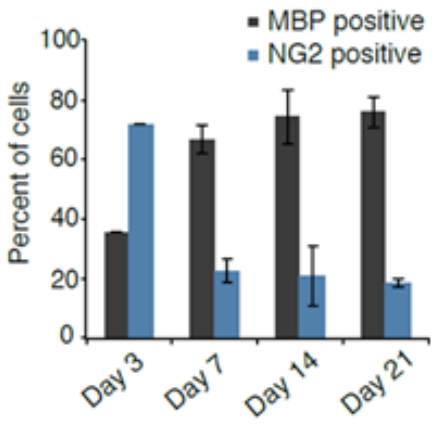


Figure 8. *Percent of oligodendrocyte precursors (NG2) and oligodendrocytes (MBP). More than 600 cells were counted per condition. Mean and standard deviation are shown for three experiments.*

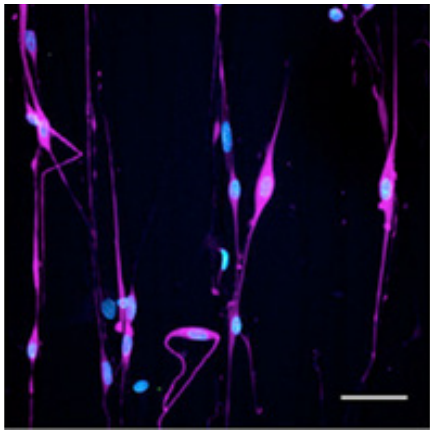


Figure 9. *Schwann cells grown for 21 days and stained with anti S-100 (purple) and Hoechst (blue). From Bechler M. E. et al. (2015) Curr. Biol. 25 (18).*

CARDIOTOXICOLOGY

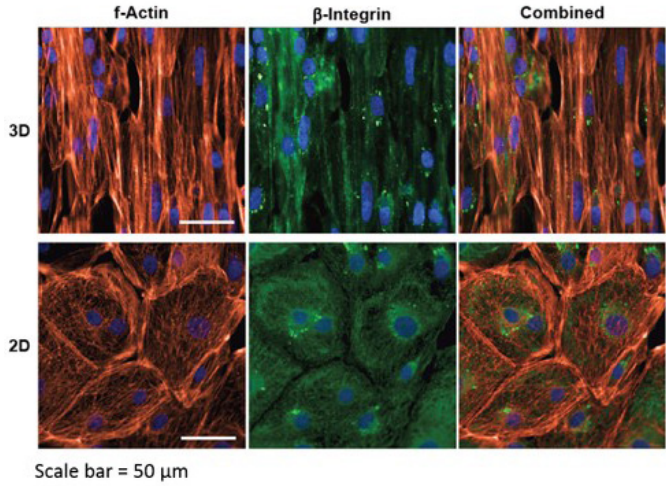


Figure 10. *Mimetix® Aligned scaffolds improve both structural and functional read-outs in cardiomyocytes, growing 3D cultures of spontaneously beating hiPSC-derived cardiomyocytes (hiPSC-CMs) in 96-well format.*

hiPSC-cardiomyocytes grown on such aligned 3D plates showed statistically significant higher Ca^{2+} transient rising slope (indicating faster kinetics), lower peak width durations, and lower amplitudes compared to standard 2D tissue culture plates. Experimental Work performed at Merk, USA.

3D DRUG DISCOVERY

Cancer cells grown in a more physiologically relevant 3D cultures have shown increased drug resistance compared to traditional 2D systems. Mimetix® scaffold has been successfully used as a matrix for numerous cancer cell models in 3D drug screening: liver, breast, ovarian as well as lung cancer models.

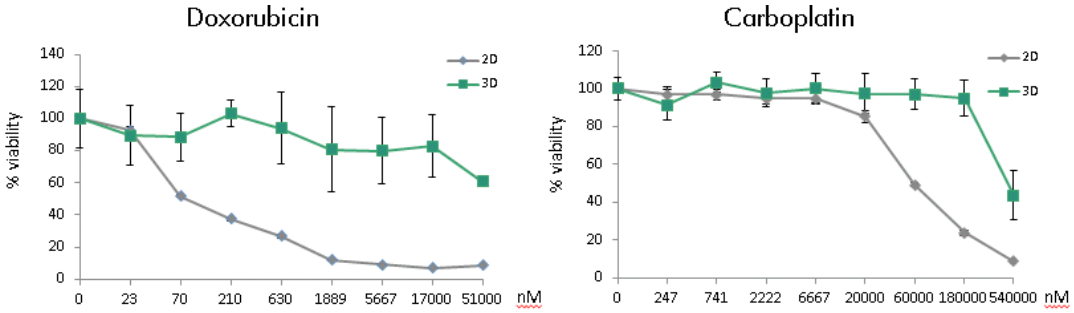


Figure 11. *SKOV-3 were seeded at 5,000 cells per well in 2D and Mimetix®. Cells were grown for 4 days in 2D and 24 days in Mimetix® (optimum schedule for each). Cells were exposed to the drug for 3 days in both schedules. Ovarian cells grown in Mimetix® are more resistant to both drugs tested. The resistance is observed when the drug is used at cytostatic concentrations too (below 5 mM).*

TOXICOLOGY STUDIES

HepG2 liver cancer cells are often used as model cultures for toxicology studies *in vitro*. Mimetix® scaffolds have proven to be provide a suitable environment for liver cells:

- ✓ **Metabolic functions** are preserved in Mimetix® for up to 28 days (Figure 12)
- ✓ **CYP activities** are similar to those in primary hepatocytes showcasing enzymatic drug metabolism *in-vitro*
- ✓ **Phase II enzymes** that are involved in solubility of drugs and hormones as well as excretable metabolites display same or increased expression in Mimetix® scaffolds compared to 2D controls (Figure 13)

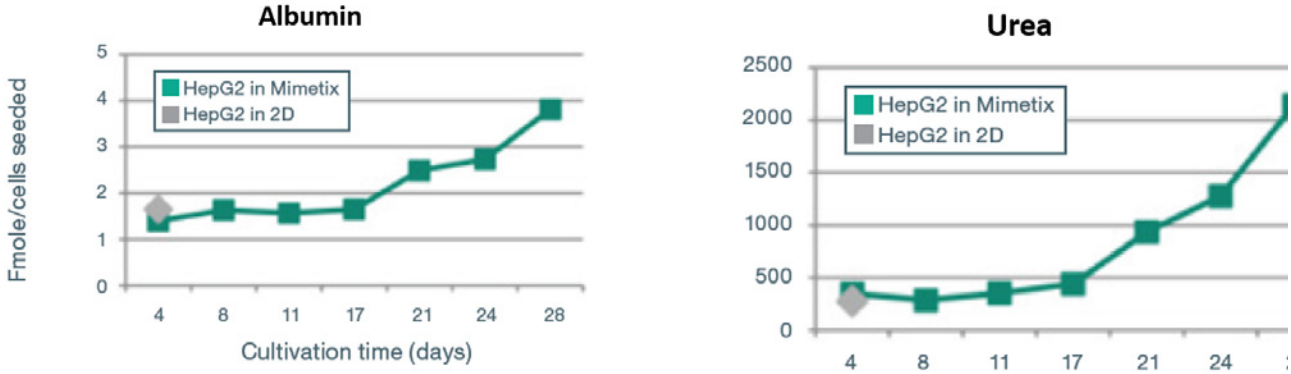


Figure 12*. HepG2 liver cancer cell culture continues to produce albumin (a sign of good hepatocyte functionality) and urea (a sign of good detoxifying properties) throughout 28 days of culture in Mimetix®.*

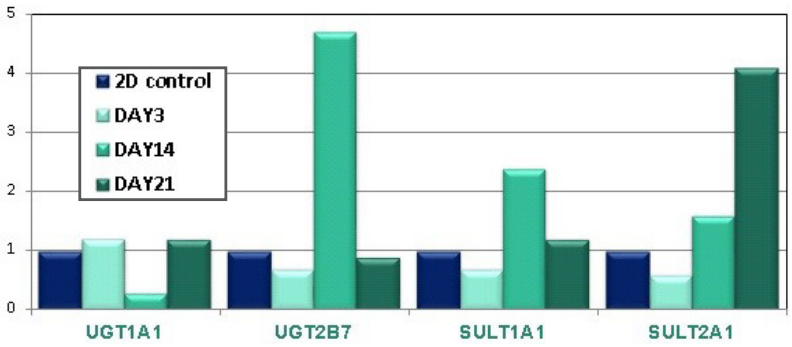


Figure 13*. Cells were seeded at a density of 10,000 cells per well in 2D and Mimetix®. RNA was extracted, cDNA was synthesized and qPCR was performed. Gene expression is calculated using the comparative Ct method and is relative to the 2D control on day3 and calibrated to GAPDH. All 4 enzymes either maintain (UGT1A1) or increase (UGT2A, SULT1A1 and SULT2A1) their expression levels over 21 days.*

CELL IMAGING IN 3D

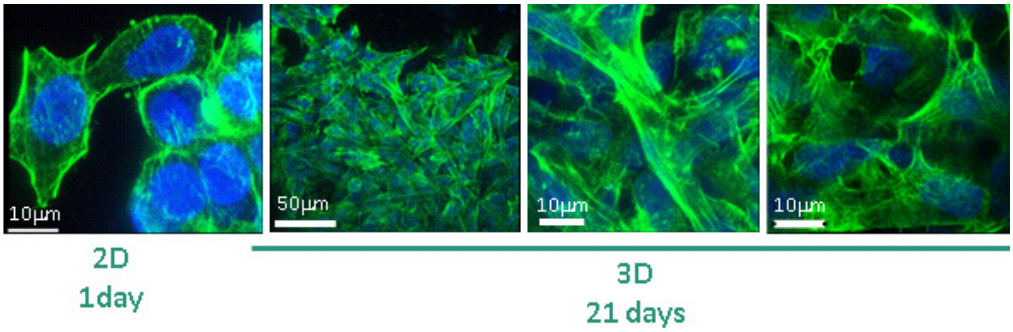


***Figure 14**. HepG2 were seeded at 10,000 cells per well and fixed. Nuclei were stained with DAPI, actin filaments with Alexa fluor 488 and imaging was performed using a Nikon Eclipse C1 confocal microscope half way down the scaffold (objective x60). Unlike in the 2D environment, liver cancer cells establish a 3D network in the Mimetix® Scaffold.*

STEM CELLS

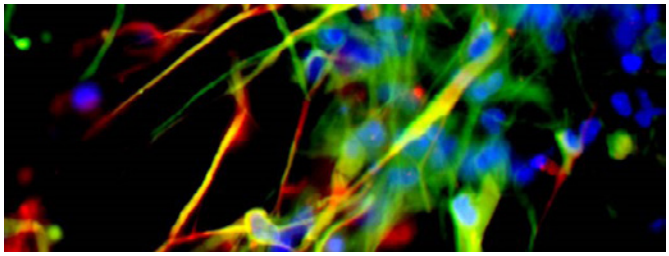


***Figure 15.** Differentiation of neural stem cells into mature neurons within the Mimetix® scaffold. Cells are stained with DCX (red) Tubulin βIII (green) DAPI (blue).*

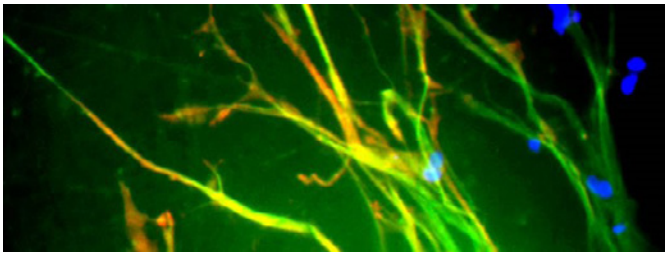


***Figure 16**. Neurite outgrowth on our Mimetix® scaffold. Cells are stained with DAPI (blue), Nestin (green) and GFAP (red). Both images: cell cultures on 12-well plate. Courtesy of Lara Stevanato, PhD, ReNeuron.*

SKIN MODEL

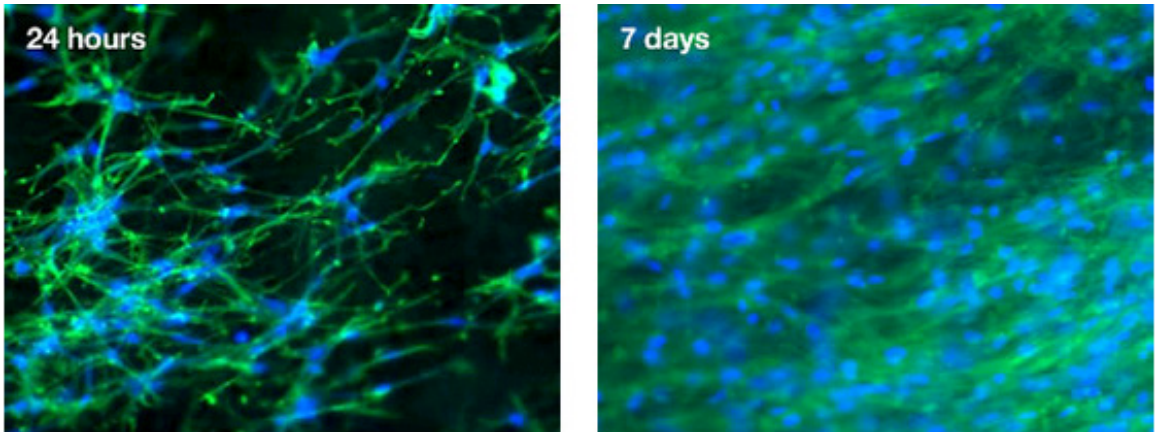


***Figure 17**. Primary dermal fibroblasts were cultured on our randomly orientated fibres scaffold and images were taken after 24 hours and 7 days. After 7 days in culture, cells had proliferated extensively to fill the pores within the scaffold.*

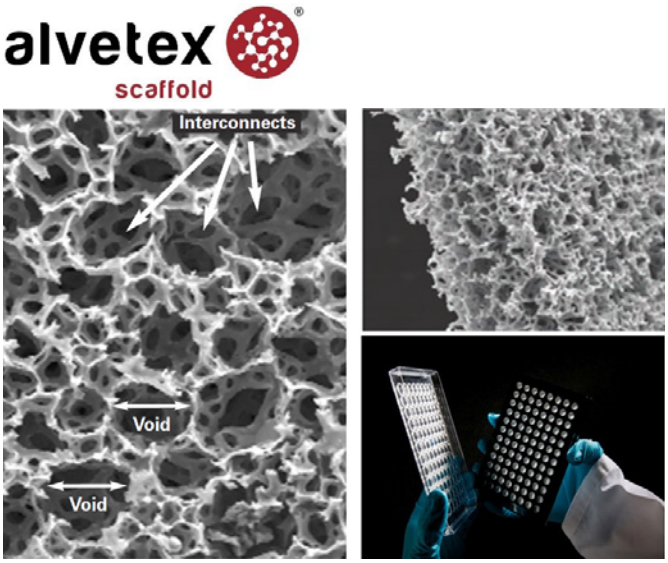


Figure 18. *In Alvetex® Scaffold, the void dimensions are ~ 36-40 µm in diameter (~ 75 cells per void) and interconnects are of ~12 -14 µm in diameter.*

Alvetex® is a highly porous (>90% porosity) cross-linked polystyrene scaffold, with a well-defined and uniform architecture, designed for 3D cell culture. It is engineered into a 200 µm thick membrane, consisting of voids linked to one another by pores (Figure 18) , which provides a 3D space into which cells can invade, proliferate and grow, thus ensuring the ultimate surroundings for optimal cell growth and functions. Alvetex® Scaffold is primarily considered for 3D culture of dissociated mammalian cells within the scaffold. Cells can penetrate throughout the whole scaffold with various proliferation rate depending on cell type. This scaffold can be pre-coated with ECM proteins, which form a web of spanning voids to encourage cells to form more complex interactions. Cells grown in Alvetex® often form a tissue-like structure due to laying down ECM that enables them to function in a more physiologically relevant manner. Cells maintain their *in vivo* morphology, behavior, and responsiveness within an *in vitro* model system. The scaffold reduces stress and aberrant responses as a result of the growth substrate. Traditionally, cultured cells normally grow on treated-polystyrene 2D surfaces as in standard cell culture plastic ware. Alvetex® presents cells with the equivalent growth substrate but in a 3D format. These materials are readily adaptable to different types of existing tissue culture plastic ware (e.g. multi-well plates, well inserts). The pre-fabricated, sterile culture device is ready to use off the shelf and can be handled in a similar manner as standard 2D plastic ware.

There are distinct advantages in using Alvetex® over existing 3D culture products which are technically more difficult to use, have a finite shelf life, and are expensive. Importantly, Alvetex® can be used for routine 3D cell culture, as an inert plastic. Alvetex® is a 3D culture product which can be treated in the same manner as traditional 2D cell culture plastic. It has been tested on a broad and growing selection of different cell types. The scaffold can be plasma-treated without any detrimental effect to its structure. Alvetex® is sterilised using gamma radiation: its manufacture is compatible with standard culture ware production. Furthermore, the use of polystyrene as a cell growth substrate is well accepted and recognised.

The Alvetex® Scaffold 96-well plate is a high-throughput format suitable for drug discovery. Plates have black walls and a clear plastic base, with Alvetex® Scaffold at the bottom of each well, thereby allowing direct luminesce read-outs in performing assays such as cell proliferation and viability.

ADVANTAGES

Many types of analysis once the 3D culture is complete:

- ✓ Imaging (histology, immunohistochemistry, confocal and electron microscopy)
- ✓ Easy retrieval of DNA, RNA and protein for gene and protein expression analysis
- ✓ Simple biochemical analysis (viability/proliferation)
- ✓ Analysis of secreted proteins in supernatant

Major cell types already used:

- ✓ **Cancer:** breast, colon, glioblastoma, lung and prostate
- ✓ **Liver:** including primary and liver carcinomas
- ✓ **Skin:** keratinocytes and full thickness models
- ✓ **Human ES cells**, mesenchymal stem cells, iPS and adipose tissue-derived stem cells (ASC)

Other commonly used cells including 3T3, CHO, HUVECs and MDCK

"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® Scaffold 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

Product Description	Pack Size	Catalogue No.
Alvetex® Scaffold 6-well inserts for 3D Culture	6 x 6-well insert	AMS.AVP004-32
	48 x 6-well insert	AMS.AVP004-48
	96 x 6-well insert	AMS.AVP004-96
Alvetex® Scaffold 12-well inserts for 3D Culture	12 x 12-well insert	AMS.AVP005-34
	48 x 12-well insert	AMS.AVP005-48
	96 x 12-well insert	AMS.AVP005-96
Alvetex® Scaffold 12-well plate for 3D Culture	1 x 12-well plate	AMS.AVP002
	10 x 12-well plate	AMS.AVP002-10
	80 x 12-well plate	AMS.AVP002-80
Alvetex® Scaffold 24-well plate for 3D Culture	1 x 24-well plate	AMS.AVP006
	10 x 24-well plate	AMS.AVP006-10
	80 x 24-well plate	AMS.AVP006-80
Alvetex® Scaffold 96-well plate for 3D Culture	1 x 96-well plate	AMS.AVP009
	10 x 96-well plate	AMS.AVP009-10
	80 x 96-well plate	AMS.AVP009-80
Alvetex® Scaffold 384-well plate for 3D Culture	2 x 384-well plate	AMS.AVP010-2
	10 x 384-well plate	AMS.AVP010-10
	80 x 384-well plate	AMS.AVP010-80
Alvetex® Scaffold Well Insert Starter Kit	6 x 6-well inserts, 6 x 12-well inserts and 1 deep petri dish	AMS.AVP-KIT-2
Alvetex® Scaffold Plate Starter Kit	1 x 12-well, 1 x 24-well 1 x 96-well plates	AMS.AVP-KIT-1
Alvetex® 2 x Well insert holder in a deep petri dish with lid for 3D culture	2 x insert holder, 1 x petri dish with lid	AMS.AVP015-2

Co-Culture and Perfusion Solutions Using Alvetex® Scaffold

Co-cultures are useful for modelling and studying the interaction and signaling between different cell types; for providing a more physiologically relevant way of demonstrating *in vivo*-like tissue morphology and function. This technique can be employed to monitor intercellular communication, cell migration dynamics, stimulation and maintenance of cell function and differentiation. Growing different cell types in 3D, inside and on the surface of Alvetex® Scaffold enables users to recreate complex tissue structures *in vitro*. A variety of cell co-culture scenarios can be set up to study different cell-cell interactions, according to the requirements of the cells and the dynamics under investigation. To further enhance the cell culture environment it is important to consider other factors such as the maintenance of the culture conditions over time. Tissues and organs of the body are continuously perfused by the blood circulatory and lymphatic systems, which together ensure a constant refreshment of nutrients and removal of waste products. The unique Alvetex® Perfusion Plates provide the opportunity for dynamic media flow and perfusion across cells cultured in 2D and/or 3D.

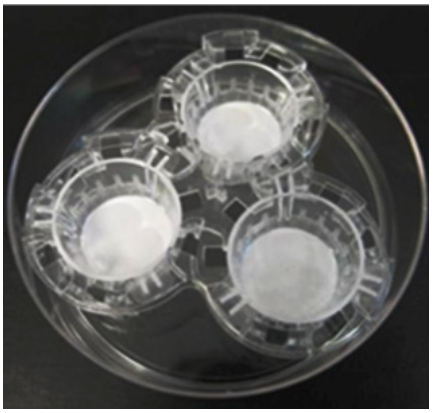


***Figure 19**. Alvetex® Scaffold inserts.*

MODULAR & FLEXIBLE ALVETEX® CO-CULTURE PLATFORM TECHNOLOGY

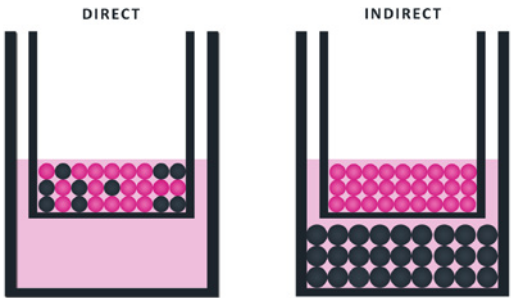


***Figure 20. Direct and indirect co-culture types.** Cell type A (black spheres) and B (pink spheres) are both growing in 3D. Direct co-culture occurs when different cell types are cultured together within the same scaffold (left), and indirect occurs when cultures are grown in two independent 3D columns (right). Contact between the two cell types is via the medium only and inter-culture communication takes place via paracrine excretions.*

BENEFITS

- ✓ Straightforward combination of co–culture in 3D
- ✓ Cells can be cultured directly or indirectly
- ✓ Flexibility of timeline of co-culture (different cell types can be added and removed at any time)
- ✓ Flow can be introduced into co-culture by using specially designed perfusion chambers

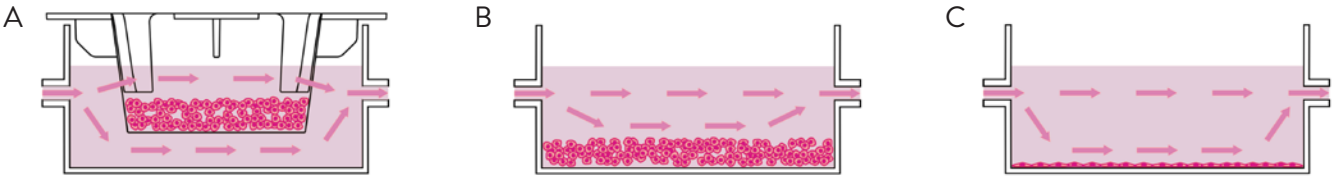


***Figure 21. Setting up different culture systems within the wells. (A)** Well inserts of different types can be fitted into the plate, including Alvetex® well inserts for 3D culture. Alvetex® well inserts have windows in their side wall to enable the flow of medium above and below the culture. **(B)** 3D cultures can also be established in the base of the well and media slowly perfused above. This arrangement is compatible with both hydrogel and scaffold-based 3D technologies. **(C)** Conventional 2D culture in the base of the well where a monolayer of cells is established and perfused with culture medium. Note: It is also possible to place tissue fragments in a well insert or base of the well and perfuse media over them for long-term maintenance in culture.*

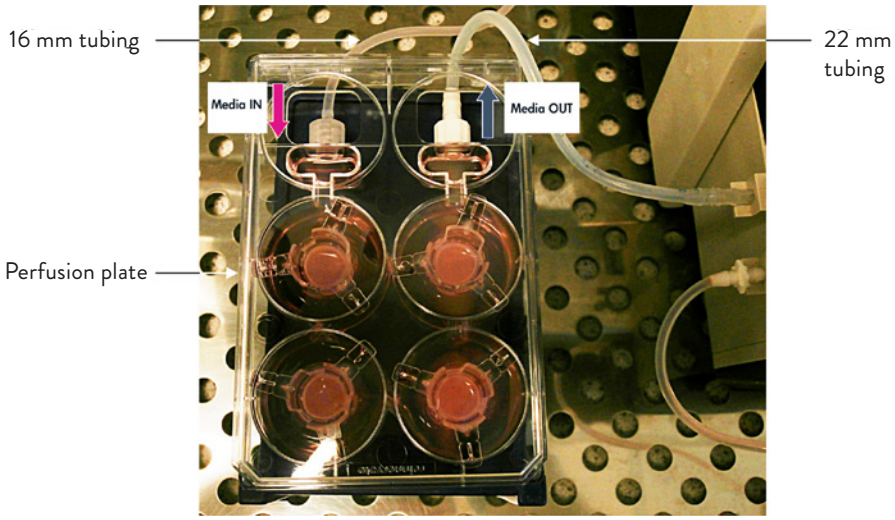


Figure 22. Photograph showing Reinnervate Perfusion Plate set-up and connected to peristaltic pump. *The plate has been raised by approximately 5 cm (placed on an empty pipette tip box). Note the difference in the tube diameters: Media IN 1.6 mm and Media OUT 2.4 mm. The example shown includes four Alvetex® well inserts within the plate with incubation medium.*

Product Description	Pack Size	Catalogue No.
Alvetex® Perfusion Plates	2 x Alvetex® perfusion plates and luer locks	AMS.AVP011-2
	10 x Alvetex® perfusion plates and luer locks	AMS.AVP011-10
Perfusion plates with Alvetex® well inserts	2 x perfusion plates and luer locks 12 x Alvetex® 6-well inserts	AMS.AVP-KIT-3
	2 x perfusion plates and luer locks 12 x Alvetex® 12-well inserts	AMS.AVP-KIT-4
	5 x perfusion plates and luer locks 48 x Alvetex® 6-well inserts	AMS.AVP-KIT-5
	5 x perfusion plates and luer locks 48 x Alvetex® 12-well inserts	AMS.AVP-KIT-6

Selected Applications of Alvetex® Scaffolds

LIVER MODEL

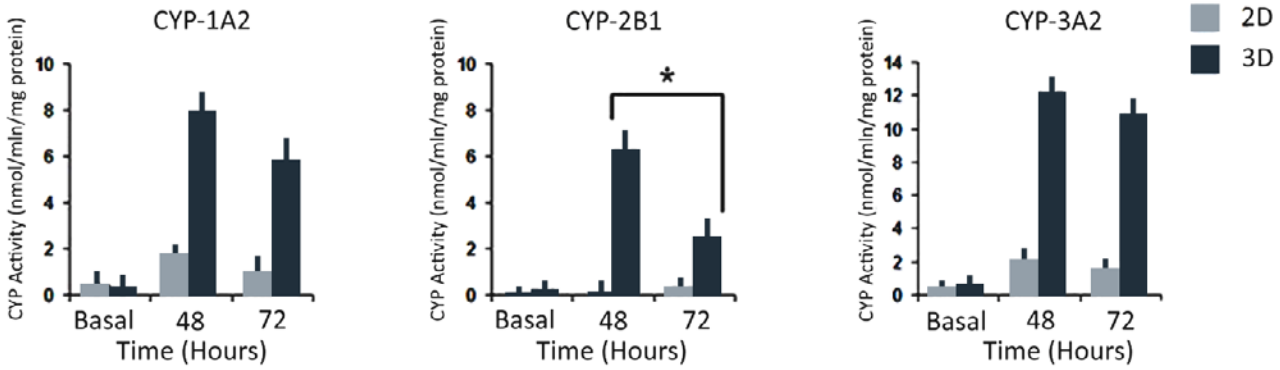


Figure 23. Function and responsiveness of 3D cultured liver cells in Alvetex® Scaffold *Rat hepatocytes were cultured in 2D (grey bars) and 3D in Alvetex® Scaffold (black bars) for 48 or 72 hours after induction and assay of different CYP isoforms. Culture in Alvetex® Scaffold resulted in enhanced inducible levels of all three enzymes tested. Figure from Bokhari M et al. (2007) Biochem. Biophys. Res. Comm. 354 (4).*

CO-CULTURE

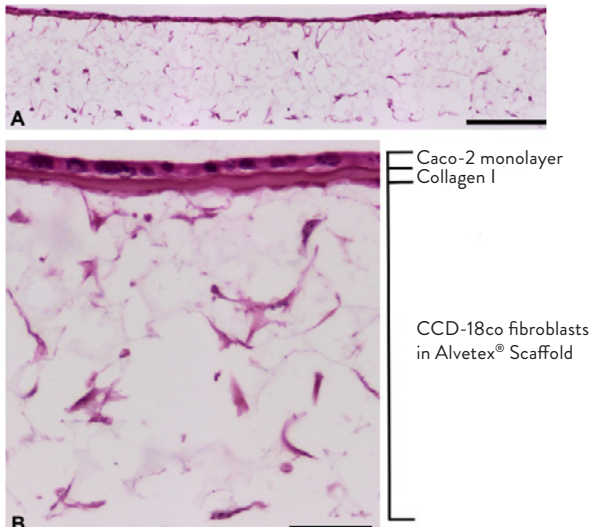


Figure 24. Co-culture of Caco-2 cells and CCD-18 fibroblasts separated by a Collagen I layer in Alvetex® Scaffold. *Low **(A)** and high **(B)** magnification brightfield micrographs of an even monolayer of Caco-2 cells form at the Collagen-coated top surface of Alvetex® Scaffold, with CCD-18Co fibroblasts underneath the same Collagen layer and distributed throughout the depth of Alvetex® Scaffold. CCD-18Co fibroblasts were grown on 22 mm diameter Alvetex® Scaffold discs presented in 6-well insert in 6-well plate format for 14 days prior to layering with Collagen I and seeding of Caco-2 cells. Co-cultures were grown for a further 5 days, after which they were fixed, embedded in paraffin wax, sectioned (10 µm) and counterstained with haematoxylin and eosin. Scale bars: 200 µm **(A)** and 50 µm **(B)**.*

NEURONAL/GLIAL CO-CULTURE

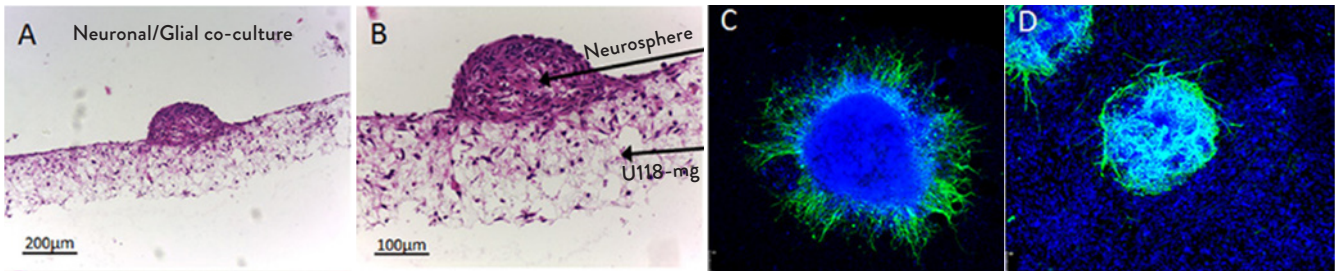


Figure 25. Studying interactions between neurons and glia in co-culture models using Alvetex® Scaffold. *Co-cultures of glial cells (U118-MG) and stem cell-derived neurons were set up to study cellular interactions and the effect of glial cells on neuritogenesis. First, 1 million U118-MG glial cells were seeded onto 10 µg/ml Laminin and poly-D-lysine coated Alvetex® Scaffold and incubated for 15 min prior to the addition of neurospheres and then cultured for a further 15 min. Co-cultures were incubated for 10 days and fixed in 4% PFA prior to imaging as before. Bright field micrographs showing low **(A)** and high **(B)** magnification images of neurosphere and glial co-cultures on Alvetex® Scaffold. Confocal imaging of neurospheres from above showed neurons producing neurites in the absence of U118-MG cells (**C**, control) and suppression of neuritogenesis in the presence of the glial cells (**D**, plus U118-MG). Cells were stained with TuJ1 (green) and DAPI (blue) to show neurons and cell nuclei, respectively. Note the uniform distribution of glia cells (**D**, DAPI stained nuclei) and how neurites tend to wrap around the neurosphere avoiding contact with the U118-MG cells **(D)**.*

Alvetex® Strata, which is a second generation of scaffold, has been designed primarily to support the growth of cells and intact tissues on the surface of the membrane. Alvetex® Strata is 200 µm thick highly porous membrane of cross-linked polystyrene presented in a well insert format. At first glance, the structure of Alvetex® Strata appears similar to Alvetex® Scaffold. However, the difference between these two materials concerns their fine structure and architecture: in Alvetex® Strata the voids and pores are significantly smaller (average 5 instead of 13 µm in diameter, respectively) compared to those in Alvetex® Scaffold. This product has multiple applications, including the ability to stably support intact viable tissues during cell culture subsequent analysis.

ADVANTAGES

- ✓ Enhanced porosity for improved nutritional support from the medium
- ✓ Modified surface topography to improve tissue attachment
- ✓ Versatility for co-culture and construction of advanced *in vitro* models

Description	Pack Size	Catalogue No.
Alvetex® Strata 6 well inserts	12 x strata 6-well inserts	AMS.STP004-12
	48 x strata 6-well inserts	AMS.STP004-48
Alvetex® Strata 12 well inserts	12 x strata 12-well inserts	AMS.STP005-12
	48 x strata 12-well inserts	AMS.STP005-48

Selected Applications of Alvetex® Strata

NEURONAL SLICE CULTURE

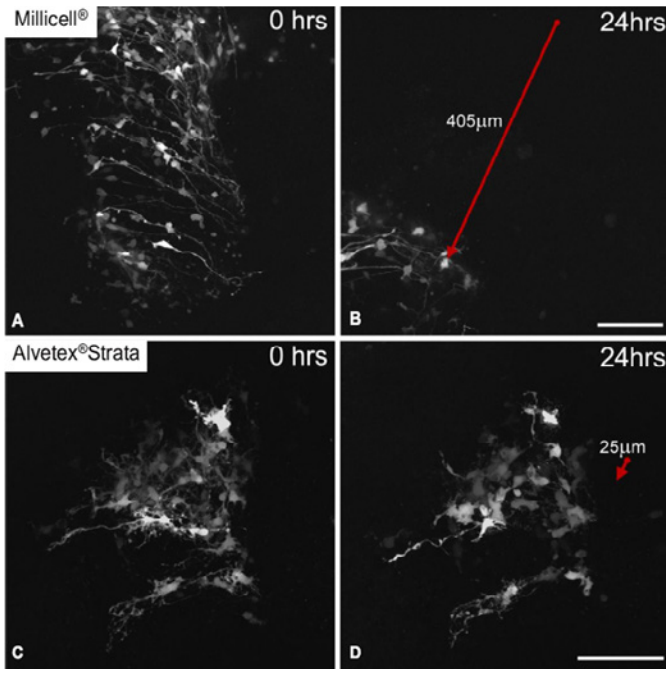


***Figure 26. Time lapse imaging of spinal cord tissue slice and demonstration of tissue slippage**. Images show the same field of view captured at time zero (**A,C**) and after 24 hours recording (**B,D**) for tissue slices maintained on either the Millicell® (**A,B**) or Alvetex® Strata (**C,D**) porous membranes. Note how the cellular information captured in the field of view from the sample maintained on the Millicell® membrane has all but slipped out of frame during the 24 hour period. In contrast, samples maintained on Alvetex® Strata remained almost completely static. The red arrows show the direction and extent of tissue slippage observed. Scale bar: 100 µm. Images courtesy of Kieran McDermott, University of Cork.*

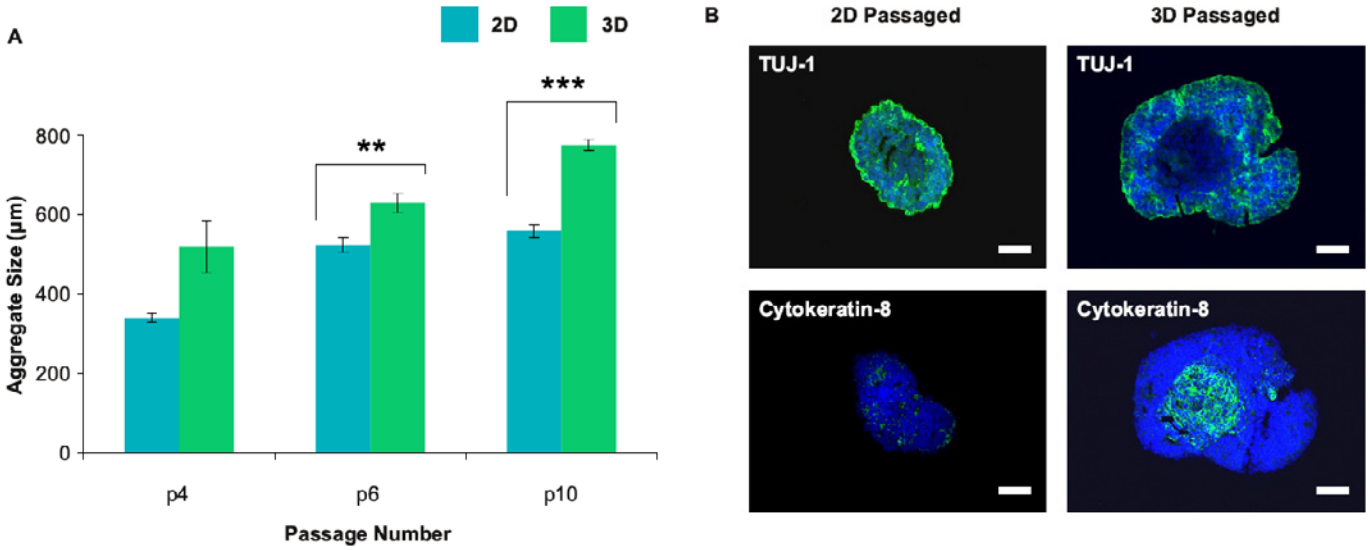


Figure 27. Passaging pluripotent stem cells in 3D results in their enhanced growth and differentiation when subsequently cultured as 3D suspended cell aggregates*. TERA2.cl.SP12 cells were propagated for 4-10 passages in 2D or 3D culture and then cultured in suspension to form aggregates. Cells maintained in 3D for 6-10 passages formed significantly larger diameter aggregates compared to cells continually propagated in 2D culture (**A**). Data represent mean, ±SEM, n=10, **p=0.01, ***p=0.001. Aggregates were immuno-stained for the neural maker TUJ-1, and the epithelial marker cytokeratin-8 (**B**). Cells passaged in 3D resulted in aggregates with greater cellular heterogeneity including intense TUJ-1 staining and areas with lower cell density expressing cytokeratin-8. Cells propagated in 2D culture formed smaller aggregates that were primarily TUJ-1 positive. Scale bars: 100 µm.*

Hydrogels

3D cell culture holds the key to bridging the gap between cell culture and cellular physiology by providing a better representation of the *in vivo* microenvironment and more importantly cell-cell and cell-ECM interactions, diffusion and fluid flow characteristics. It allows for better translation of *in vitro* studies to *in vivo* applications. Currently existing products, such as extracted and reconstituted basement membrane (BME), have associated with them a diverse array of unknown cues which can be highly disadvantageous, particularly when trying to isolate the effects of specific factors. In addition, these products generally have a variable composition as well as variable mechanical properties.

Hydrogels are result of the efforts to create a cell microenvironment that mimics the dynamic native ECM, which have been driven by the clinical demand for tissue and organ repair and replacement. They are formed of synthetic and/or natural extracellular matrices, cross-linked networks, and interconnected pores possessing high water retention. Interesting hydrogels features allow to utilize them as scaffolds for tissue engineering because it is easy to adjust their physico-chemical (electrical charge and pore size) and mechanical (stiffness and tensile strength) properties to the levels that are desirable for tissue scaffolds, cell encapsulation, immobilization and drug delivery.

3D culture systems for use with stem cells (SCs) are gaining popularity as matrix grown cells have more *in vivo*-like gene expression, better differentiation potential, proliferation and cellular function, and are more physiologically relevant *in vitro* models. Alginate is a convenient, easy to use hydrogel that has been used to develop successful 3D cell culture systems for a range of different cells types including tumor cells and chondrocytes. MAPTrix™ HyGel is an animal-free hydrogel that provides a well controlled and reproducible extracellular environment for 3D cell culture.

ALGINATE

Transformed cells, such as tumor cells, have the characteristic feature of anchorage- independent growth, unlike normal cells. Some normal cells, such as chondrocytes, are also capable of anchorage-independent growth, and the phenotypic expression of these cells is known to be stronger compared with monolayer cultures. Soft agar culture is a method in which cultures are grown with cells suspended in soft agar gel, and has been used conventionally as a method to detect the ability of cells to undergo anchorage-independent growth. As agar solidifies on cooling, the temperature must be maintained at approx. 37 °C while preparing the seed culture plate. Also, since special reagents are required when harvesting the cells in the gel, the resulting culture is not suitable for analysis of cell function.

In contrast, alginate, which is an anionic polysaccharide derived from cell walls of brown algae, forms a gel in the presence of calcium and liquefies to a solution upon addition of a calcium chelating agent. Alginate gel has been a choice for 3D cell culture because only cultured cells can be easily harvested. The Alginate 3D Cell Culture Kit is a convenient, easy-to-manufacture kit optimized to produce alginate gel beads. This product has been used to develop successful 3D cell culture systems for a range of different cells types including tumor cells and chondrocytes due to the ease at which cultured cells can be harvested. Alginate is typically used in biomedicine including wound healing, drug delivery and tissue engineering applications.

ADVANTAGES

- ✓ Easy to use
- ✓ Convenient
- ✓ Viability and cytotoxicity screening
- ✓ Compatible with fluorescent and confocal microscopy
- ✓ Allows immunohistochemistry
- ✓ DNA and RNA can be extracted for gene analysis
- ✓ Non-invasive study of cell signaling and communication

Figure 28. *Coagulated alginate beads.*

Description	Pack Size	Catalogue No.
Alginate 3D Cell Culture Kit	1 x kit	AMS.CSR-ABC-KIT
Sodium alginate solution	25 mL	AMS.CSR-ABC-AL
Calcium chloride solution	100 mL	AMS.CSR-ABC-CA
Sodium citrate solution	100 mL	AMS.CSR-ABC-CI

Selected Applications of Alginate

Hep G2 CELLS (HEPATOCYTE CARCINOMA)

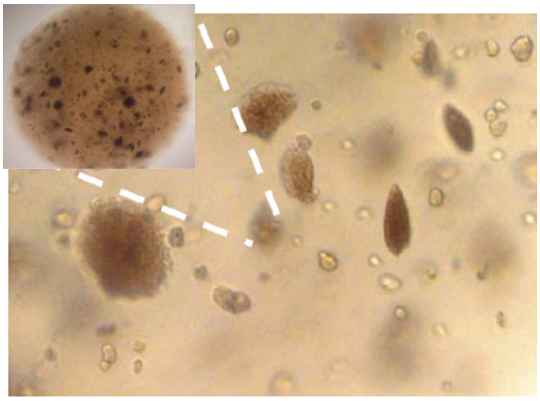


Figure 29. *HepG2 cells cultured in alginate beads for 9 days (5×10^5 cells/mL, 10 beads/well, DMEM containing 10% FBS).*

Saos - 2 CELLS (OSTEOGENIC SARCOMA)

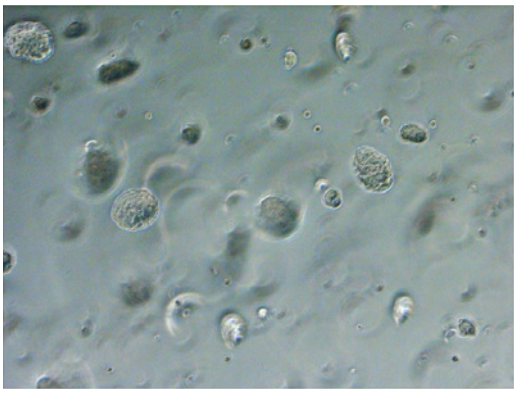


Figure 30. *Saos-2 cells cultured in alginate beads for 14 days (2×10^5 cells/mL, 10 beads/well, DMEM/F-12 containing 10% FBS).*

NORMAL CHONDROCYTES

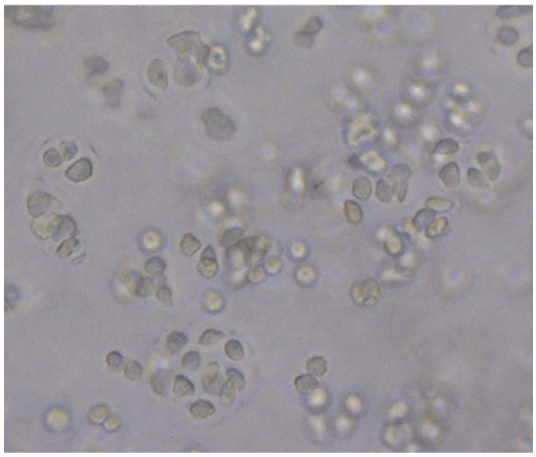


Figure 31. *Normal porcine chondrocytes prepared from articular cartilage of knee joint cultured in alginate beads (2x10^6 cells/mL, DMEM/F-12 containing: 10%FBS, 100 ng/mL IGF-I and 25 µg/mL L-ascorbic acid).*

MAPTrix™ HyGel

MAPTrix™ HyGel is a recombinant mussel adhesive protein-based biosynthetic three-dimensional extracellular matrix (ECM) line of products that are tailored to mimic biochemical and mechanical properties of native ECM. These hydrogel products are composed of two components: MAPTrix™ ECM, a mussel adhesive protein-based extracellular matrix (ECM) mimetic, and MAPTrix™ Link: a multi-arm polyethylene glycol derivative.

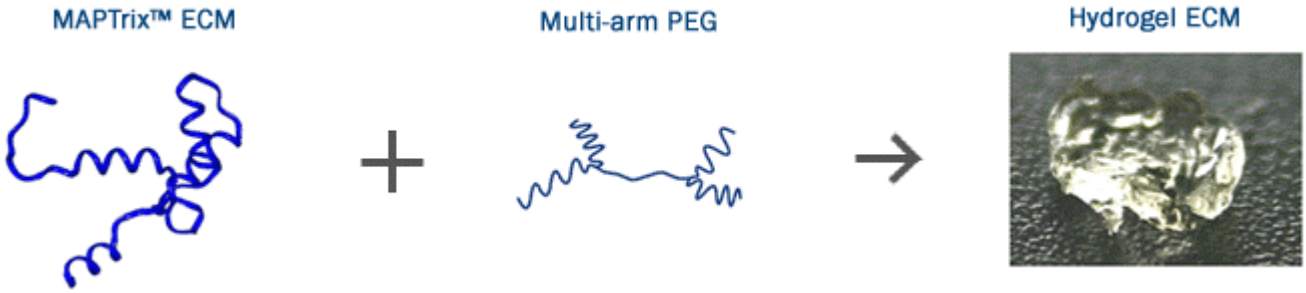


Figure 32: *Schematic view of MAPTrix™ HyGel composition.*

Use of MAPTrix™ HyGel generates a well-controlled and reproducible extracellular microenvironment for 3D cell culture and related applications. Hydrogel biofunctionality should be engineered predictably and precisely by tailoring biochemical functionality with MAPTrix™ ECM. By altering the gelation factors the scaffold structure can be designed specifically for your application.

Find out more about MAPTrix™ recombinant protein features, technology and selected applications on p. 28-34.

ADVANTAGES

- ✓ Ready to use formula to create biochemically-defined hydrogel *in situ*
- ✓ Easy to use stable under refrigerator conditions for 6 months: no freezing required
- ✓ Fully compatible with existing cell culture protocols

Selected Application of MAPTrix™ HyGel

MCF10A MAMMARY EPITHELIAL CELLS

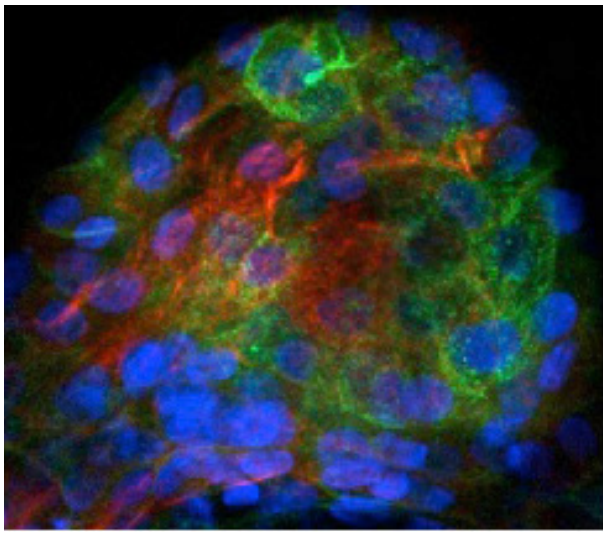


Figure 33*. MCF10A mammary epithelial cells forming an aggregate in MAPTrix™ HyGel. The thick section is about 100 µm. This image demonstrates a potential for morphogenesis, differentiation in basal/ myoepithelial cells (CD49f -green) and luminal cells (muc1 - red), nuclei are labeled with DAPI. Image courtesy of Pierre Savagner, Institute of Cancer Research of Montpellier.*

MAPTrix™ HyGel protein derivative	Mimetic motif	Catalogue No.
Fibronectin	RGD	36105Y
	GRGDSP	36107Y
	REDV	36124Y
	PHSRN-RGDSP	36125Y
	PHSRN	36104Y
Laminin	YIGSR	36414Y
	IKVAV	36224Y
	KAFDITYVRLKF	36442Y
	RQVFQVAYIIIKA	36204Y
	NRWHSIYITRFG	36226Y
	TWYKIAFQRNRK	36229Y
	RKRLQVQLSIRT	36232Y
	RYVVLPR	36411Y
	RNIAEIIKDI	36460Y
Collagen I	GLPGER	36501Y
	GFPGER	36504Y
	DGEA	36506Y
Collagen IV	GEFYFDLRLKGDK	36623Y
	TAGSCLRKFSTM	36621Y
	TAIPSCPEGTVPLYS	36631Y
Cadherin	LFSHAVSSNG	36702Y
	ADTPPV	36703Y
	LRAHAVDING	36708Y
Vitronectin	KKQRFRHRNRKGYRSQ	36802Y
	FRHRNRKGY	36801Y
Other	VAEIDGIEL	36831Y
	FHRRIKA	36902Y
	TTSWSQCSKS	36931Y

Key to catalogue numbers on page 34

Extracellular Matrix Proteins

The extracellular matrix (ECM) is the non-cellular environment of the cell that directs multicellular organization and provides structural support for tissues. The ECM provides anchorage for cells and connects directly to the cell cytoskeleton through trans-membrane receptors. These physiological interactions control vital cell functions, such as proliferation, differentiation, migration, polarity, and survival. These processes are regulated through modulation of the cells' epigenetic program and signal transduction cascades.

Basement membranes are continuous sheets of specialized extracellular matrix that form an interface between endothelial, epithelial, muscle, or neuronal cells and their adjacent stroma. Basement membranes are degraded and regenerated during development and wound repair. They not only support cells and cell layers, but they also play an essential role in tissue organization that affects cell adhesion, migration, proliferation, and differentiation. Basement membranes provide major barriers to invasion by metastatic tumor cells.

ECM proteins have revolutionized *in vitro* and *in vivo* cell models by providing optimal environmental conditions to promote physiologically relevant cellular structure and function. These proteins have been used to:

- ✓ Develop several organotypic models using 3D culture
- ✓ Provide barriers
- ✓ Evaluate metastatic potential
- ✓ Improve cellular implantation and evaluate angiogenesis *in vivo*
- ✓ Maintain stem cells in an undifferentiated state
- ✓ Induce stem cell differentiation

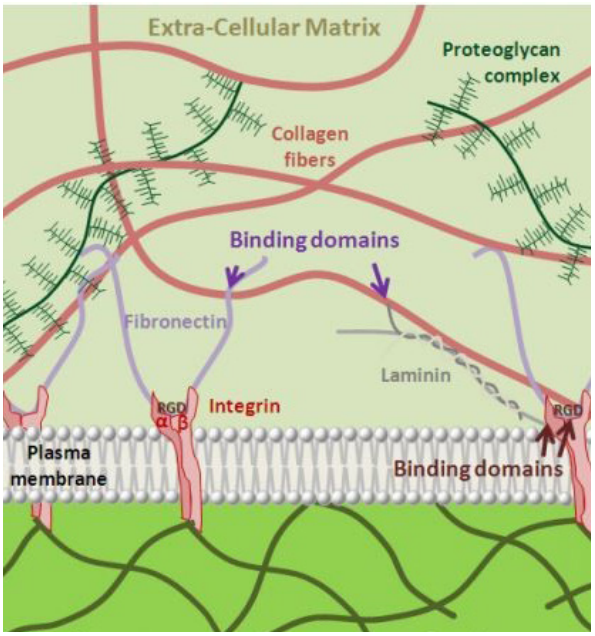


Figure 34. *Composition and structure of ECM.*

iMATRIX-511 RECOMBINANT HUMAN LAMININ

iMatrix-511 is an innovative cell culture matrix compatible with a wide variety of cell types, and exceptionally well suited for pluripotent stem cells. This product is comprised of recombinant Laminin-511 E8 protein fragments which permit ES/iPS cells to be maintained in xeno free culture conditions, enable the passaging of single cells, and provide **greater adhesion than full-length Laminin, Vitronectin or Matrigel.**

There are more than 12 Laminin isoforms, all of which promote stem cell growth. Laminin-511 is a major component of the basement membrane, which is expressed in early development of the embryo and can be used as a matrix for pluripotent (ES/iPS) stem cells, as it binds to integrin on stem cell surfaces. Laminin-511 is more efficient at enhancing cell growth than other Laminins such as Laminin-111 (Matrigel) (Figure 35). However, Laminin-511 is a large protein (800 kDa) composed of three chains, making it difficult to produce recombinantly. In order to overcome this challenge, Laminin-511 proteins were fragmented to find the smallest integrin-binding component: hES cells were found to adhere more strongly to the E8 fragment than to the full length protein.

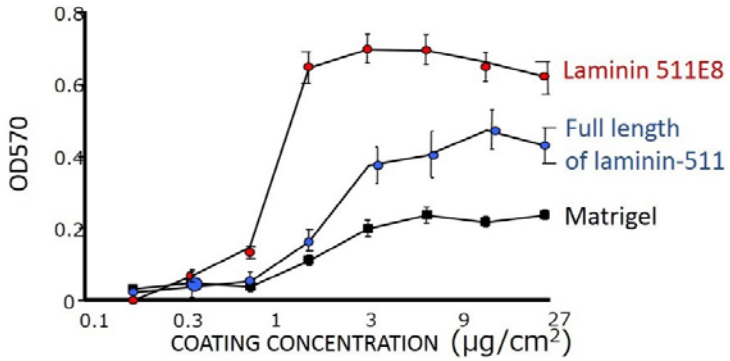


Figure 35. *The Binding activity of Laminin 511 E8 Fragment against ES cell was better than full length 511 and traditional substrate. The same function was confirmed in human iPS cells.*

iMatrix-511 silk has the same product specifications as iMatrix-511, but it is more cost effective. iMatrix-511 silk is expressed by silkworm cocoons as opposed to CHO cell lines. This makes the manufacturing process and, thus, the product cheaper.

Description	Quantity	Catalogue No.
Laminin iMatrix-511 E8	350 µL(175 µL × 2 tubes)	AMS.892 011
Laminin iMatrix-511 E8	1050 µL (175 µL × 6 tubes)	AMS.892 012
Laminin iMatrix-511 silk E8	1050 µL (175 µL × 6 tubes)	AMS.892 021
Laminin iMatrix-411 E8	350 µL (175 µL × 2 tubes)	AMS.892 041
Laminin iMatrix-411 E8	1050 µL (175 µL × 6 tubes)	AMS.892 042

StemFit®

For the best results, we strongly recommend iMatrix-511 be used together with StemFit® medium

Selected Application of iMatrix-511 Recombinant Human Laminin

HUMAN iPS CELLS

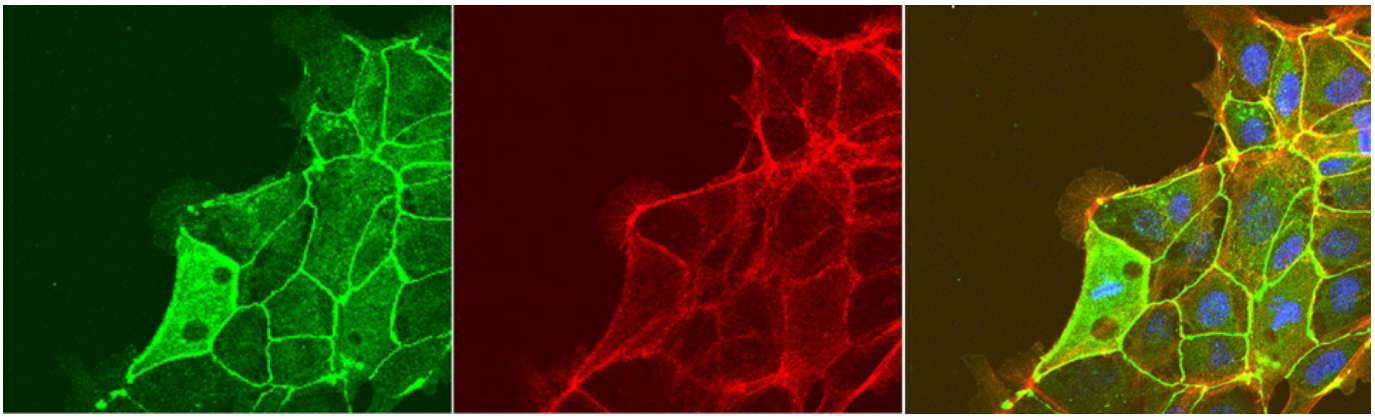


Figure 36. *Human iPS cells on Laminin-511 E8 (coated Laminin concentration 0.4 µg/cm²): ZO-1, Beta-Actin. Objective lens x40.*

COLLAGEN NATURAL AND RECOMBINANT PROTEINS

Collagen is the main structural protein in the extracellular space in the various connective tissues in animals. As the most abundant protein in mammals, it makes up 25% to 35% of the whole body protein content. Collagen, in the form of elongated fibrils, is mostly found in fibrous tissues such as tendons, ligaments and skin. It is also abundant in corneas, cartilage, bones, blood vessels, the gut, intervertebral discs and the dentin in teeth. Collagen constitutes one to two percent of muscle tissue, and accounts for 6% of the weight of strong, tendinous muscles. Fibroblasts are the most common cells that create Collagen.

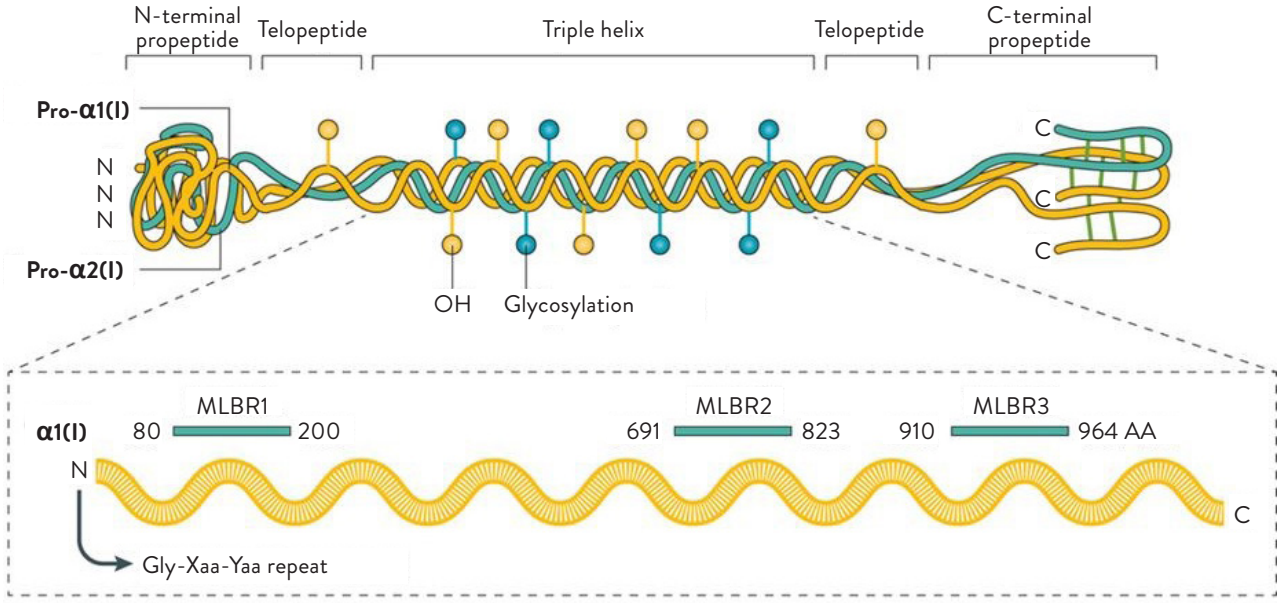
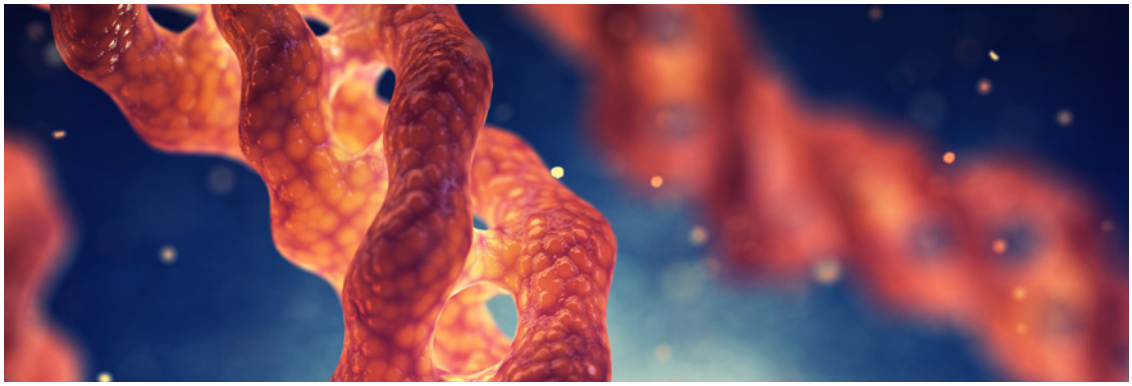


***Figure 37**. Collagen structure. From Marini J. C. et al. (2017) Nature Rev. Disease Primers 3, 17052.*

Type I Collagen is a major structural component of skin, bone, tendon, and other fibrous connective tissues, and differs from other Collagens by its low lysine hydroxylation and low carbohydrate composition. Type I Collagen is a hetero-trimer composed of two α1 chains and one α2 chain, which spontaneously form a triple helix scaffold at neutral pH and 37 °C. This phenomenon can be exploited to promote cell attachment, proliferation, differentiation, migration, and tissue morphogenesis during development. Cells cultured in 3D Collagen gels simulate the *in vivo* cell environment better than traditional 2D systems. This allows type I Collagens to be very useful in studying cell function and behavior, and the effects of diseases on the mechanical properties of the ECM and the interactive cells.

Type III Collagen provides structure and strength to connective tissue. It is found in many places in the body, especially skin, lung, intestinal walls and the walls of blood vessels. Collagen III is initially produced as proCollagen, which is then modified by the cell using specific enzymes to allow the formation of a stable molecule and then later allow crosslinking to other molecules outside the cell. Type III Collagen is typically used as a thin coating on tissue culture surfaces and acts as a substrate scaffold to enhance cell attachment, adherence and proliferation.



All Attachin™ Collagen products are isolated from specific tissues and are purified using a validated manufacturing process that insures inactivation of possible prion and/or viral contaminants. Attachin™ Collagens are then sterilized by membrane filtration and confirmed negative for bacterial and fungal contaminants. Identities and purities of Collagens are determined by SDS-PAGE gel electrophoresis.

Peptide	Species	Pack Size	Catalogue No.
Cell culture grade Collagen I	Bovine	4 mg/ml x 12.5 ml	1202
	Porcine	4 mg/ml x 12.5 ml	1203
Recombinant Collagen I	Human	2 mg	4796-2
		10 mg	AMS.PBV10415r-10
Attachin™ Collagen I	Bovine	10 mg/ml x 20 ml	AMS.Q1BC0200
		6 mg/ml x 50 ml	AMS.Q1BC0500
		3 mg/ml x 100ml	AMS.Q1BC1000
		5 mg/ml x 35ml	AMS.Q1BCIG35
Attachin™ InstaGel Collagen I	Bovine	5 mg/ml, 35 ml	AMS.Q1BCIG35
Collagen I coated plate	Bovine	1 pack	90252
	Rat	1 pack	90253
	Human	1 pack	90254
	Mouse	1 pack	90255
6-well Collagen I coated plate	Rat tail	5 pack	CC-6
12-well Collagen I coated plate	Rat tail	5 pack	CC-12
24-well Collagen I coated plate	Rat tail	5 pack	CC-24
96-well Collagen I coated plate	Rat tail	5 pack	CC-96
T-25 Collagen I coated flask	Rat tail	5 pack	CC-25
T-75 Collagen I coated flask	Rat tail	5 pack	CC-75
T-225 Collagen I coated flask	Rat tail	1 pack	CC-225
Recombinant Collagen III	Human	1 mg	AMS.PBV10416r-1
		5 mg	AMS.PBV10416r-5
Attachin™ Collagen III (85% Collagen III, 15% Collagen I)	Human	1 mg/ml x 10ml	AMS.Q3HC0100
Attachin™ Collagen IV	Human	5 mg	AMS.Q4HCL050
Attachin™ Collagen V	Bovine	0.2 mg/ml, 2.5 ml	AMS.Q5BC0025

FIBRONECTIN PROTEINS

Fibronectin is an extracellular matrix protein that is found abundantly in blood and connective tissue. Its expression is associated with the epithelial to mesenchymal transition of metastatic cells, and tumor cells with stem cell-like properties. Fibronectin performs essential functions in Collagen fibrillogenesis, as either a general cell adhesion molecule or a modulator in binding between cell surfaces and the extracellular matrix. Fibronectin matrix assembly is essential for normal vertebrate development and is thought to contribute to the generation of tumor metastases by supporting the establishment and persistence of premetastatic niches. Fibronectin is secreted as a disulphide-linked dimer of 230-270 kDa, comprised of three types of repeating modules that mediate interactions with extracellular matrix components (including Fibronectin itself), and cells *via* integrins and other Fibronectin receptors. Thus, Fibronectin can be used for coating tissue culture surfaces or as a medium additive to promote cell adhesion and proliferation.

Description	Pack Size	Catalogue No.
Fibronectin-1, Human recombinant Protein	100 µg	AMS.90143

VITRONECTIN PROTEINS

Vitronectin is an extracellular, soluble, disulphide-linked dimer composed of a 75 kDa and a 65 kDa peptide chain with a total molecular weight of 140 kDa. Vitronectin is a major plasma glycoprotein that promotes cellular adhesion and spreading. It also inhibits the membrane damaging effect of the terminal cytolytic complement pathway and binds to several serpin serine protease inhibitors. Vitronectin, along with Collagen IV, Fibronectin, and Laminin can support robust, long term proliferation of undifferentiated human embryonic stem cells. Vitronectin can be used for coating tissue culture surfaces to promote cell adhesion, proliferation and differentiation, or as an additive for serum-free media.

Description	Pack Size	Catalogue No.
Vitronectin, human plasma	100 µg	4087-100
Recombinant human Vitronectin	100 µg	AMS.PBG10479-U100
Recombinant human Vitronectin	500 µg	AMS.PBG10479-U500
Animal-free recombinant human Vitronectin	100 µg	AMS.PBG10507-U100
Animal-free recombinant human Vitronectin	500 µg	AMS.PBG10507-U500

E-CADHERIN PROTEINS

E-Cadherins are a family of transmembrane proteins found on epithelial cells. E-cadherins are crucial for cell to cell adhesion. They play an important role in development and maintenance of tissue structure, and cell migration. Loss of function of E-cadherins is associated with increased tumor metastasis. E-cadherin function is dependent on extracellular interactions with Calcium ions. E-cadherins have a small intracellular domain, a small transmembrane domain, and most of the protein is found in the extracellular domain. One peptide is made of around 750 amino acids. Calcium ions interact with the extracellular domains of two peptides originating from two different cells. This leads to the formation of tightly bound dimer that keeps the two cells together.

Description	Pack Size	Catalogue No.
Human CellExp™ E-Cadherin /ECAD /Cadherin-1/CD324 Protein	25 µg	7420-25
Human E-cadherin protein	1 mg	AMS.ECD-H5222-1mg
Human E-cadherin protein	100 µg	AMS.ECD-H5222-100ug
Recombinant protein of human cadherin 1, type 1, E-cadherin	20 µg	TP320731

OSTEOPONTIN PROTEINS

Osteopontin is an adhesion protein found throughout the body. It was initially discovered for its prevalence and involvement in bone morphogenesis and maintenance (thus the name). Since then, Osteopontin has been found to play a crucial role in other processes such as cell attachment and wound healing. It promotes cell survival by regulating apoptotic signaling pathways. Osteopontin interacts with many integrin receptors such as α4β1, α9β1 and α9β4.

Description	Pack Size	Catalogue No.
Recombinant human Osteopontin	50 µg	AMS.PBG10345-U050
Recombinant human Osteopontin	100 µg	AMS.OPN-H5227-100ug
Recombinant human Osteopontin	1 mg	AMS.OPN-H5227-1mg

THROMBOSPONDIN PROTEINS

Thrombospondin is an adhesive glycoprotein that mediates cellular interactions that take place in the extracellular matrix (cell to cell and cell to matrix interactions). It can bind various extracellular matrix proteins such as fibrinogen, Fibronectin, Laminin and Collagen V, and integrin α5β1. Thrombospondin is a large protein (~120 kDa) that expresses the RGD motif throughout its domain, allowing extensive interactions between this protein and the extracellular matrix. It plays a role in angiogenesis, migration and apoptosis.

Description	Pack Size	Catalogue No.
Thrombospondin, human recombinant	10 µg	4805-10
Thrombospondin, human recombinant	25 µg	AMS.PBV10420r-25
Thrombospondin, human recombinant	50 µg	AMS.PBV10419r-50
Thrombospondin, human recombinant	1 mg	AMS.PBV10419r-1

POLY-L-LYSINE AND POLY-D-LYSINE

These highly positively charged amino acid chains are commonly used as a coating agent to promote cell adhesion in culture on TCT plastic or glass surfaces. Poly-D-Lysine is resistant to enzymatic degradation and promotes the proliferation and differentiation of a variety of neuronal cell lines.

Description	Pack Size	Catalogue No.
Poly-D-Lysine with Laminin coating solution	2.5 ml	AMS.PR12725
Poly-D-lysine 70 000-HBr research grade	25 mg	33224.01
Poly-L-Lysine solution, 10x	10 ml for 100-200 slides	AR0003
Poly-L-lysine 70 000-HBr research grade	25 mg	33225.01

MAPTrix™ RECOMBINANT ANIMAL-FREE PROTEINS

Mussel Adhesive Protein based matrix (MAPTrix™) recombinant extracellular matrix (ECM) acts as bio-mimetic for traditional basement membrane extracts. MAPTrix™ replaces traditional ECM with genetically incorporated bioactive peptides (recognition peptides) that provide an environment for the maintenance of cells under serum, and feeder-free conditions.

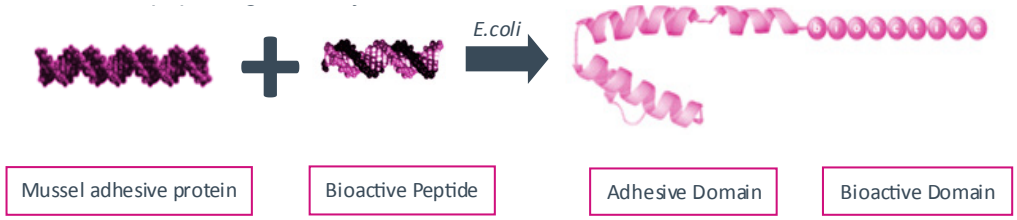


Figure 38. *Bioactive peptide genetically fused to muscle adhesive protein (MAP).*

MAPTrix™ technology for extracellular matrix (ECM) based coatings or surface modification is simple, convenient, and highly reproducible. You can readily engineer a synthetic ECM surface that binds to adhesion receptors such as integrins to promote cell adhesion and spreading. MAPTrix™ utilises mussel adhesive protein to create the first combinatorial synthetic ECM library for engineering integrin specific surfaces.

These surfaces mimic the native extracellular environment. Mussel adhesive protein is highly desirable for use in a variety of biological and medical applications due to its strong, wet, adhesive, non-toxic, biodegradable, and low immunogenicity properties.

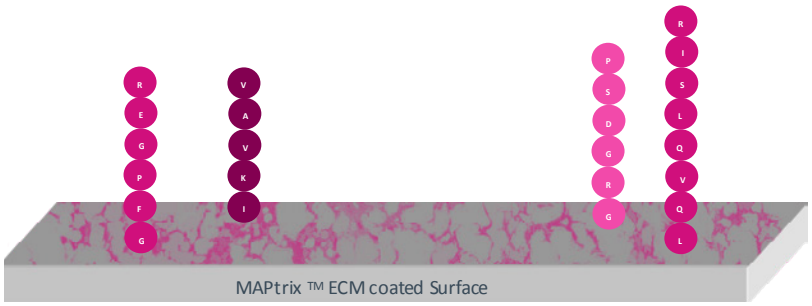


Figure 39. *Schematic diagram of MAPTrix™ ECM. The genetically engineered bioactive domains are "sticking out" of the matrix, making cell surface integrins and bioactive domain interactions easy to occur.*

MAPTrix ™ bio-mimetics are already used in: stem cell technology, tissue engineering scaffolds, drug delivery, cell surface modification and coating of medical devices.

ADVANTAGES

- ✓ Biochemically defined & animal free
- ✓ Reproducible & reliable protein coating
- ✓ Low cost
- ✓ Ready to use
- ✓ Improved cell morphology and cell proliferation
- ✓ Adhere to USP guidelines
- ✓ FDA recommendations compliant
- ✓ Eliminates risk of animal or viral infectious agents in cell cultures

READ OUTS

- ✓ Defined media conditions
- ✓ Defined adhesion conditions
- ✓ Adhesion assays
- ✓ Proliferation assays
- ✓ Other ECM-dependent functional assays

Mix & Match Motifs to Create *in vivo*-like Environment

You can mix & match various motifs from MAPTrix™ ECM mimetics in order to create *in vivo* – like environment for your cells.

ADVANTAGES

- ✓ Simple
- ✓ Convenient
- ✓ Highly reproducible
- ✓ Lot to lot consistency
- ✓ Soluble in a variety of buffers (including water) under a wide range of pH (pH= 2.0 - 9.5)

APPLICATIONS

- ✓ Tissue engineering scaffolds
- ✓ Drug delivery
- ✓ Coating of medical devices
- ✓ Surface modification

Compatible with *in vitro* techniques (in situ hybridization, immunoassays…)

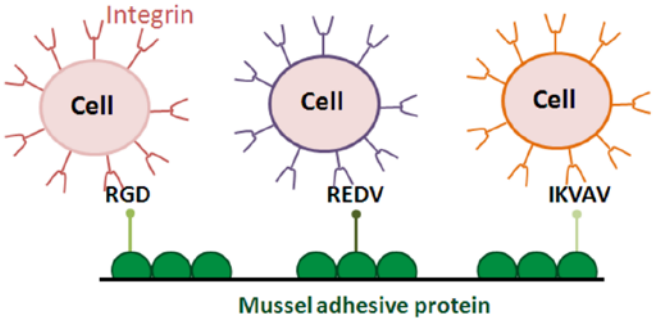


Figure 40. *Use of appropriate binding domain promotes cell adhesion, providing a simple and highly reproducible in vivo like environment for cells.*

Selected Applications of MAPTrix™

MESENCHYMAL STEM CELL CULTURE

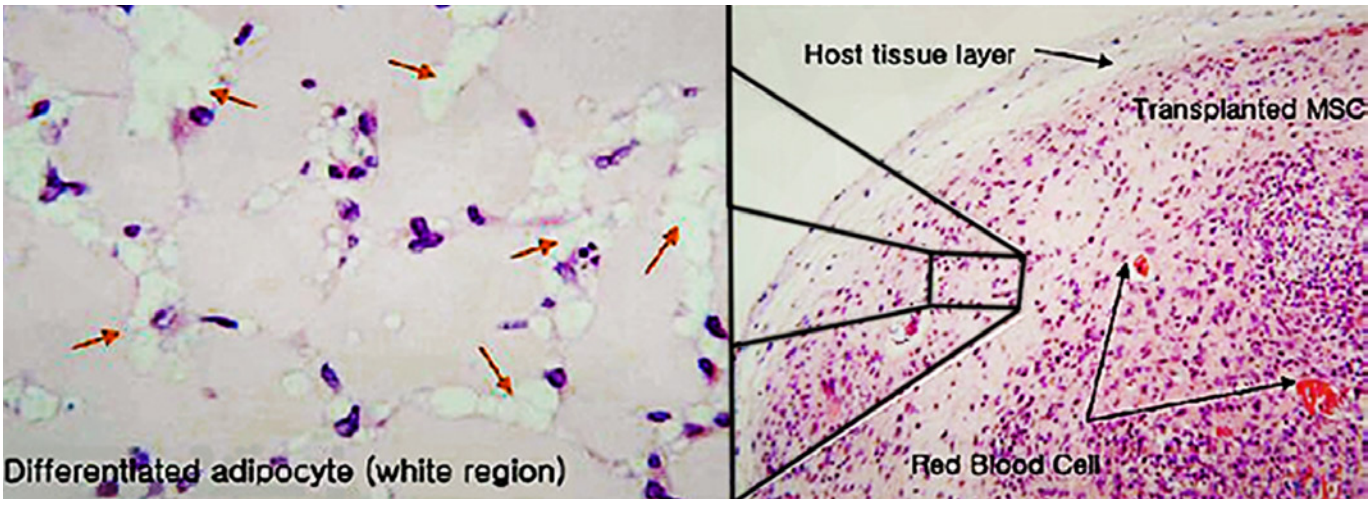


Figure 41. *Mesenchymal stem cells from male mouse adipose were encapsulated and cultured in hydrogel containing MAPTrix™ Collagen type I mimetic for 7 days. The encapsulated MSCs were then transplanted to the abdominal cavity of a female mouse for one month before analysis (If Y chromosome is detected in the abdominal cavity tissue, it indicates that the tissue came from the encapsulated stem cells).*

See also figure 45 on page 33

Fibronectin-Derived Peptides

Fibronectin (FN) is a high molecular weight glycoprotein that consists of three types of repeating amino acid units: type I, type II, and type III. The structure of Fibronectin depends on whether it is secreted in plasma or synthesized by resident cells. Cellular Fibronectin contains the alternatively spliced extra domain A and/or extra domain B. In addition, a third alternatively spliced domain, the IIICS domain (for rodents: the V-region), can be included, but regulations for its inclusion have not been fully discovered yet. Fibronectin naturally exists as a dimer, consisting of two nearly identical monomers. Two regions in each Fibronectin subunit possess cell binding activity: III9-10 and III14-V.

The primary receptor for adhesion to Fibronectin commonly involves the RGD motif of repeat III10 through integrins such as α5β1. However, this integrin-ligand interaction is only sufficient for cell attachment and spreading. Additional signaling through the cell surface proteoglycan such as syndecan-4 is required for focal adhesion formation and rearrangement of the actin cytoskeleton into bundled stress fibres. This binding occurs primarily via the Hep II domain (containing the FN type III repeats 12-14) in the C-terminal region of Fibronectin.

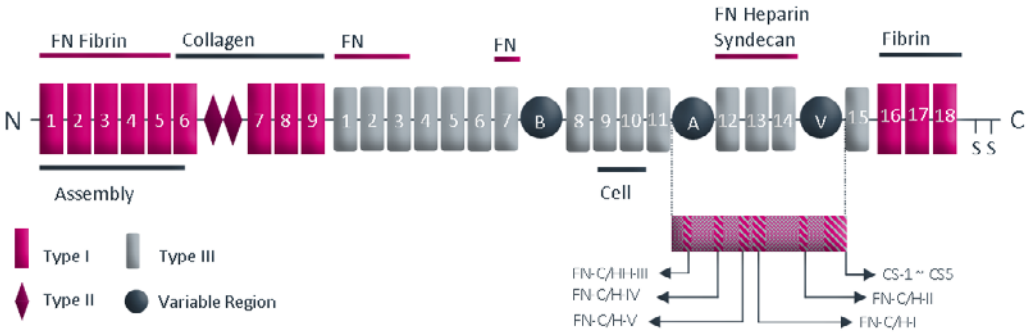


***Figure 42**. Fibronectin structure.*

Domain	Peptide Motif	Catalogue No.
Type III-5	KLDAPT	16103X
Type III CS-1	PHSRN	16104X
Type III-10	RGD	16105X
Type III-10	GRGDSP	16107X
FN-C/H-III	YRVRVTPKEKTGPMKE	16109X
FN-C/H-1V	SPPRRARVT	16110X
Type III-13	ATETTITIS	16111X
FN-C/H-V	WQPPRARI	16116X
FN-C/H-II	KNNQKSEPLIGRKKT	16119X
Type III CS-1	EILDVPST	16120X
Type III CS-5	REDV	16124X
Type III	PHSRN-RGDSP	16125X
Type III-5	KYILRWRPKNS	16101X
Type III CS-1	WTPPRAQITGYRLTVGLTRR	16202X
Type III-10	EDGIHEL	16108X
Type III-13	ANGQTPIQRYIK	16114X
FN-C/H-III	YKPDVRSYTITG	16113X
Type IIICS-1	VVIDASTAIDAPSNL	16122X

Key to catalogue number on page 34

Collagen-Derived Peptides

Collagens serve as scaffolds for the attachment of cells and matrix proteins. Collagen is the major insoluble fibrous protein in the extracellular matrix and connective tissue. In fact, it is the single most abundant protein in the animal kingdom. There are at least 16 types of Collagen, but 80 – 90% of the Collagen in the body consists of types I, II, and III. These Collagen molecules form long thin fibrils. Type IV, in contrast, forms a two-dimensional reticulum. Several other types associate with fibril-type Collagens, linking them to each other or to other matrix components. The various Collagens and the structures they form help tissues withstand stretching. Collagens are also highly biologically active with many other ligands. For example, Collagens provide integrin- and heparin-binding motifs. α2β1 integrin recognizes GXO/SGER such as GFPGER or GFOGER for endothelial cell binding, activation and angiogenesis.

Integrin binding sites for αvβ3 have antitumor activity, and may inhibit the activation of human neutrophil or the proliferation of capillary endothelial cells. Integrin binding sites in the NC1 domains have anti-angiogenic properties mediated by the α1β1 or αvβ3 integrin binding.

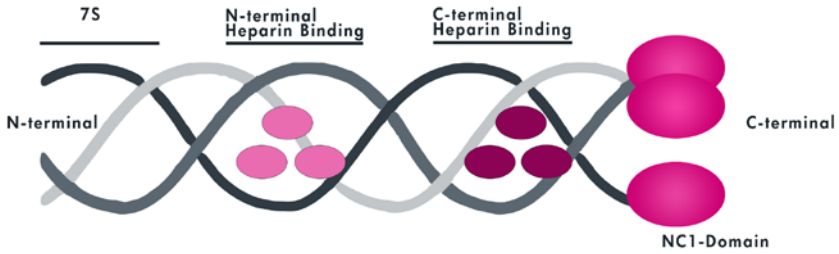


Figure 43. *Collagen Structure.*

Domain	Peptide Motif	Catalogue No.
Type I α1	GLPGER	16501X
	KGHRGF	16502X
	GFPGER	16504X
	DGEA	16506X
	GPAGKDGEAGAQG	16507X
	GTPGPQGIAGQRDVV	16512X
Type IV α1	TAGSCLRKFSTM	16621X
	GEFYFDLRLKGDK	16623X
Type IV α3	TAIPSCPEGTVPLYS	16631X
	TDIPPCPHGWISLWK	16632X
	ISRCQVCMKKRH	16635X

Key to catalogue number on page 34

Laminin-Derived Peptides

Laminins (heterotrimers composed of α, β, and γ chains), are multifunctional glycoproteins present in basement membranes. Integrins, dystroglycan, syndecans, and several other cell surface molecules are cellular receptors for Laminins. The globular domains located in the N- and C-terminus (Figure 44) of the Laminin α chains are critical for interactions with the cellular receptors. Integrin α6β1 binds to most of the Laminin isoforms. Integrin α3β1 interacts with Laminin-5 and -10/11 more specifically than the other isoforms. Integrins α1β1, α2β1, and α7β1 show binding activity to Laminin-1 and -2. Interaction of integrin α6β4 with Laminin-5 forms hemidesmosomes in the skin. α-dystroglycan strongly binds to the Laminin α1 and α2 chains and moderately interacts with the α5 chain.

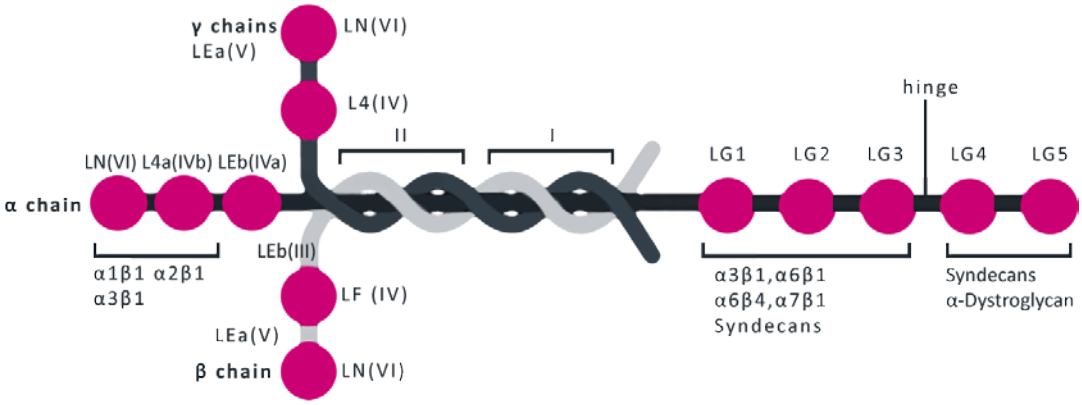


***Figure 44**. Laminin structure.*

Domain	Peptide Motif	Catalogue No.
α1 chain	RQVFQVAYIIIKA	16204X
	IKVAV	16224X
	AASIKVAVSADR	16225X
	NRWHSIYITRFG	16226X
	TWYKIAFQRNRK	16229X
	RKRLQVQLSIRT	16232X
α3 chain	PPFLMLLKGSTR	16288X
	KNSFMALYLSKGRLVFALG	16293X
α5 chain	GIIFFL	16369X
β1 chain	RYVVLPR	16411X
	YIGSR	16414X
	LGTIPG	16421X
γ1 chain	KAFDITYVRLKF	16442X
	SETTVKYIFRLHE	16452X
	RNIAEIIKDI	16460X

Key to catalogue number on page 34

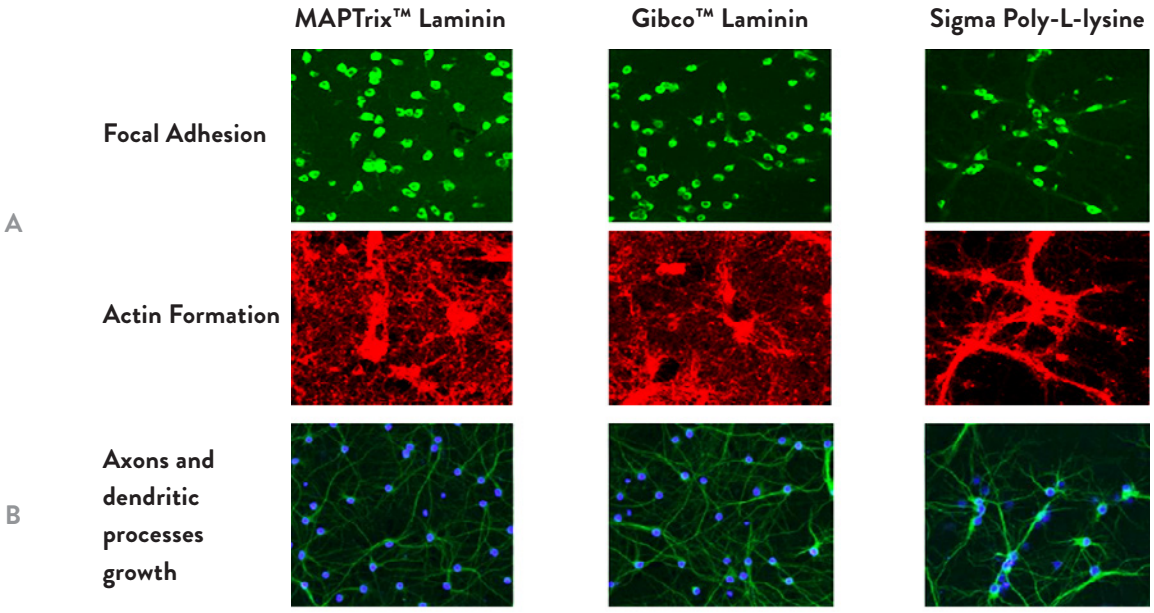


Figure 45. *MAPTrix™ Laminin bio-mimetic is comparable to native Laminin and poly-L-lysine in supporting neuronal adhesion and outgrowth. (**A**) Primary nerve cells form similar focal adhesions on both MAPTrix™ bio-mimetic (left panels) and native Laminin (middle panels). (**B**) Hippocampal neuronal cells outgrowth is also equally supported by MAPTrix™ and native Laminin. Both Laminins were superior to poly-L-Lysine (right panels) in supporting adhesion and axon dendritic growth.*

Additional Adhesion Peptides

Some extracellular matrix components are adhesion-modulatory proteins which interact with the main ECM components or integrins. These proteins include vitronectin, nidogen, Tenascin, and SIBLINGs (small integrin-binding ligand, N-linked glycoprotein) such as bone sialoprotein (BSP). These components can also influence the cellular behaviorby regulating cell signaling (directly or indirectly).

Cadherins are calcium-dependent cell adhesion proteins which are involved in many morphoregulatory processes including the establishment of tissue boundaries, tissue rearrangement, cell differentiation, and metastasis. The extracellular domain of E-cadherin tends to bind in a homophilic manner; although heterophilic binding does occur under certain conditions. The binding of extracellular cadherin is the basis for cell-cell adhesion, tends to be prevalent at adherin junctions, and is structurally associated with actin bundles.

ADDITIONAL ADHESION PEPTIDES ORDERING INFORMATION

Domain	Peptide Motif	Catalogue No.
E-cadherin ECD1	SHAVSS	16701X
	LFSHAVSSNG	16702X
	ADTPPV	16703X
E-cadherin, Ca^{2}+ binding	DQNDN	16706X
N-cadherin ECD1	HAVDI	16707X
	LRAHAVDING	16708X
	LRAHAVDVNG	16709X
Vitronectin HVP	FRHRNRKGY	16801X
	KKQRFRHRNRKGYRSQ	16802X
Somatomedin B	RGDV	16803X
Affinity Tag for Recognition		
C-terminal	DYKDDDK	16981X
C– and N-terminal	DYKDDDK	16982X
Fc binding motif	(Arg-Thr-Tyr)4-K2-K-G	16983X
	HWRGWV	16984X
Nidogen G2	LNRQELFPFG	16811X
	SIGFRGDGQTC	16812X
Tenascin-C	VAEIDGIEL	16831X
	VFDNFVLK	16832X
Elastin	VGVAPG	16851X
	PGVGV	16852X
Bone Sialoprotein (BSP)	KRSR	16901X
	FHRRIKA	16902X
CCN (connective growth factor)	TTSWSQCSKS	16931X
Fibrinogen	HHLGGAKQAGDV	16953X
	KRLDGSV	16952X
Osteopontin	SVVYGLR	16961X
	SLAYGLR	16962X
Thrombospondin	LALERKDHSG	16971X

Key to catalogue number below

KEY TO MAPTRIX™ PRODUCTS CATALOGUE NUMBERS

Catalogue No. ending with X =	Pack Size
Replace X with: 2	2.5 mg protein, aqueous solution at 0.5 mg/mL
Replace X with: 4	10 mg protein, aqueous solution at 1 mg/mL
Catalogue No. ending with Y =	**Pack Size**
Replace Y with: 1	10 mg MAPTrix™ ECM + 50 mg Linker
Replace Y with: 2	20 mg MAPTrix™ ECM + 100 mg Linker
Replace Y with: 3	50 mg MAPTrix™ ECM + 100 mg Linker
Replace Y with: 4	100 mg MAPTrix™ ECM + 500 mg Linker

Assays for Physiologically Relevant Cell Cultures

CELL BEHAVIOR ASSAYS

Cell Behavior Assays Types	
Cell behavior assays cover a broad range of physiological functions:	
Cell Adhesion	Cell adhesion is the binding of a cell to a surface, such as an extracellular matrix or another cell, using cell adhesion molecules. Cell adhesive interactions play important roles during many normal physiological processes such as embryonic development and wound repair, and also during the progression of diseases such as cancer. Cell adhesion is mediated by the specific interactions of cell surface receptors with extracellular glycoproteins. The best characterized cell adhesion receptors are the integrins. Cellular adhesion through integrins links the cytoplasm of cells and is involved in signal transduction.
Cell Migration	Cell migration is the movement of cells in response to a chemical stimulus; this is also known as chemotaxis. Traditionally, cell migration assays evaluate cell migration based on the cells' ability to traverse an uncoated membrane with pores, in response to a chemotactic gradient. The cells must undergo cytoskeletal remodelling to fit into the pores and pull them through to the underside of the membrane. Alternatively, Oris™ assays quantify migration or invasion through extracellular proteins into the empty centre of each micro-plate well.
Cell Invasion	Cell invasion is cell migration through a physiological barrier in response to a chemo-attractant. This recapitulates cell movement within a physiological environment which is composed of extracellular matrix proteins. Here, the membranes are coated with a layer of extracellular matrix proteins, and the cells must traverse this barrier through a combination of protein degradation and cellular locomotion.
Organoid and Spheroid based Assays + Solutions	Detailed information about organoid culture protocols and reagents can be find in our Organoid Culture Handbook. Here we demonstrate spheroid formation assay.

The quantification of cellular responses is the bedrock of cell biology. Assays here enable precise evaluation in physiological context of variety of cell types:

- ✓ Adhesion
- ✓ Migration
- ✓ Invasion

MAPTrix™ ADHESION ARRAYS

For many cell types, the exact requirements to generate a physiologically-relevant microenvironment are unknown. MAPTrix™ biomimetics are xeno free solutions based on mussel adhesion protein technology to provide microenvironmental signals (see also p. 28-34). Using a series of MAPTrix™ arrays, cellular requirements can be screened. Below are standard arrays containing MAPTrix™ bio-mimetics for the most frequently used extracellular matrix proteins. Custom arrays for specific requirements – containing MAPTrix™ with bio-mimetics for extracellular proteins and/or growth factors are also available.

MAPTrix™ arrays provide a high-throughput platform to screen for the behavior of cells under a variety of microenvironmental signals. They can be used to find out which signals are required or to study the different behavior of cells in response to them. MAPTrix™ arrays can be used to select specific bio-mimetic to be incorporated into other environments such as cell culture media or as essential signal provided in addition to existing artificial or natural scaffolds.

BASIC ECM MIMETIC SCREEN

- 96-well array for cell adhesion assays
- 24 different ECM mimetics (quadruplets)
- Derived from Collagen, Fibronectin, & Laminin

READ OUTS

- ✓ Adhesion assays
- ✓ Proliferation assays
- ✓ Other ECM-dependent functional assays

BENEFITS

- ✓ Easy read-out using any plate reader or imaging technology
- ✓ Flexible platform to study adhesion, proliferation or other cellular process depending on the microenvironment
- ✓ Easy comparison between microenvironmental signals: the recombinant MAPTrix™ backbone is identical between all wells
- ✓ MAPTrix™ found to be ideal on arrays can be bought for future use as stand-alone products for any assay or as part of defined media

Description	Pack Size	Catalogue No.
MAPTrix™ Screen for Cell Adhesion Assay, 96 well plate coated with 24 different ECM mimetics (quadruplets)	2 plates per case	ANN901

Bulk pricing available for orders over 10 plates

Further plates in development

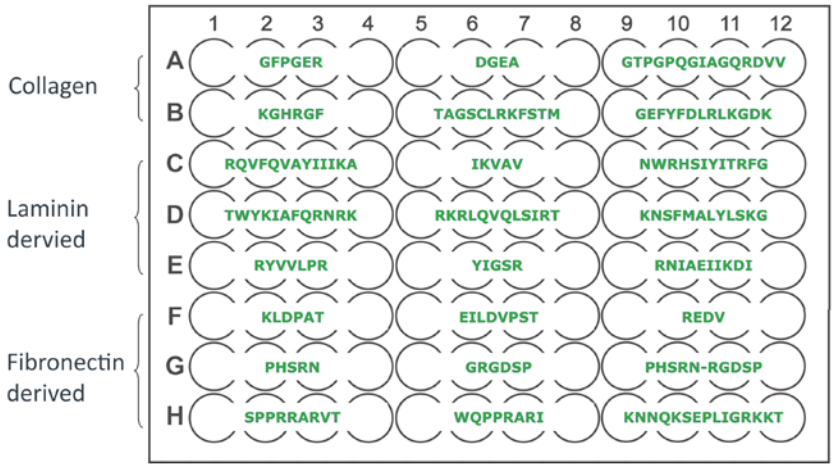


***Figure 46**. Schematic representation of a layout of a standard MAPTrix™ adhesion array. 96-well plate is coated with 24 different ECM mimetics derived from Collagen (6), Laminin (9) and Fibronectin (9) in quadruplets.*

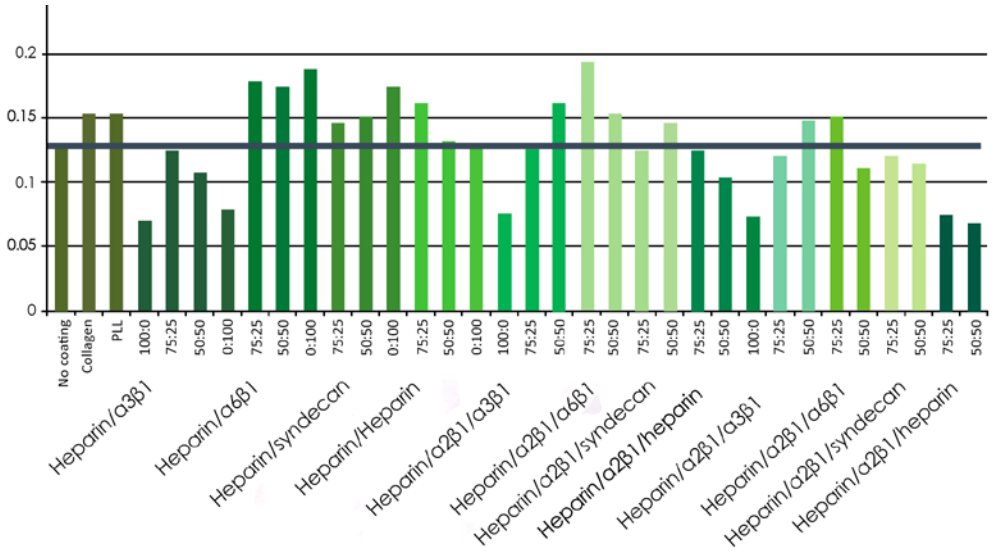


***Figure 47**. 49 Primary HUVEC cells were incubated on MAPTrix™ ECM arrays for 48 hours in serum-free conditions. Cell counts were normalized against average cell counts on Non-coating (BD Falcon). Each bar represents the mean value of three wells.*

MAPTRIX™ SCREEN-LAMININ

- 12-well array for cell adhesion assays
- 10 different ECM mimetics derived from Laminin
- Recombinant MAPTrix™ Technology (details overleaf)

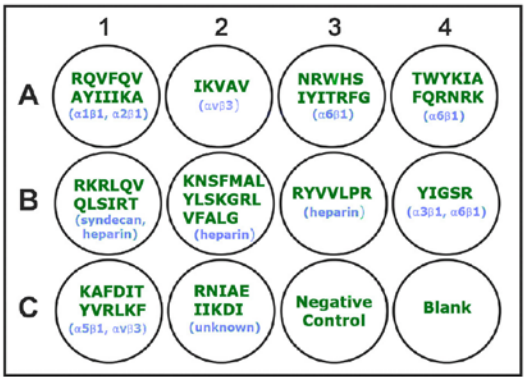


***Figure 48 .** Layout of a standard MAPTrix™ 12-well array*

Description	Pack Size	Catalogue No.
MAPTrix™ Screen for Cell Adhesion Assay, Laminin mimetic coated 12 well plate	2 plates per case	LNN101

Bulk pricing available for orders over 10 plates

Further plates in development

ORIS™ AND ORIS™ PRO CELL MIGRATION AND INVASION ASSAYS

The migration of cells is known to occur during a vast array of both normal biological processes, such as the development of an organism, immune response and wound healing, or marking pathological events, such as cancer metastasis, cardiovascular disease, atherosclerosis and arthritis. The need to better understand the process of cell migration has led to the development of improved methods of investigation including culturing methods, live cell trackers and detection methods. Improvements in both the investigative methods and throughput technologies will allow for rapid expansion of investigation in the area of cell migration research.

The Oris™ Cell Migration and Invasion Assays are designed with a unique cell seeding stopper, what eliminates the need for artificial membrane inserts. Their innovative design generates highly reproducible results using microscopes, plate readers or high content imaging systems.

Oris™ Pro Cell Migration and Invasion Assays are designed with a biocompatible gel (BGC) eliminating the need for artificial membrane inserts. They are reproducible, sensitive, and flexible assays that can be used to monitor cell migration or invasion. Formatted in a well plate, these assays use non-toxic BGC to form a cell-free zone. After seeding cells into the well plate, the BCG self-dissolves permitting cells to migrate or invade through Collagen into the well centres. The Oris™ Pro Assays enable the use of automated liquid handling equipment for cell seeding and allows for unlimited access to wells from cell seeding through to data readout. The Oris™ Pro Cell Migration Assay is designed to be used with any commercially available stain or labelling technique. Researchers can capture and quantify real-time and endpoint cell migration data using inverted microscopes, high content screening and high content imaging instruments.

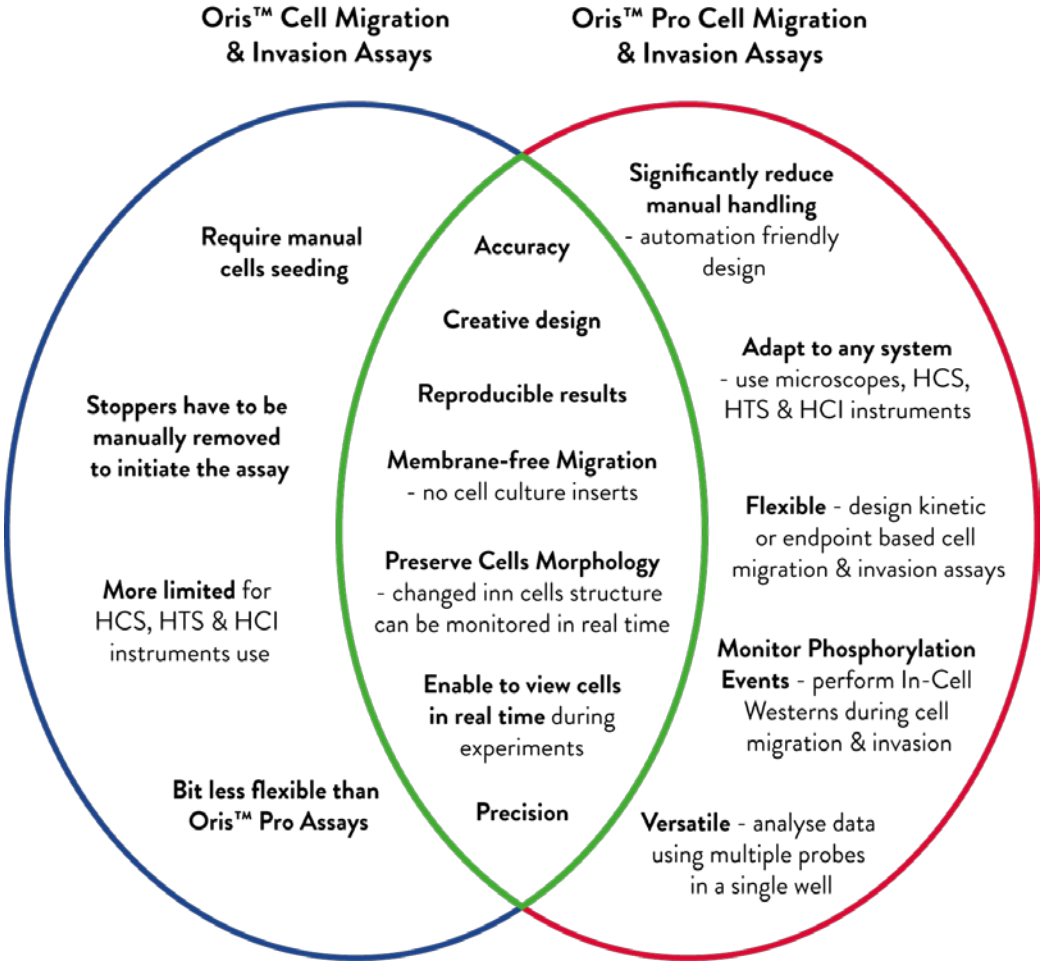


***Figure 49**. Comparison of Oris™ and Oris™ Pro Cell Migration and Invasion Assays features.*

Migration Assays

Oris™ and Oris™ Pro Cell Migration Assays were designed to address the limitations of the scratch assay, which was so far commonly used to study cells migration *in vitro*, and can be utilized to detect chemokines, perform morphological analysis and mimic a 2D closure/wound assay. The scratch assay is performed by creating a cell-free gap, or "scratch" on a confluent cell monolayer upon which cells at the edge of the opening move inward to close the scratch. Cells migration can be assessed by comparing images taken at the onset of the scratch creation and at user-defined intervals during scratch closure. However, methods for creating the scratch vary from lab to lab and results can be highly variable. Furthermore the process of scratch formation has been shown to damage the underlying ECM. Moreover in wound healing studies the scratch assay results in high variability as it wounds the cells monolayers causing release of factors from the dead and dying cells. Both Oris™ and Oris™ Pro Cell Migration Assays have greater reproducibility than the scratch assay due to uniformly sized Detection Zones and in addition ECM is not damaged by silicone stopper or BGC gel.

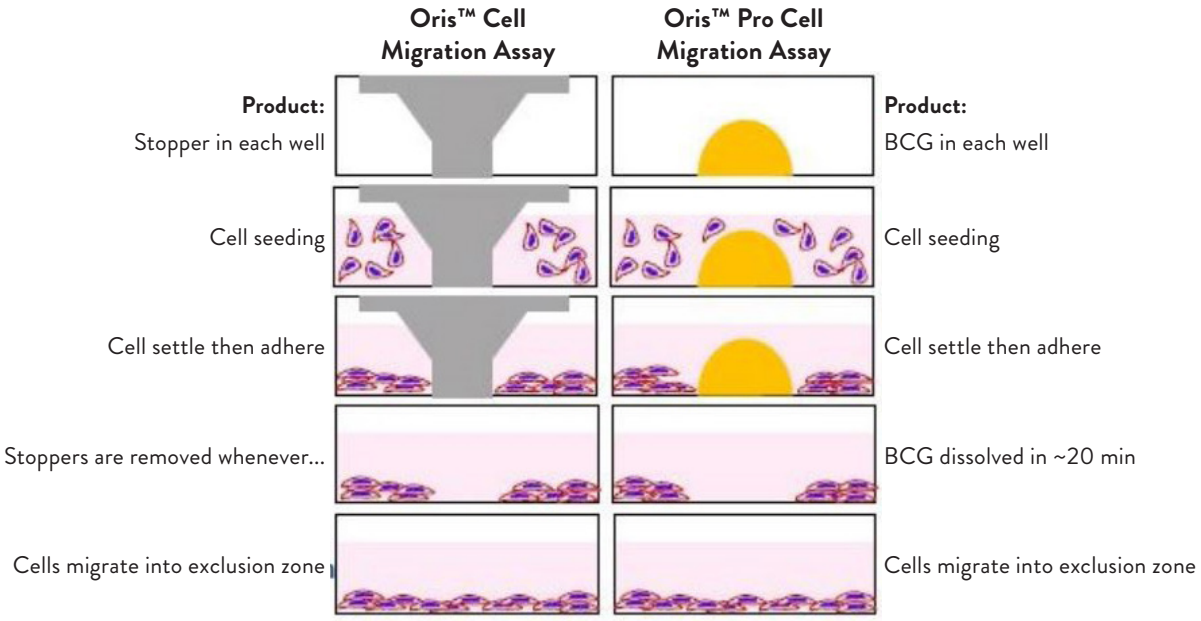


Figure 50. Schematic depiction of the concepts underlying behind Oris™ and Oris™ Pro Cell Migration Assays. *Unique Exclusion Zone Technology creates a central cell free zone in each well which enables quantification of cells migration. The initial size and shape of the cell free zone ensures robust statistics. It also facilitates the quantification of fluorescent staining with plate readers for those who do not have access to appropriate microscopes. In case of Oris™ Assays cells are seeded around a physical barrier, which is created by silicon stoppers. These stoppers can remain in place after cells seeding for a desired length of time, facilitating extended adhesion times for the cells that need it. On the other hand Oris™ Pro Assays offer a different solution. Cells are seeded around a spot of non-toxic BCG that dissolves after media and cells are added.*

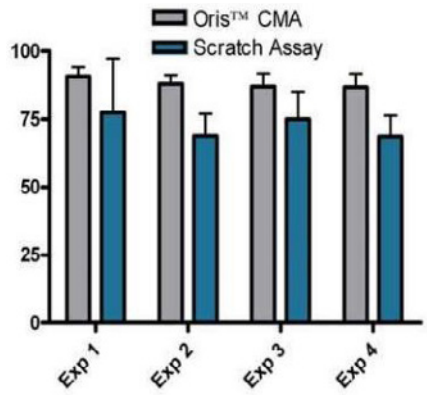


Figure 51. *Graph comparing effectiveness of the Oris™ Cell Migration Assay (CMA) and scratch assay in determining cells migration. Assays were performed in parallel over four experiments. Data are presented as average percent closure ± SD (n≥12).*

Selected Applications of Oris™ and Oris™ Pro Cell Migration Assays

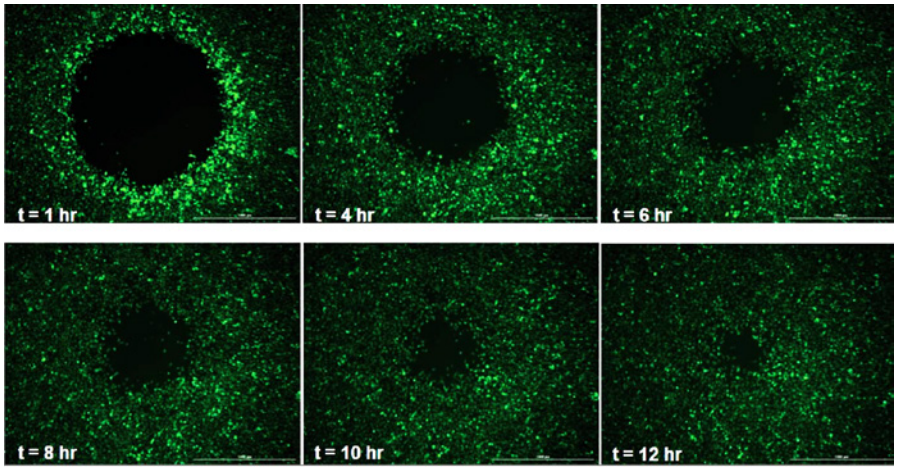


Figure 52. Seeding density and incubation time optimization using Oris™ Pro Cell Migration Assay. *Representative images from a well containing 50K HT-1080 cells per well in a 96-well plate format imaged kinetically every 2 hours for 12 hours.*

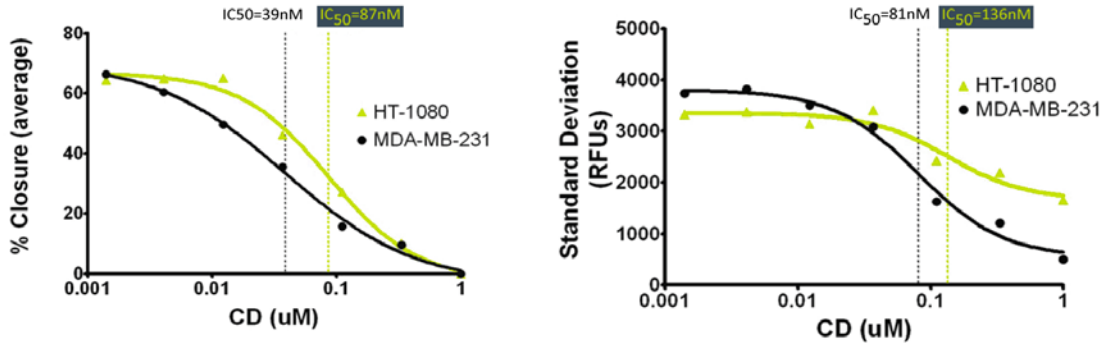


Figure 53. Dose response curves generated from the data obtained using Oris™ Pro Cell Migration Assay. *MDA-MB-231/GFP and HT-1080 CT cell were imaged in the 96-well microplate format after treatment with CD in dose response format* ***(A)*** *Image J analysis plots percent closure vs. inhibitor concentration while* ***(B)*** *Gen5 analysis plots standard deviation vs. inhibitor concentration.*

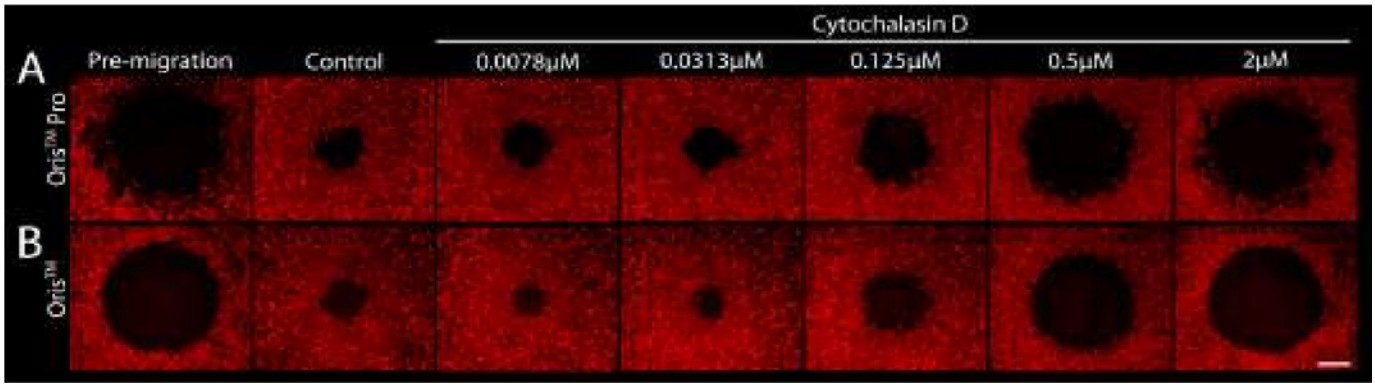


Figure 54. *Determination of IC_{50} values for the actin polymerization inhibitor, Cytochalasin D, on the migration of HUVECs using the Oris™ Pro (A) and Oris™ (B) Assays. Cells were labeled with TRITC-phalloidin.*

Invasion Assays

Oris™and Oris™ Pro Cell Invasion Assays are simple quantitative kits to monitor cells invasion and morphological changes in a more physiological relevant environment. These 96-well invasion assays enable cell movement through a 3D Collagen I extracellular matrix offering quantifiable and reproducible data from well to well and plate to plate. With no Transwell™or artificial membrane obscuring visualisation of the cells, invasion rates and morphology changes can be measured as end-point assays or in real time. Oris™ and Oris™ Pro Cell Invasion Assays can be used with a variety of instruments, from microscopes, plate readers and to high content imaging systems contributing to a significant reduction in handling time for set up and analysis.

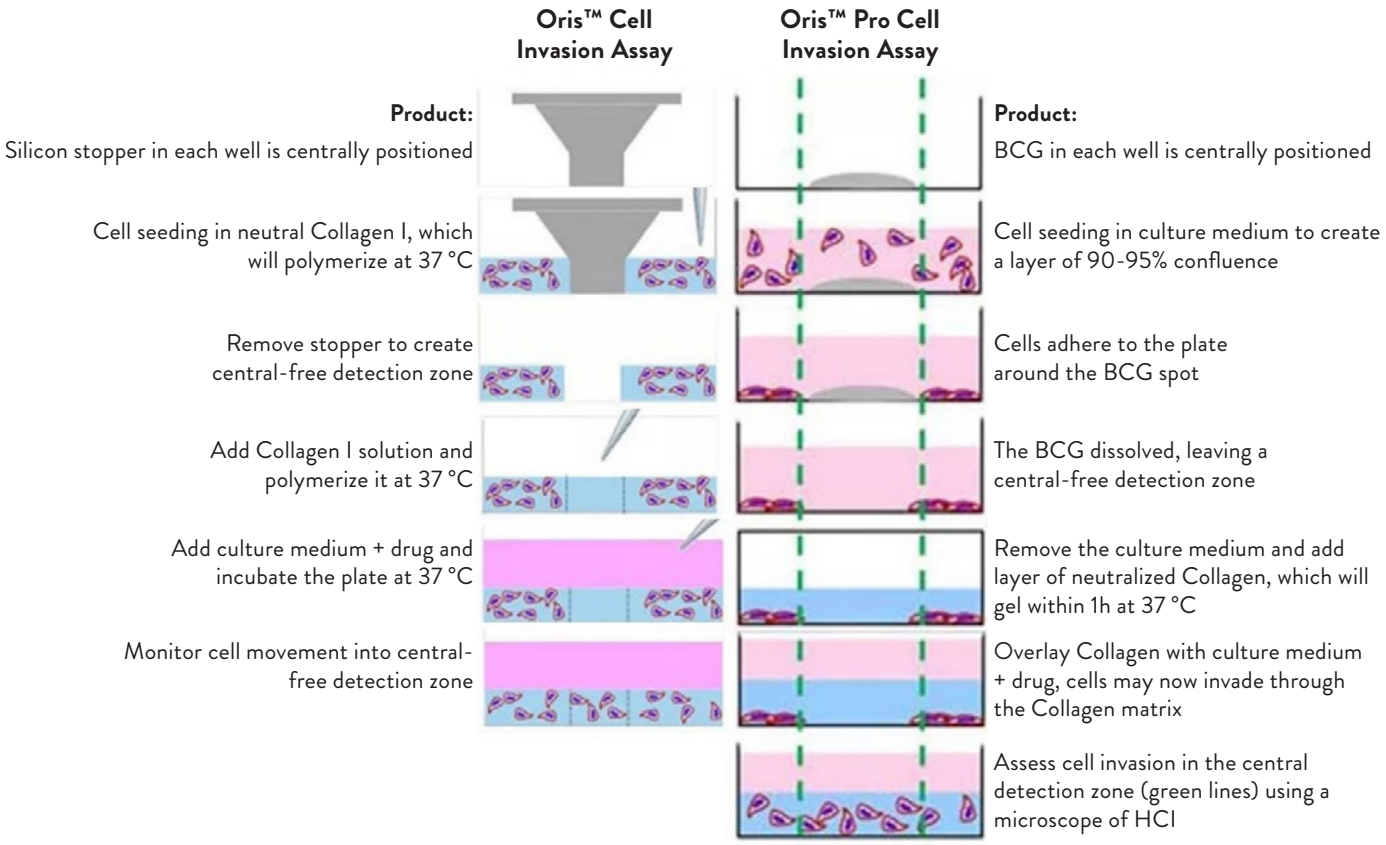


Figure 55. *Schematic depiction of the concepts underlying behind Oris™ and Oris™Pro Cell Invasion Assays.*

BENEFITS

- ✓ Enhanced Efficiency– Screen more compounds in a fully automatable 384-well format
- ✓ Increased Reliability– Obtain robust and reproducible data from high content imaging/high content screening (HCI/HCS) instrumentation
- ✓ Generate More Useful Data– Use multiplexed staining to simultaneously measure cell movement, morphology and phenotypic changes

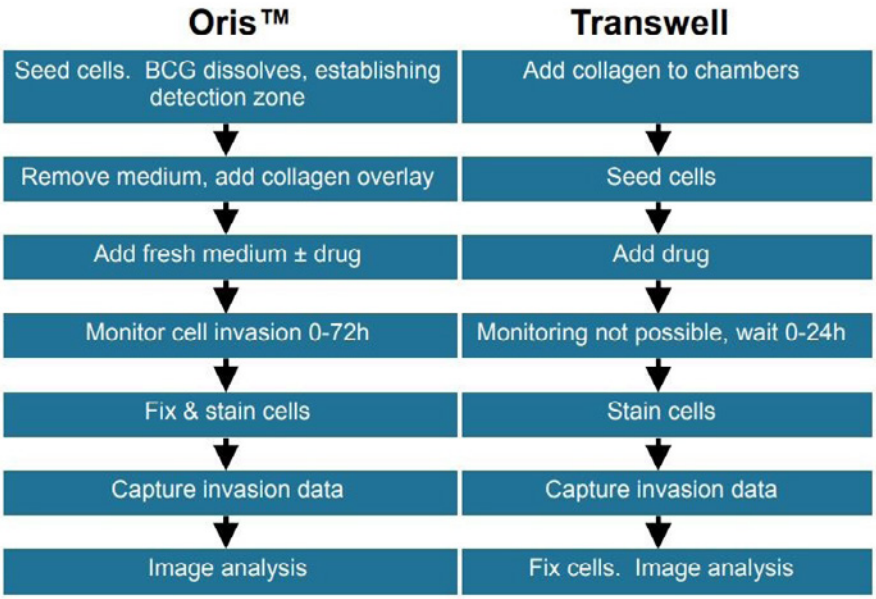


Figure 56. *Comparison of the workflows for Oris™ Pro Cell Invasion Assay and Transwell Invasion Assay.*

Selected Applications of Oris™ and Oris™ Pro Cell Invasion Assays

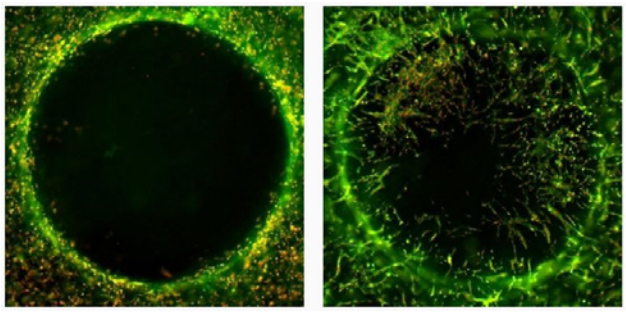


Figure 57. *Using the Oris™ 3D Embedded Invasion Assay, cells are suspended in a thick layer of Collagen I surrounding a 2 mm circular central cell free Collagen I detection zone (left image). After several days of incubation, invading cells move into the detection zone (right image) where they can be unambiguously quantified. Images are false-colour composites of the detection zone stained for nuclei (red) and actin (green).*

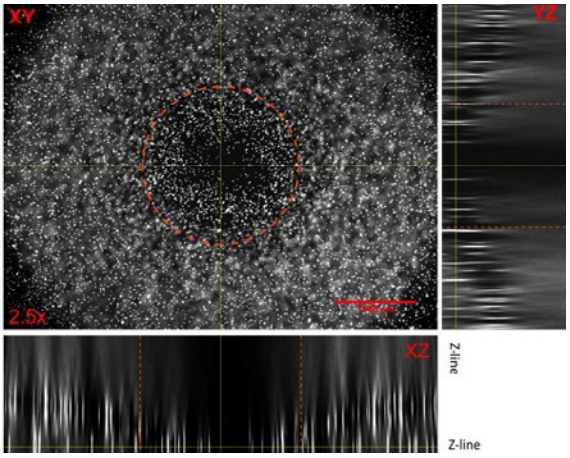


Figure 58. Image analysing using image J to generate orthogonal view. *Z-stack images of HT-1080 cells taken during Oris™ 3D Embedded Invasion Assay, were analysed using Image (NIH) software. Representative image shown above was taken at 2.5x magnification. Stacks of the image were taken at optimal Nyquist rate 21.39 µm. Image was processed to obtain orthogonal views of the XZ plane (bottom panel) and YZ plane (right panel). The Z-lines were set at the zero plane for each well. Total height of Z-stacks is 1519 µm. An outline of the 2 mm diameter detection zone (red dashed lines) serves as a reference point for cell invasion which has been superimposed onto all images accordingly.*

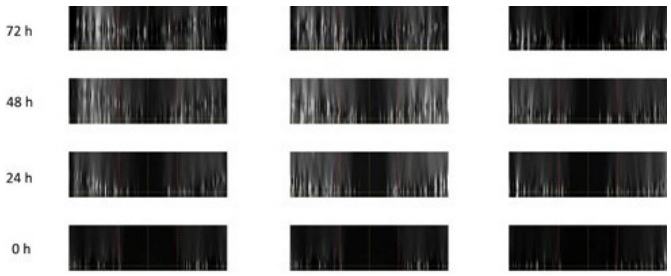


Figure 59. Effect of Collagen overlay density as a function of time in cell invasion assay: Increasing the concentration of the Collagen overlay restricts the invasion of HT-1080 cells. *An Oris™ 3D Embedded Invasion Assay formed with HT-1080 cells overlaid with 1, 2 or 3 mg/mL Collagen and assayed at 0, 24, 48, and 72h. The results demonstrate an increase in cell invasion as a function of time. Lower levels of invasion were seen in the higher concentration Collagen matrices. At 24h, cell movement into detection zone (dashed vertical lines) was observed. At 48h and 72h, significant invasion into the Z-plane and detection zone was observed with 3 mg/mL allowing the least amount of cellular movement. Representative images shown were taken at 2.5x magnification (12 replicates per condition) and are 1519 µm in Z-height.*

Oris™ and Oris™ Pro assays are available with tissue culture treated plates, Collagen I coated plates, Fibronectin coated plates or a TriCoated version that contains all 3 surfaces.

Cell Migration Assays	Catalogue No.			
	1 x 96 well plate	5 x 96 well plate	1 x 384 well plate	5 x 384 well plate
Oris™ Pro Cell Migration Assay - Tissue Culture Treated	PROCMA1	PROCMA5	PRO384CMA1	PRO384CMA5
Oris™ Pro Cell Migration Assay - Collagen I Coated	PROCMACC1	PROCMACC5	PRO384CMACC1	PRO384CMACC5
Oris™ Cell Migration Assay Kit - Tissue Culture Treated	CMA1.101	CMA5.101		
Oris™ Cell Migration Assay - Collagen I Coated	CMACC1.101	CMACC5.101		
Oris™ Cell Migration Assay - Fibronectin Coated	CMAFN1.101	CMAFN5.101		
Oris™ Cell Migration Assay - TriCoated	CMATR1.101	CMATR5.101		

Cell Invasion Assays	Catalogue No.		Description
	1 x 96 well plate	5 x 96 well plate	
Oris™ Pro 96-well Invasion Assay	PROIA1	PROIA3	The Invasion Assays come with a Collagen I overlay that creates a 3D extracellular matrix for cell invasion. Cell invasion into the Detection Zone can be assessed by performing a Z-stack analysis. The Plus variation provides a higher concentration matrix.
Oris™ Pro 96-well Invasion Assay Plus	PROIAPLUS1	PROIAPLUS3	
Oris™ 3D Embedded Invasion Assay	EIA1	EIA3	
Oris™ 3D Embedded Invasion Assay, starter pack for 48 wells	EIAST		

Cell Migration Kits	Catalogue No.		Description
	1 x 96 well assay	5 x 96 well assay	
Oris™ Universal Cell Migration Assembly Kit	CMAU101	CMAU505	The Universal Cell Migration Assembly Kit includes the cell plate separately from the stoppers which should be inserted according to the protocol provided. This enables you to coat the plate with a specific protein combination suitable for your target cell (important for neurons for example). The FLEX allows for more control over how many tests can be performed in a single experiment.
Oris™ Universal Cell Migration Assembly Kit—FLEX (4 x 24 stoppers)	CMAUFL4		

SPHEROID AND ORGANOID BASED ASSAYS + SOLUTIONS

One of the most successful and popular approaches to creating physiologically relevant cell cultures has been spheroid based solutions. In their simplest format, a small number of cells can be seeded on a low-attachment surface to induce the clustering of cells into spheroids. In most cases, several other signals will be required to generate the correct micro-environment: these can come from other cell types in the culture system, addition of extracellular matrices, or any other reagents added. Spheroid based approaches are shown to quantify the proliferation and invasion of cancer cells. The formation of these spheroids can be assisted by specialised basement membrane extract to enhance the adhesion of the cells to each other. This ensures the ability to quantify the size of each spheroid in a 96-well plate and enhance the throughput of these assays. Addition of another specialised basement membrane extract enables invasion from the central spheroid, again enabling quantitative approach to this complex and important process in cancer biology.

Spheroid based kits are the next step in the evolution of 3D culture. These 96-well kits utilise specially formulated extracellular matrix to enhance the generation of multicellular spheroid models for quantification of proliferation and invasion. While these assays were developed primarily for cancer cells, they could be used with other cell types with invasive properties such as endothelial cells.

The 96-well spheroid formation plates are a flexible, standardized, high-throughput format to study cell proliferation and invasion. Cells are added to 3D Culture-qualified, specialized matrices for spheroid formation and invasion with no transfer steps required. This allows for simple and easy standardisation.

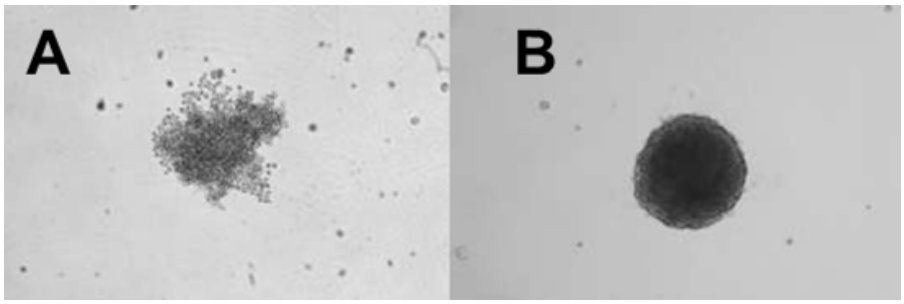


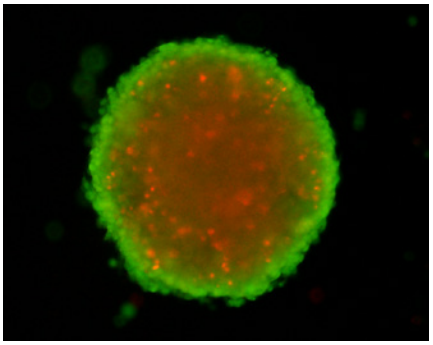
Figure 60. *MDA-MB-231 Breast cancer cells in spheroid culture for 3 days on U-bottom Low-Adhesion plates form (**A**) loose colonies in media only (**B**) tight spheroids in media with 1 X Spheroid Formation ECM.*

READ OUTS

- ✓ Proliferation
- ✓ Viability
- ✓ Invasion
- ✓ Immunofluorescence
- ✓ Live imaging

Selected Application of Spheroid Based Assays

Figure 61. *PC-3, Human prostate cancer spheroids increase in size with increasing cell seeding densities, exhibiting physiological tumor gradients resulting in viable cells on the surface and dead cells in the core. Cells assembled into spheroids over a 72 hour period and were treated with 2 µM Calcein AM which is metabolically converted to Calcein (green-viable) and with 1 µg/ml Propidium iodide (red-dead).*



Lipidure®- COAT Low-Attachment Solutions

Spheroid cell culture is typically based on the spontaneous formation of an aggregate of cells in an environment where cell-cell interactions dominate over cell-substrate interactions. This can be achieved by using low-attachment cell culture conditions. Obtained spheroids can be next passaged into organoids.

Lipidure®-COAT plates and dishes are a top of the range products for spheroid formation, with the Lipidure coating providing a superior low-attachment solution for the formation of single spheroids in each well of multi-well plates. Using Lipidure®-COAT dishes, it is possible to undertake large scale assays to provide sufficient materials for applications such as Western blotting or gene expression microarrays.

LIPIDURE®-COAT SOLUTIONS HAVE SEVERAL ADVANTAGES OVER OTHER TECHNOLOGIES:

1. Low-adhesion surface promotes cell aggregation & spheroid formation.
2. Uses a biocompatible MPC Polymer containing Phosphoryl Choline (which is found in cell membranes)
3. Completely synthetic, containing no substances of biological origin.

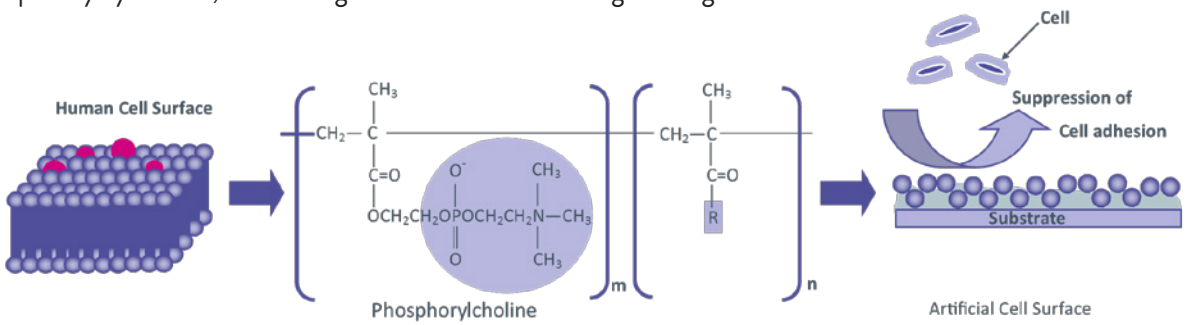


Figure 62. *Schematic depiction of Lipidure®-COAT low-attachment solution.*

BENEFITS

- ✓ Easy to handle
- ✓ Excellent reproducible results
- ✓ A superior low-attachment solution
- ✓ Compatible with variety of cell based assays
- ✓ Multiple formats for simple up-scaling

READ OUTS

- ✓ Bright field imaging (clear plates)
- ✓ Fluorescence (black plates)
- ✓ Luminescence (white plates)

Selected Applications of Lipidure®- COAT

LOW CELL BINDING PROPERTY OF LIPIDURE®-COAT

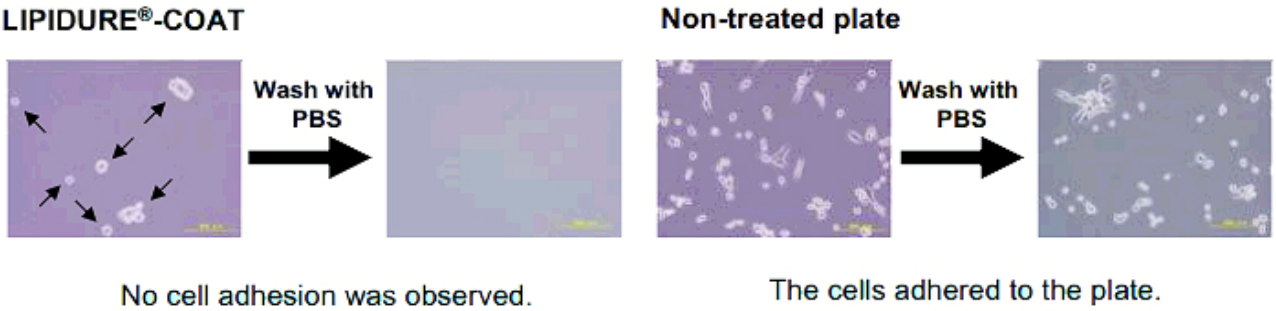


Figure 63. *NIH 3T3 (Fibroblast, mouse) spheroids were grown for 4 days on Lipidure®-Coat Multi-Dish A-6MD and uncoated plate, then washed with PBS and followed with low cell binding properties evaluation. Taken images have shown that obtained spheroids adhered to the bottom of the plate uncoated with Lipidure® powder.*

EMBRYOID BODY FORMATION

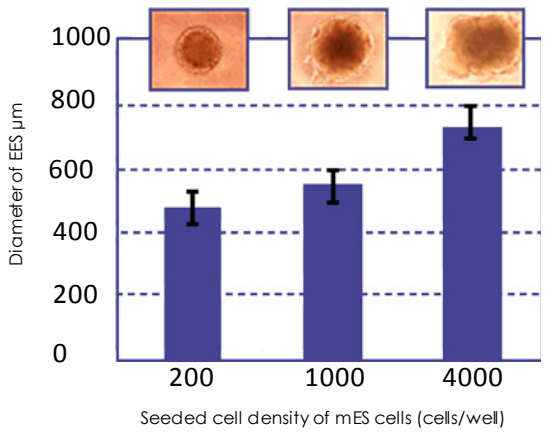


Figure 64. *Example of embryonic bodies formed using variable starting densities of the murine ES cell line 129SV on Lipidure®-Coat Plate A-U96.*

NEUROSPHERE FORMATION

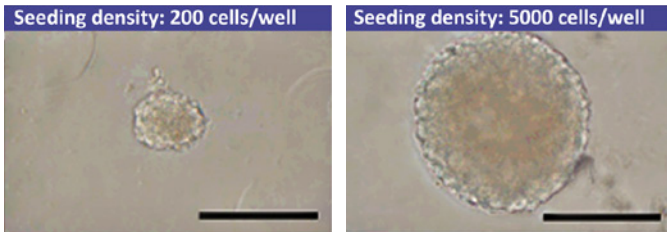


Figure 65. *Neurospheres formed by Rat Hippocampus Neuronal Cells at different cells seeding density by day 5 on Lipidure®- COAT Plate A-U96. Scale bar—200 µm. Photo supplied by Dr Ljima, Kyusyu University.*

SUPERIOR FORMATION OF SINGLE SPHEROID ON U-BOTTOM PLATE

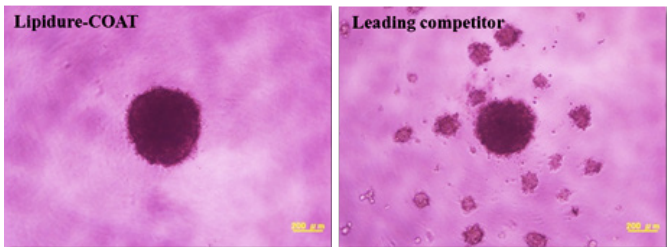


Figure 66. *A single spheroid is generated in the Lipidure®-COAT well, while numerous satellite spheroids are found in the competitor's plates. This phenomenon shows that a lot of cells adhered to the competitor's "low-adhesion" surface.*

Description	Pack Size	Catalogue No.
Lipidure®-Coat Low Adhesion Plate A-U96 (96 well U-bottom plate)	6 plates	AMS.LCP-A-U96-6
Lipidure®-Coat Low Adhesion Plate A-V96 (96 well V-bottom plate)	6 plates	AMS.LCP-A-V96-6

Proteins Regulating Cell Transcription

We offer the most widely used factors for regulating transcription used with physiologically relevant cell cultures. They are involved in crucial signaling pathways such as proliferation, growth and differentiation. Thus, manipulating your culture using these factors you can control cell fate to a very high level.

To see the full range of proteins we offer please refer to our website, contact us or look through our *Organoid Culture Handbook.*

WNT

Wnt proteins are a family of cysteine-rich secreted polypeptides (more than 16 mammalian family members) involved in several important cell functions such as cell-cell communication, proliferation, migration, polarity, survival and self-renewal. Wnt3a particularly plays an important role in the ability of organoids to expand. Additionally, loss of activation of Wnt expression is associated with alteration of cell fate, morphogenesis and mutagenesis.

Wnt3a is highly unstable in serum-free medium, its half-life is about 2 hours. Our stabilizer significantly reduces aggregation, allowing Wnt to maintain activity for 30 hours in serum-free culture conditions (Figure 67). With the presence of Wnt protein stabilizer, purified Wnt3a protein can support even colon organoid cultures (requiring strong Wnt activity).

David Keller from Nexus Personalized Health Technologies in ETH Zurich purchased Wnt3a from us. Here is what he says about our Wnt3a products:

"Your Wnt3a in the TOP/FOP Flash Reporter Assay showed higher activity at the same concentration than the industry leader"

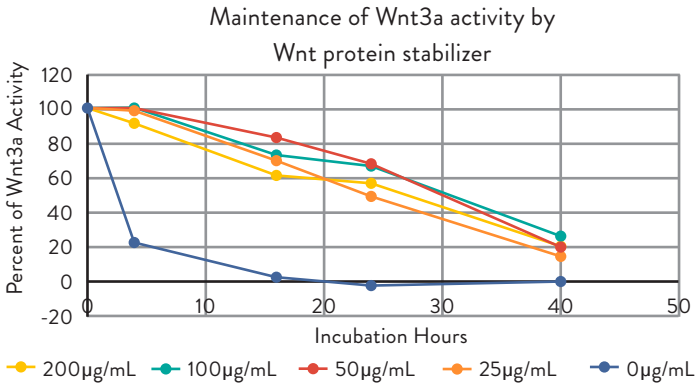


Figure 67. *Wnt3a half-life in serum free medium increases from around 2 hours in absence of a stabilizer (blue line) to 30 hours with increasing concentrations of stabilizer. Activity without incubation is set as 100% and background reading is set at 0%. The readings of Wnt3a activity from incubated (37 °C) samples are calculated as percentage of Wnt3a without incubation. Wnt3a activity was measured using TOP-Flash Reporter Assay. Measurements performed on NIH3T3 Wnt NIH3T3 Wnt reporter stable cell line.*

AMSBIO offers a range of Wnt human and mouse recombinant proteins in low (75%) and high (85 – 90%) purity. These human recombinant proteins are purified from HEK293 cells while the mouse proteins are expressed in CHO cells. Both are suitable for various cell based assays and treatments.

Description	Purity	Pack Size	Catalogue No.
Mouse Recombinant Wnt3a	75%	2 µg	AMS.rmW3aL-002
		10 µg	AMS.rmW3aL-010
	85-90%	2 µg	AMS.rmW3aH-002
		10 µg	AMS.rmW3aH-010
Human Recombinant Wnt3a	75%	2 µg	AMS.rhW3aL-002
		10 µg	AMS.rhW3aL-010
	85-90%	2 µg	AMS.rhW3aH-002
		10 µg	AMS.rhW3aH-010

Add "–stab" at the end of the catalogue number to get the Wnt protein with its stabilizer (eg. AMSBIO.rmW3aL-002-stab)

R-SPONDIN-1

Roof plate-specific Spondin-1 (R-Spondin-1 or RSPO1), also known as CRISTIN3, is a 27 kDa secreted activator protein that belongs to the R-Spondin family. R-Spondins positively regulate Wnt/β-catenin signaling, most likely by acting as a ligand for LGR4-6 receptors and an inhibitor for ZNRF3. R-Spondin-1 induces proliferation of intestinal crypt epithelial cells, increases intestinal epithelial healing, and supports intestinal epithelial stem cell renewal. R-Spondin-1 is a critical ingredient used in the maintenance and proliferation of mouse and human organoid progenitor stem cells.

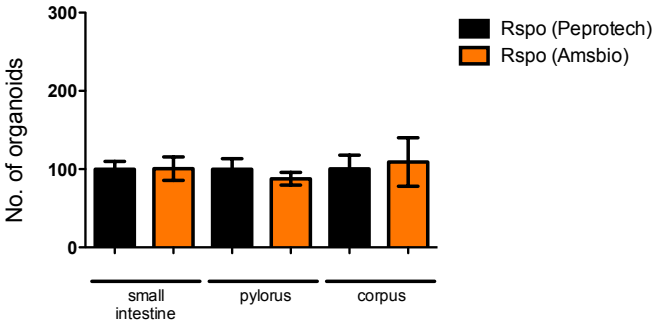


Figure 68. *Organoid counts of small intestine and gastric (pyloric and corpus) organoids at 4 days in culture using RSPO1 from AMSBIO and competitor. Data courtesy of Dr Nick Barker, A* STAR Institute of Medical Biology, Singapore.*

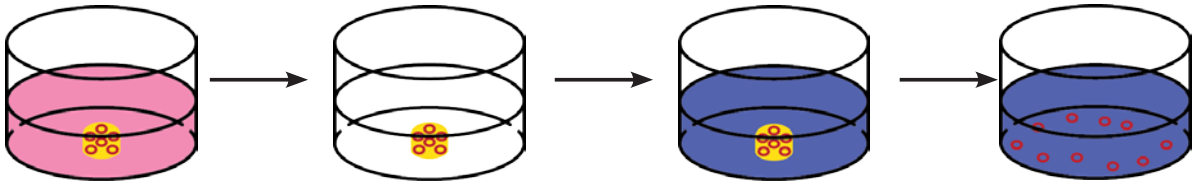
Description	Pack Size	Catalogue No.
Human Recombinant R-Spondin-1	10 µg	7189-10
	50 µg	7189-50
	5 µg	AMS.PBG10386-U005
	20 µg	AMS.PBG10386-U020
	50 µg	AMS.RS1-H4221-50ug
	1 mg	AMS.RS1-H4221-1mg
Human CellExp™ R-Spondin-1	10 µg	7482-10
	10 µg	AMS.PBV11103r-10
Mouse Recombinant R-Spondin-1	50 µg	AMS.RS1-M5220-50ug
	1 mg	AMS.RS1-M5220-1mg
Purified recombinant protein of Human R-Spondin homolog (Xenopus laevis)	20 µg	TP723385

Cell Harvesting and Storage

SPHEROID AND ORGANOID HARVESTING SOLUTION

Spheroid and Organoid cultures exhibit cellular behaviors and morphologies similar to those seen *in vivo*. However, the adaptation of these models for studying biochemical processes has been impeded by the challenge of separating intact organoids and spheroids from extra-cellular proteins comprising the hydrogel. Commonly, proteases are employed to degrade these extracellular proteins, however, proteases also degrade proteins on the cell surface and protease activity may carry over into subsequent cultures or lysate preparations. Spheroid and Organoid Harvesting Solution provides a ready to use, non-enzymatic method for depolymerizing extracellular matrix proteins to allow for harvesting of intact organoids and spheroids for passaging, cryopreservation, or biochemical analysis.

HARVESTING PROTOCOL



Count organoids to determine split for passaging

Aspirate Organoid Culture Medium and wash with cold (4 °C) PBS

Add cold (4 °C) Organoid Harvesting Solution (3700-100-01) - 30 min with gentle rocking

ECM depolymerizes leaving intact organoids

BENEFITS

- ✓ Ready to use
- ✓ Non-enzymatic chelating solution
- ✓ Depolymerizes basement membrane matrix for harvesting organoids from culture
- ✓ Gentle for cells: preserves original morphology

APPLICATIONS

- ✓ Organoid passaging
- ✓ Sample preparation (PCR, Western Blot, and Immunohistochemistry)

Description	Pack Size	Catalogue No.
Spheroid & Organoid Harvesting Solution	100 ml	AMS.3700-100-01

DETACHIN™ CELL DETACHMENT SOLUTION

Detachin™ Cell Detachment Solution is a superior alternative to Trypsin/EDTA for gentle detachment of *in vitro* cultured adherent cells. It provides a quick, consistent, safe, and efficient method for cell detachment and dissociation from all known tissue culture plastic ware. Detachin™ contains protease and Collagenase activities in an isotonic, phosphate buffer solution with EDTA. It has been tested successfully on many different primary cells and cell lines including: bone marrow cells, stem cells, fibroblasts, hepatocytes, mouse germ cells, keratinocytes, macrophages, chick embryo cells, neuronal cells, vascular endothelial cells, A-375, BHK, CHO, COS, D54, HEK293, HeLa, L-929, M-24, MG-63, MRC-5, NIH-3T3, NT-2 cells, U-251, Vero, Sf9 insect cells, and many others.

BENEFITS

- ✓ Gentle and rapid detachment
- ✓ Maximum cell viability over Trypsin
- ✓ Effective on a wide range of cells
- ✓ No mammalian or bacterial by-products
- ✓ No need to wash detached cells
- ✓ Stable at 4 °C for 2 months
- ✓ Convenient and cost effective variety of formats
- ✓ Unused Detachin™ can be preserved for longer term storage

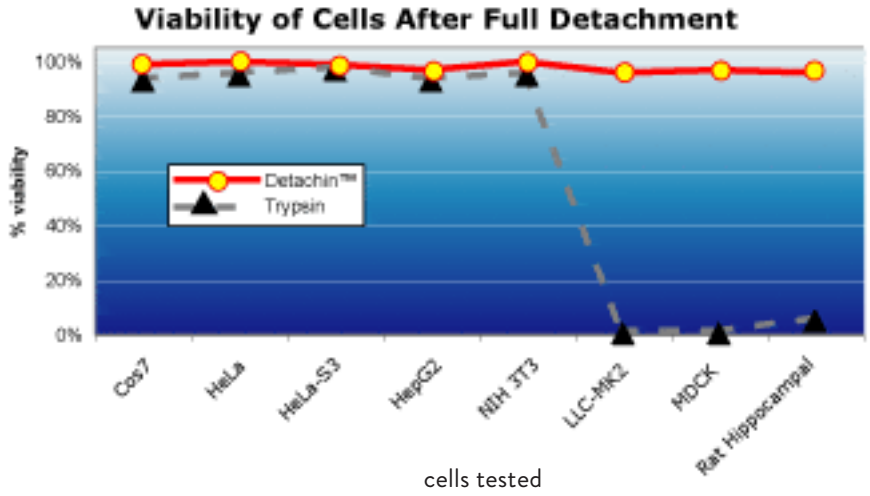


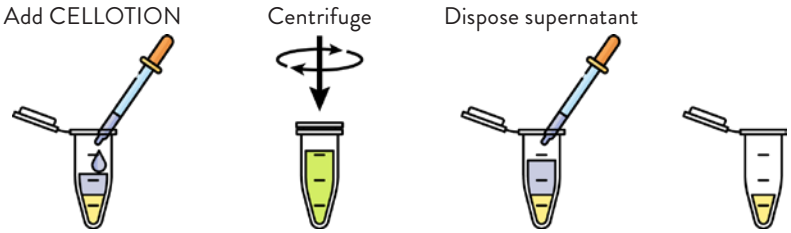
Figure 69. *Comparison of various cells viability after full detachment using Detachin™ vs. Trypsin.*

Description	Pack Size	Catalogue No.
Detachin™	100 ml	T100100
Detachin™	10 x 100 ml	T100110
Detachin™ 6-PAC	6 x 50 ml	T100106

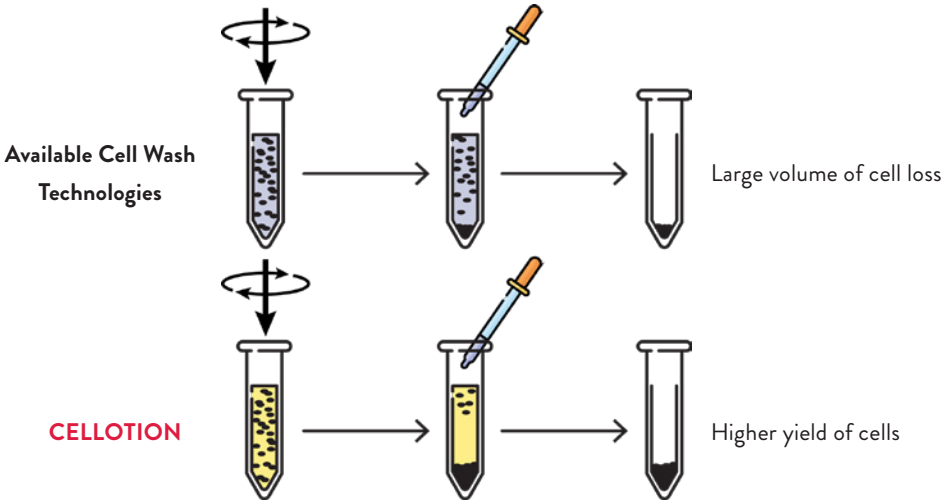
CELLOTION CELL WASH AND RECOVERY SOLUTION

Cellotion is a safe and chemically defined cell washing solution for increased cell recovery. The unique formulation of Cellotion is serum, proteins and sugars free, and can be applied to cells, tissues and organs washing. A series of cell washes during cell isolation or post-thawing manipulation leads to significant cell loss. Washing with Cellotion can result in 40% loss of cells, what ensures better yields of recovered cells. Cellotion can be used as a substitution for general use cell wash solution such as PBS buffer, saline or culture medium with standards cell wash procedures.

PROTOCOL



EFFICIENCY OF CELLOTION



BENEFITS

- ✓ Significantly increased cell yield after washing procedures while maintaining cell viability
- ✓ Chemically defined solution with serum and animal derived component fee formulation
- ✓ Simple protocol

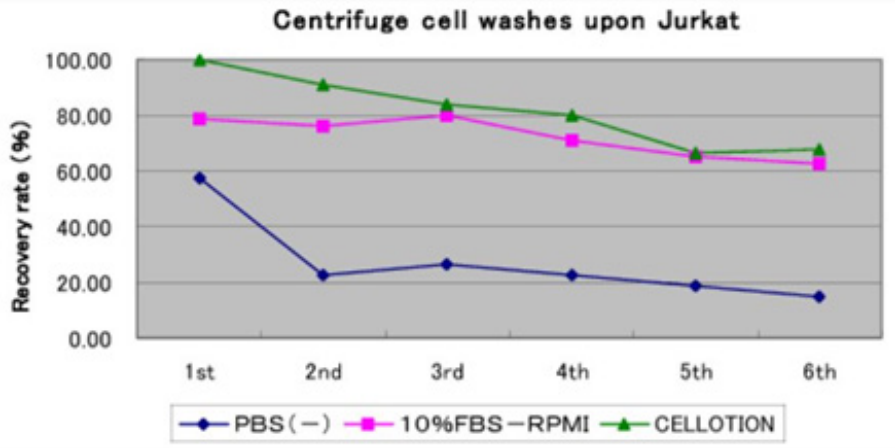


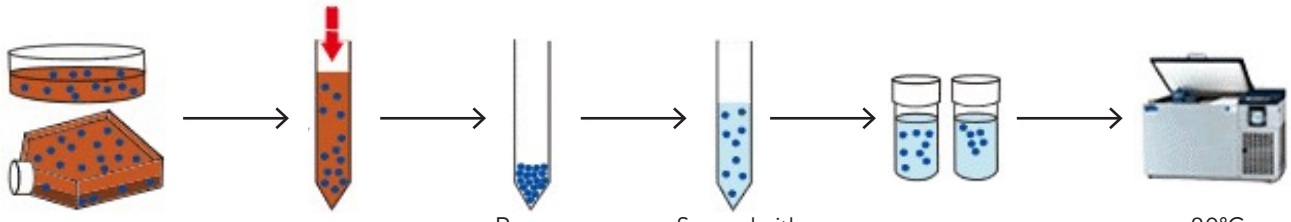
Figure 70. *Comparative analysis (multiple washing procedures) of Cellotion Cell Wash and Recovery Solution vs. general use cell wash technologies.*

Description	Pack Size	Catalogue No.
Cellotion	100 ml	11898

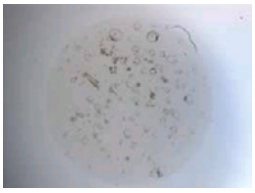
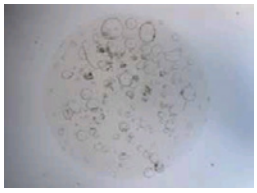
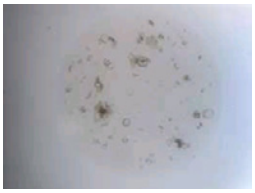
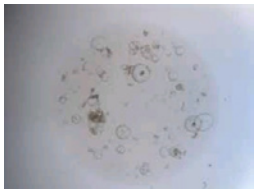
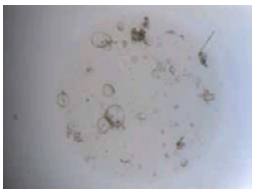
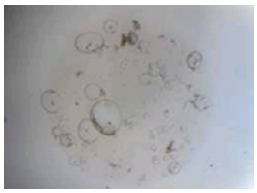
CELLBANKER® FREEZING MEDIA SERIES

CELLBANKER® is a series of easy to use cells cryopreservation media. CELLBANKER® enables long term storage of different type of cells maintaining consistent high cells viability regardless of their sensitivity due to superior protection against cell stress during freeze/thaw cycles. As cells freezing medium, CELLBANKER® does not require a gradual temperature decrease in programmed freezer nor storage in liquid nitrogen to guaranty efficient cells storage. This makes CELLBANKER® more affordable and accessible than other cells freezing media. CELLBANKER® solutions are simple to use and allow to achieve the highest cells viability while maintaining cells natural functions.

CRYOPRESERVATION PROTOCOL



Human Liver Organoids Stored in CELLBANKER®

	Day 2	Day 4	Organoid Growth Following Freezing
CELLBANKER® 1			☑
CELLBANKER® 2			☑
STEM-CELLBANKER® GMP grade			☑

Images courtesy of Robert Arnes, Huch Lab

"Day 2 and day 4 images clearly show that organoids recovered and grew well in these three CELLBANKER® freezing media"

-Meritxell Huch, Gurdon Institute, Cambridge, UK

CELLBANKER®1

The first product of the CELLBANKER® series, CELLBANKER® 1, was launched in 1992 and now has a significant history of reliable, consistent and high viability recoveries post-cryopreservation.

Contains serum, DMSO, glucose, salts and buffer.

Description	Pack Size	Catalogue No.
CELLBANKER 1	20 ml	11889
CELLBANKER 1	4 x 20 ml	11884
CELLBANKER 1	100 ml	11888

CELLBANKER®2

CELLBANKER® 2 is a serum free cells freezing medium, which formulation is optimised for serum free cultured cells and peptide/protein expressing cells. As it does not contain any animal derived products it is recommended for all applications where risks of contamination must be avoided.

Contains no animal derived products, fully defined.

Description	Pack Size	Catalogue No.
CELLBANKER 2	20 ml	11892
CELLBANKER 2	4 x 20 ml	11893
CELLBANKER 2	100 ml	11891

STEM-CELLBANKER® (GMP Grade) - DMF with FDA

STEM-CELLBANKER® is a chemically defined, xeno free freezing medium manufactured in compliance with JPN, EU, US, and PIC/S GMP guidelines - optimized for stem cells and iPS cells storage as well as other valuable cells.

Available in DMSO and DMSO free formulations STEM-CELLBANKER® is completely free of serum and animal derived components and contains only European or US Pharmacopoeia graded ingredients. STEM- CELLBANKER® is ready to use and requires no special devices, such as a controlled rate freezer, in order to achieve consistently high cell viability following resuscitation from cryopreservation, even over extended long-term storage. STEM-CELLBANKER® significantly increases cell viability while maintaining cell pluripotency, normal karyotype and proliferation ability after freeze-thaw. It is an optimal freezing. Cryopreservation of cells using STEM-CELLBANKER® is an optimal solution for basic research and in the clinical application of cell therapy products.

Description	Pack Size	Catalogue No.
STEM-CELLBANKER® - GMP	20 ml	11897
STEM-CELLBANKER® - GMP	4 x 20 ml	11894
STEM-CELLBANKER® - GMP	100 ml	11890
STEM-CELLBANKER® - GMP - DMSO Free	20 ml	11897F
STEM-CELLBANKER® - GMP - DMSO Free	4 x 20 ml	11894F
STEM-CELLBANKER® - GMP - DMSO Free	100 ml	11890F

HSC-BANKER® - GMP Grade

Hematopoietic Stem Cells cryopreservation medium, HSC-BANKER®, is an optimized serum free GMP grade medium specially formulated for hematopoietic stem cells.

The results of comparative study on the cryopreservation of hematopoietic stem cells, performed by the Cord Blood Bank of Japanese Red Cross Society, revealed that HSC-BANKER® is at least an equivalent to conventional freezing media containing DMSO and dextran.

Contains no animal derived products, chemically defined, GMP manufactured.

Description	Pack Size	Catalogue No.
HSC-BANKER	15 ml	11900

Custom Screen in 3D Services

We have recently partnered with University of Strathclyde in Glasgow (UK) to launch a new company, called ScreenIn3D (www.ScreenIn3D.com), to conduct Screening and Profiling Services for Drug Discovery, taking advantage of the latest advances in microfluidics and 3D cell culture to develop a novel microfluidic screening platform. ScreenIn3D service is the first application platform launched by ScreenIn3D, which is targeted at improving anticancer drug treatment and accelerating development of new personalised medicine solutions using patient derived multicellular tumor spheroids/organoids (comprising cancer cells, stromal cells, cancer stem cells and/or immune cells).

ScreenIn3D has been chosen by Society for Laboratory Automation & Screening (SLAS Europe 2018) as an exciting innovation technology company. ScreenIn3D has also won Product Innovation Award at Basel Life 2018, were it was one of 60 organizations showcasing cutting-edge technologies for research, science and innovation.

SCREEN IN 3D

Here are some of the **main features** of using our screening services:

- ✓ Obtain spheroids in the absence of matrices or scaffolds!
- ✓ Long term culture of 3D multicellular spheroids (up to 4 weeks), physiologically relevant co-culture models are available as well
- ✓ Make the most out of limited starting material: 10,000 cells needed to produce 100 spheroids, even in the case of primary cells
- ✓ Compact spheroids from primary cells form within 2 to 3 days
- ✓ Drug concentration gradients applied due to precise control of convective and diffusive mass transport
- ✓ Well characterized, patient-derived, and stable cell line cancer models (and many more under development)
- ✓ CRISPR cell lines representing more than 15 clinically relevant mutations are available (specific mutations available on request)
- ✓ Compatibility with robotic handling and microscopy interfacing
- ✓ Cost-effective screening: up to 8 concentration points per device with high statistical relevance
- ✓ Single and combination drug and radio-chemo combination available
- ✓ 100x more cost-effective than ULA plate assay

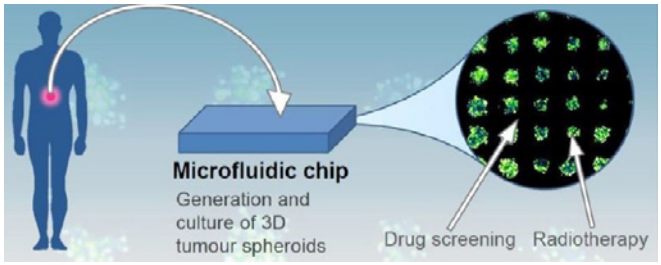


Figure 71. *From patient biopsies to drug screening using microfluidic platform.*

Our microfluidic platform offers precise control over the cellular microenvironment and maximizes use of precious limited human cancer samples.

We also **provide multiparametric, label-free and endpoint measurements for drug screening and profiling assays such** as:

- ✓ Measurement of changes in tumor spheroids shape and size
- ✓ Viability and proliferation measurement of spheroids
- ✓ Assessment of spheroid temporal evolution after drug treatment
- ✓ Assessment of apoptotic events
- ✓ Analysis for cytokine profiling
- ✓ Possibility of biomarker evaluation pre/post drug treatment
- ✓ Immunofluorescence

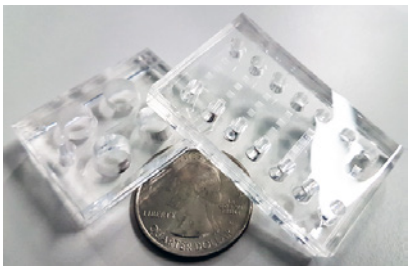
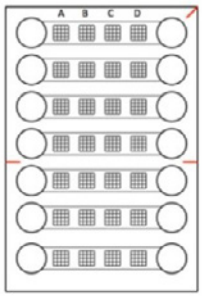
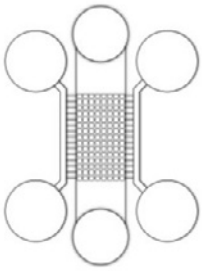


Figure 72. *Microfluidic devices.*



SINGLE CONCENTRATION PLATFORM



GRADIENT CONCENTRATION PLATFORM

- 5-8 points Conc. Resp. Curves
- Customer selected concentration values
- Preferred for cell lines

- 5-8 points Conc. Resp. Curves
- Linear concentration gradient (values 1-100% of MAX)
- Preferred for biopsy and limited/rare tissue samples

Figure 73. Schematic presentation of single and gradient concentration platforms.

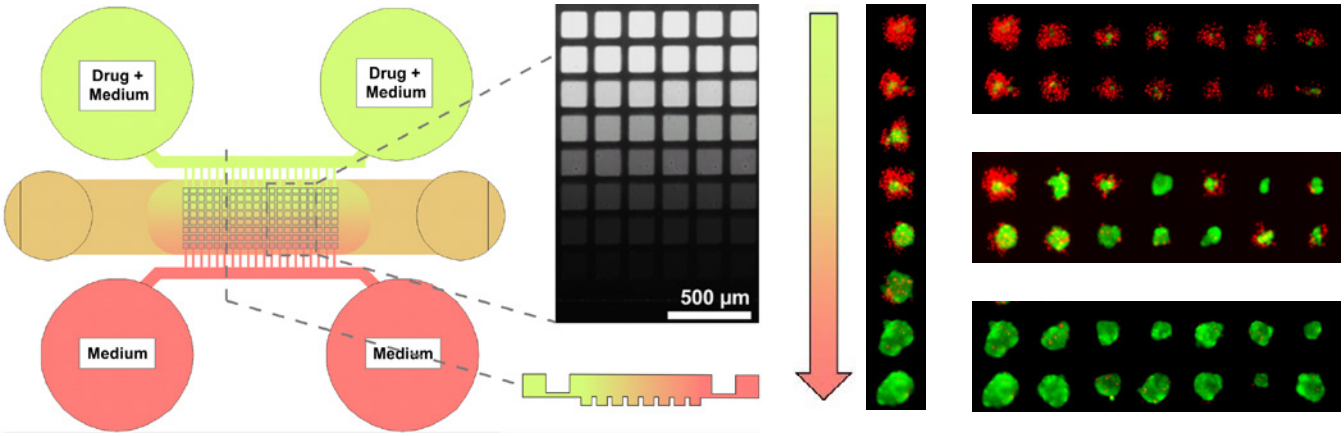


Figure 74. *Schematic representation of drug concentration and spheroid size gradient control.*

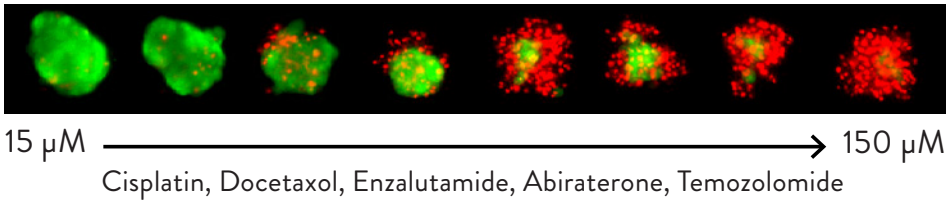


Figure 75. Assessment of spheroids viability. *With increasing concentration of commonly used drugs in cancer treatments the damage of spheroids progress, leading eventually to their death, what is indicated by increasing levels and intensity of red fluorescent coming from propidium iodide (a dye, which is unable to penetrate the cell membrane of healthy cells).*

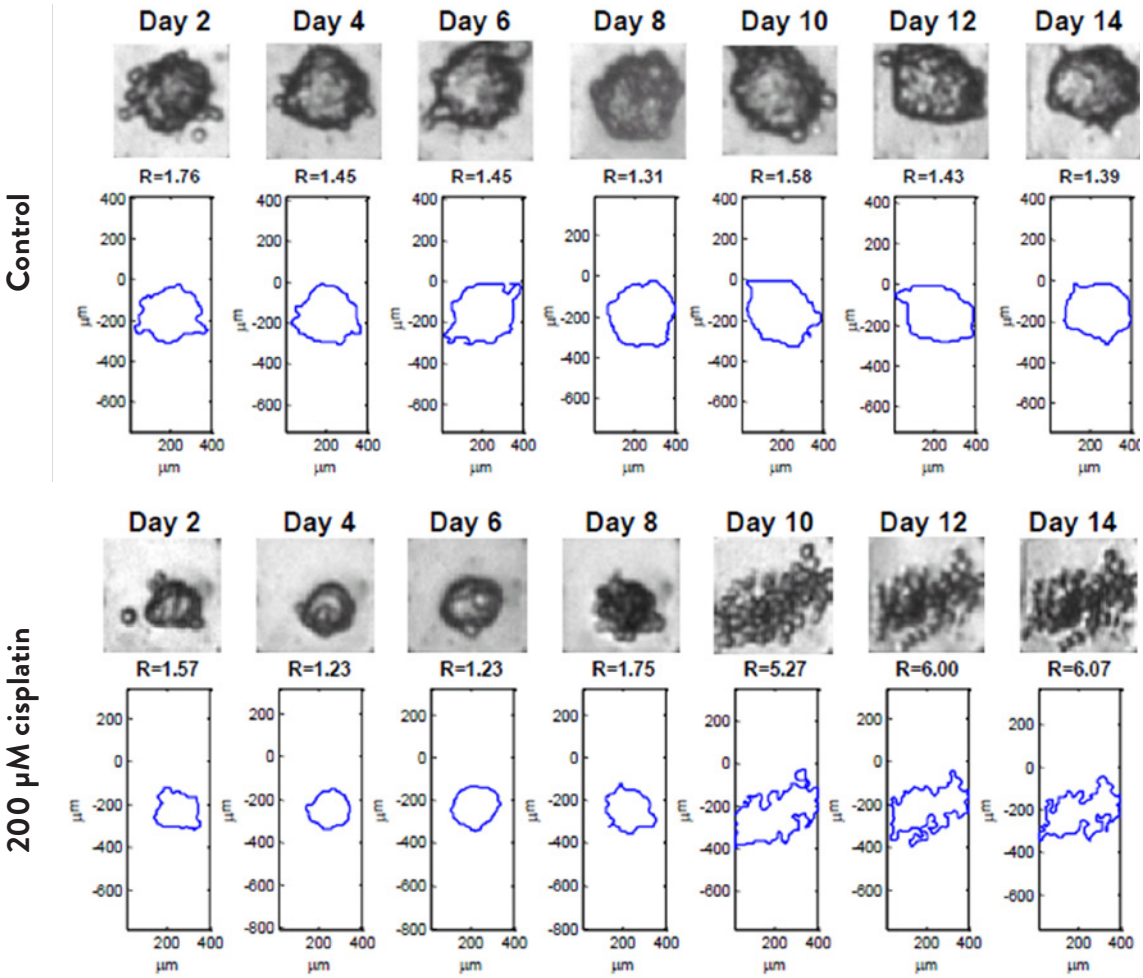


Figure 76. *Assessment of tumor spheroid size and volume shrinkage post cisplatin exposure using custom software image analysis tools.*

WE CAN:

- ✓ **Offer** a full consultation service to allow you start testing treatments in 3D
- ✓ **Help** design and perform screening experiments using physiologically relevant models
- ✓ **Assist** in deciding what the best assays and end-points are, which will enable to inform on follow-on in scientific or clinical considerations

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V2.6

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