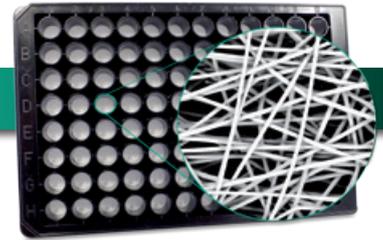


Mimetix tissue culture plates for 3D cell-based assays: ONCOLOGY



MIMETIX
CELLS IN 3D



Your new predictive tool in oncology drug discovery

There is a significant need for more predictive *in vitro* efficacy assays in oncology to reduce both the number of costly drug failures in clinical trials and the number of animals used in pre-clinical testing. Performing certain cell-based assays in 3D can improve their relevance as models. Cells grown in a 3D micro-environment have different morphology to those grown in 2D on tissue culture plastic and are in contact with other cells which influences tissue-specific gene expression, cell growth and the uptake and metabolism of drugs. It is known that the tumour physiological environment is intimately connected with cancer growth and progression. The Mimetix® 96-well tissue culture plate provides an ideal architectural environment to support the growth of cells in 3D, i.e. as a tissue rather than a flat layer, in a format that is easy to automate in the laboratory.

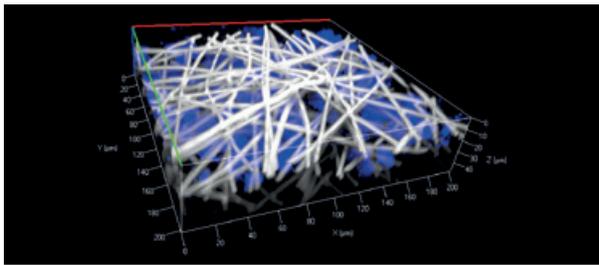
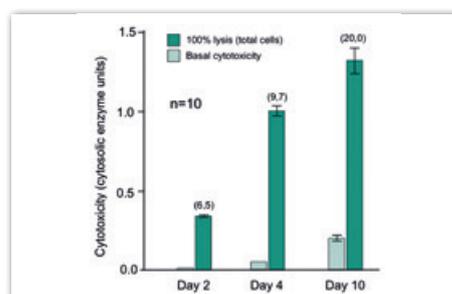


Image 1: MCF7 human breast cancer cells growing in 3D in the Mimetix scaffold

- Mimetix scaffolds are created by electrospinning the medical-grade polymer poly(L-lactide) (PLLA) into microfibrils, which are highly consistent with regard to fibre diameter and pore size, resulting in excellent reproducibility of cell-based assays.
- The scaffold depth of 50 µm is thick enough to provide the benefits of 3D cell morphology, yet thin enough for microscopic imaging.

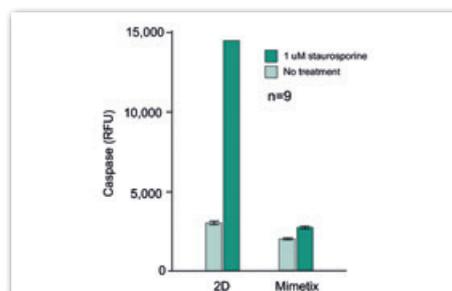
Cell proliferation

Over a 10-day culture period, total cell number increased steadily. The measurements were very consistent (CVs of <10%); comparable variability to that seen in 2D experiments (Data from Avanticell Science Ltd.)



Response to apoptotic drugs

The human breast cancer cell line HMT3909S8 is known to be resistant to drug-induced apoptosis in 3D hydrogel culture. When grown in Mimetix scaffolds and challenged with 1 µM staurosporine, the cells were significantly more resistant to apoptosis than those grown in 2D.



Experimental:

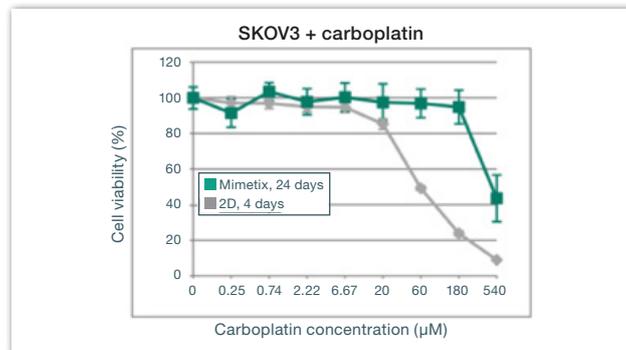
- HMT3909S8 human breast cancer cells are available from AvantiCell Science Ltd (cat no. BCL-HL-051) and were seeded at 15,000 cells/well in a Mimetix 96-well plate.
- Basal toxicity and total cell number after lysis was measured by an LDH assay.
- Apoptosis was induced by adding 1 µM staurosporine 24 h prior to read-out at day 10 and measured as activity of caspase 3/7.

[1] Weaver VM, Lelievre S, Lakin JN, Chrenek MA, Jones JCR, Giancotti F, Werb Z, Bissell MJ "B4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium". *Cancer Cell* 2002, Vol 2 (3), pp. 205-216.

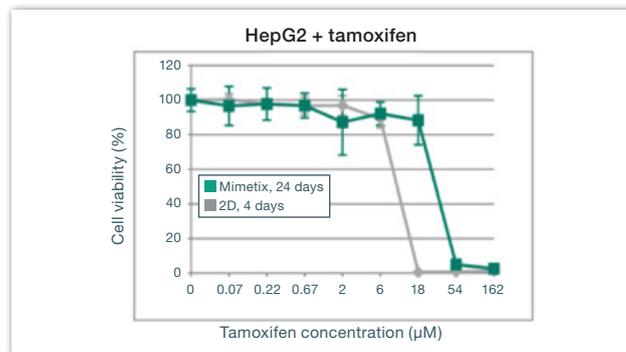
Response to cytotoxic drugs: Mimetix 3D scaffolds vs. 2D

Cancer cell lines pre-cultured in 3D in the Mimetix scaffold for 21 days show lower sensitivity to two commonly used cytotoxic drugs, carboplatin and tamoxifen, relative to cells pre-cultured for 1 day in conventional 96 well plates. Cells did require some time to adjust to the 3D environment and no difference in drug response was seen between cells grown for 1 day in conventional vs. Mimetix scaffold plates before treatment (data not shown). This indicated that with these drugs there was no significant issue with drug adsorption to the scaffold. The difference in response to drugs may be due to a range of factors related to the 3D micro-environment.

SKOV3 cells show an 8-fold increase in resistance to carboplatin when grown in the Mimetix scaffold (IC_{50} increases from 60 μ M to 500 μ M).



HepG2 cells show a 3-fold increase in resistance to tamoxifen when grown in the Mimetix scaffold (IC_{50} increases from 12 μ M to 36 μ M).



Experimental:

- SKOV3 ovarian carcinoma cells and HepG2 hepatocellular carcinoma cells (available from ATCC) were seeded at 5,000 cells/well and 10,000 cells/well, respectively, in Mimetix scaffolds and conventional 96 well plates.
- Cells were pre-cultured for 1 day in conventional plates, and for 21 days in the Mimetix scaffold before being dosed with the drugs carboplatin and tamoxifen for 3 days.
- Cell viability was determined using CellTiter Blue[®] from Promega.

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
- Material does not degrade or alter over the course of an experiment
- Thin scaffold provides benefits of 3D cell morphology and behaviour, yet allows microscopic imaging



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