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Mimetix® electrospun scaffolds for 3D culture of human epithelial breast cancer cells

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Background:

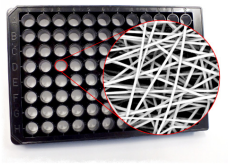
There is significant need for more predictive *in vitro* efficacy and toxicology assays to reduce both the number of costly drug failures in clinical trials and the number of animals used in pre-clinical testing. A range of 3D cell-based assay techniques, in which cells demonstrate different morphology and behaviour to cells grown in conventional 2D, are being evaluated for use in drug discovery programmes. In addition to providing a more physiologically relevant environment for cells, a 3D cell-based assay format should provide highly consistent performance and be readily compatible with standard automated handling and imaging equipment.

The objective of this study was to test if the Mimetix® electrospun scaffold is a suitable tool for the culture of human epithelial breast cancer cells in 3D, which can be used in drug discovery programmes for in vitro toxicity and efficacy assays. Cell viability and growth in 12-well plates and 96-well plates was shown to be highly consistent (cv < 10%) and cells were shown to be more resistant to drug-induced apoptosis following 10 days of proliferation in the Mimetix scaffolds than on 2D tissue culture plastic, indicating that they had assumed a 3D phenotype.

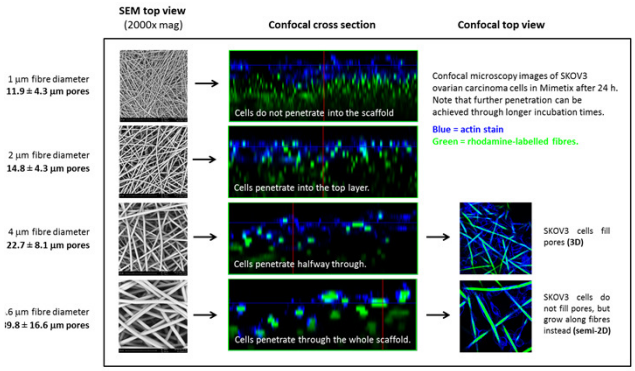
The Mimetix scaffold:

- Mimetix® electrospun scaffolds are designed to mimic the architecture of the extracellular matrix and thereby support the three-dimensional growth of cells.
- Fibres are made of the FDA-approved medical-grade polymer poly-L-lactide (PLLA).
- Mimetix is available with fibre diameters of 4 µm and 2 µm, corresponding with pore sizes of 10-18 µm and 15-31 µm, respectively.

- Mimetix can be supplied as…
  - ➢ a 96-well plate with scaffolds welded into the base (see image), which was used for all quantitative cell-based assays in this study
  - ➢ a 12-well plate with removable scaffold discs, which was used for all confocal experiments presented
  - ➢ a 6-well plate with hanging inserts

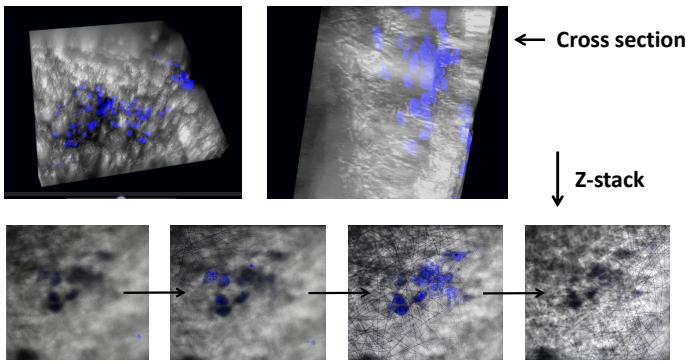


Question 1: What is the optimal pore size?



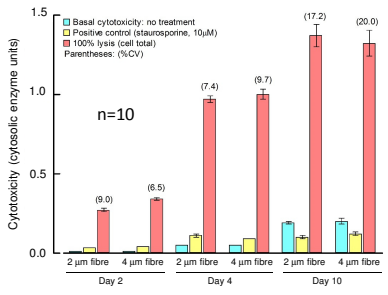
A preliminary study using SKOV3 ovarian cancer cells investigated the penetration of cells into scaffolds of different fibre diameters (correlating with different pore sizes) by confocal fluorescence microscopy. It was shown that scaffolds with fibre diameters of 2/4/6 µm allowed for cell penetration, but in the case of the 6 µm scaffold cells were found to grow on top of/along the fibres rather than within the pores in a semi-2D manner.

Question 2: Do the cells penetrate into the scaffold?



Next, the penetration of HMT3909S8 human breast cancer cells, used for all further experiments in this study, into a 2 µm fibre diameter Mimetix scaffold was investigated. Z-stacks obtained by confocal fluorescence microscopy (nuclei counterstained) demonstrated that the **cells migrated into the scaffold and grew in different planes.**

Question 3: Do the cells proliferate in the scaffold?

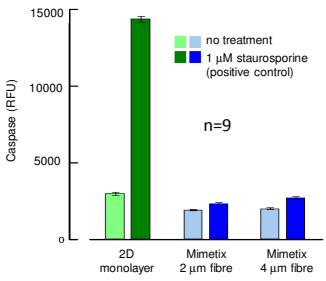


Basal toxicity, drug-induced cytotoxicity (10 µm staurosporine) and total cell number after lysis was measured by an LDH assay.

Total cell number increased over the testing period of 10 days. **The measurements were very consistent (CVs of < 10%)** up to day 10. On day 2 and 4, basal cytotoxicity was low and cells sensitive to induced cytotoxicity, but basal cytotoxicity/CVs were significantly increased by day 10.

The experiment suggests that a culture duration of 4-6 days is optimal.

Question 4: Do the cells assume a 3D phenotype?



The human breast cancer cell line HMT3909S8 is known to be resistant to apoptosis in 3D culture [1].

To see if this can be observed for cells cultured in Mimetix, 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.

As the graph shows, the cells did indeed become more resistant towards drug-induced apoptosis when grown in 3D, demonstrating the suitability of Mimetix as a tool for the 3D culture of human breast cancer cells.

Conclusions:

In this study we cultured the human epithelial breast cancer cell line HMT3909S8 within our Mimetix scaffold made of electrospun PLLA microfibres, designed to be biomimetic of the extracellular matrix. Cell viability was highly consistent from well to well (CV <10%) for the first 6 days of culture. Cells were observed throughout the scaffold by confocal microscopy. Epithelial breast cancer cell lines are known to be more resistant to apoptosis in 3D than 2D [1]. The HMT3909S8 human epithelial breast cancer cell line was found more resistant to drug-induced apoptosis following 10 days of proliferation in the Mimetix scaffolds than on 2D tissue culture plastic, indicating that the cells had assumed a 3D phenotype.

References:

[1] Weaver VM, Lelievre S, Lakins 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.

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