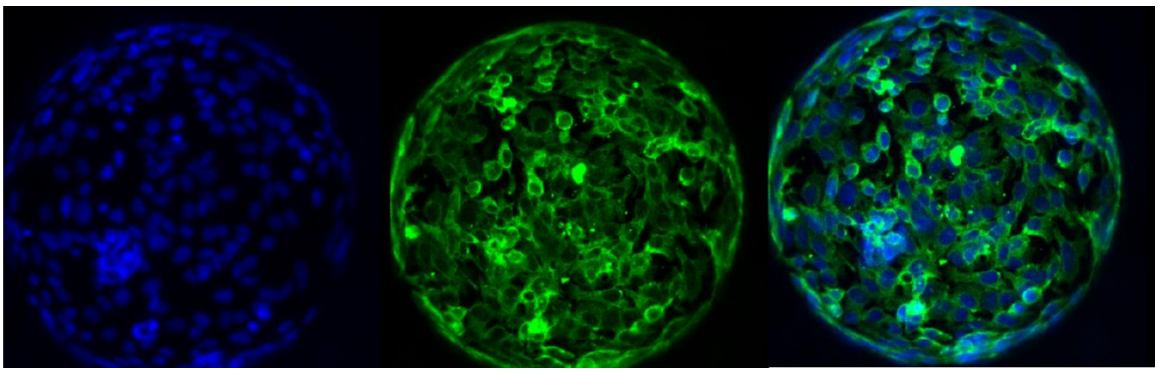
300MICRONS
Flexible 3D Cell Culture Solutions

STATARRAYS©: flexible 3D cell culture solutions



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Application Note: determination of the IC_{50} of Terfenadine with a human hepatocyte cell line (Hep G2) in STATARRAYS© microcavity array plates MCA96-16.224

In the last decade, it has become apparent that three-dimensional cell culture approaches are indispensable to observe cellular organotypic functions in vitro. Among the methods to generate 3D-aggregates are those that rely on scaffolds whereas others are scaffold-free. Compared to other techniques, the microcavity array technology offers several advantages. Firstly, the size of the microcavities, and therefore the aggregate size, is highly reproducible. From single cell microcavity dimensions up to organoid dimensions, the technology is highly flexible so that different aggregate sizes can be realized (fig. 1)

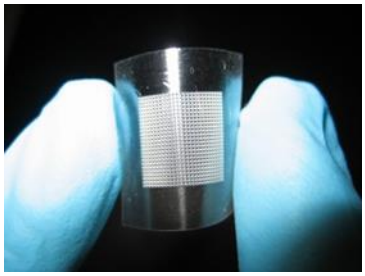

Fig. 1: DYNARRAYS© polymer film-based microcavity array.

Moreover, the number of microcavities for the format of choice can be adjusted to customer needs. Two examples of microcavity arrays in 96 well format are shown in fig. 2.

Fig. 2: Microtiter plate-based microcavity array platform STATARRAYS© with 169 or 10 microcavities per well, respectively.

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The microcavity array plates of 300MICRONS are innovative tools to generate homogeneous adherent or non-adherent, uniform three-dimensional cellular aggregates. The following protocol describes the use of the microcavity array plates with a human hepatocyte cell line (Hep G2) to determine an organotypic IC_{50} value.

Protocol:

1. Deaeration of the microcavity array:

Use an alcohol series of isopropanol with the following steps: 100%, 70%, 50%, 30%, PBS.

▫ Pipet 200µl of solution into each well to be seeded with cells by revers pipetting the solution three to five times into the well. Try to wash out obvious bubbles in the microcavities.

▫ Afterwards, remove the solution by turning over the plate and tapping it on a soft cloth

▫ Do that for all steps of the alcohol series and then finally for PBS.

▫ Try to remove excess fluid from the well area of the plates with only 10 microcavities by using a Pasteur pipette and vacuum pump device for example. This will lead to a better focusing of the cell pellet above the microcavity area.

▫ Once this has been done, don't let the plate get dry, otherwise the procedure has to be repeated.

2. Collagen coating:

▫ Prepare a stock solution of 2 mg/ml collagen I in 0.1% acetic acid. From this stock solution mix 4.5 µl + 33 µl sterile water for the coating of one well. Pipet the solution into the well and incubate at 4°C over night (4 h at least).

▫ Afterwards, wash three times with PBS using the technique explained under deaeration.

3. Inoculation of cells:

▫ For e.g. cell lines, such as Hep G2, use 0.5 Mio cells/well of a 96-well plate with 169 microcavities, depending on your experimental purpose.

▫ The process can be facilitated by centrifugation at 10 to 50 g prior to incubation.

- After the inoculation, cover the plate with the lid and incubate at standard conditions
- Terfenadine dilutions were made from a 50 mM stock in DMSO
- Incubate the cells for 48 hours
- Measure viability with e.g. Resazurin or ATPlite.

Results

When cultured in 3D, even cell lines display a more organotypic behavior compared to monolayer culture.

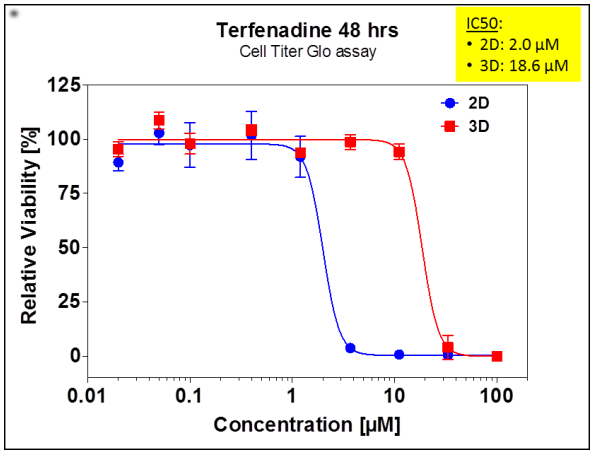

Fig. 3: IC_{50} of Terfenadine determined in the Hep G2 cell line cultured in 2D or 3D (microcavity array plates). With courtesy of Dr. R. Class, Dr. Daniel Müller, Pharmacelsus GmbH, Saarbrücken.

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When the IC_{50} of Terfenadine is determined in 3D and compared to 2D one can observe that the reduced inhibitory concentration of Terfenadine resembles more closely the in vivo situation no matter how it is determined (fig. 3 and 4).

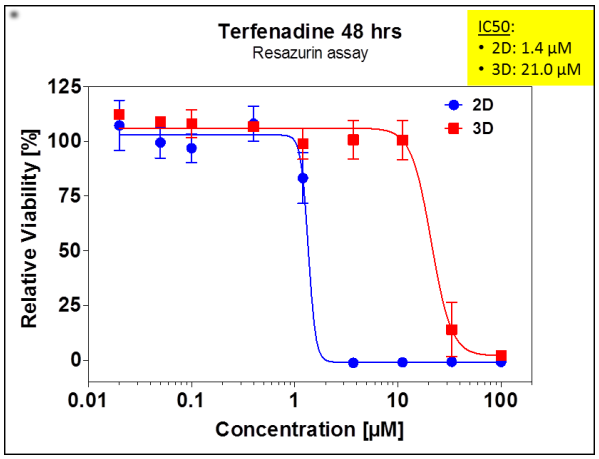

Fig. 4: IC_{50} of Terfenadine determined in the Hep G2 cell line cultured in 2D or 3D (microcavity array plates). With courtesy of Dr. R. Class, Dr. Daniel Müller, Pharmacelsus GmbH, Saarbrücken.

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Please request a quote via the contact form of the company homepage of 300MICRONS GmbH (www.300microns.com).