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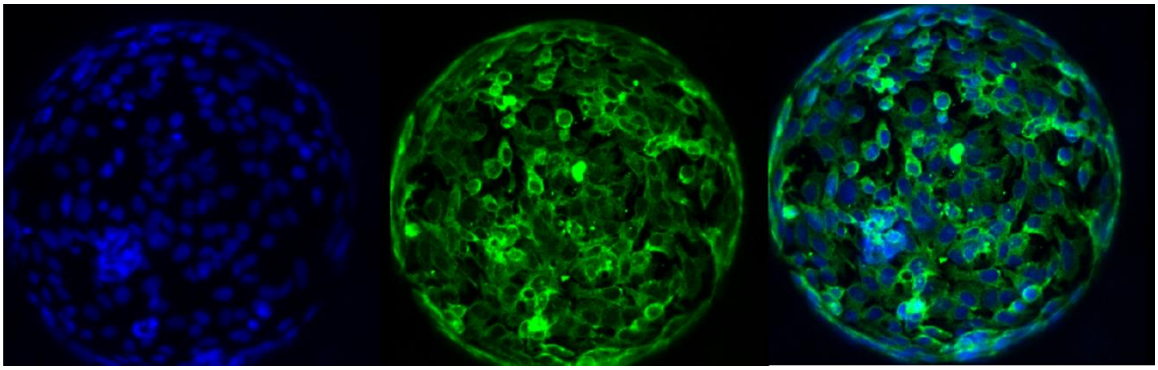
Flexible 3D Cell Culture Solutions

DYNARRAYS© - Microcavity Arrays



300MICRONS GmbH
Daimlerstr. 35
D-76185 Karlsruhe

Tel. +49 (0)721-94247-891
Fax. +49 (0)721-94247-893
www.300microns.com
info@300microns.com

VAT registration number according to §27a: 35005/31139
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Application Note: Maintaining the stem cell fate in DYNARRAYS© microcavity array systems

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)

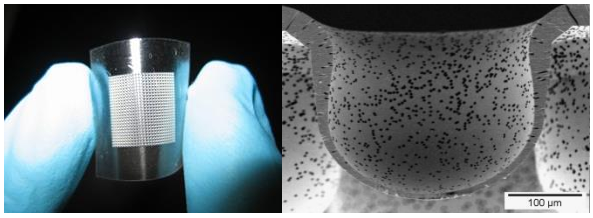

Fig. 1: Left: DYNARRAYS© polymer film-based microcavity array. Right: porous microcavity with 3 µm pores. Pore density $1 - 2 * 10^6$.

Moreover, the number of microcavities for the format of choice can be adjusted to customer needs.

Microcavity arrays can be used in dynamic as well as static applications. For the latter one, two examples of microcavity arrays in 96 well format are shown in fig. 2.

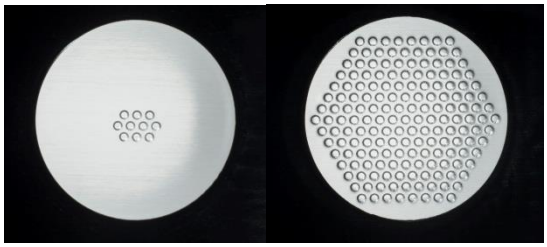

Fig. 2: STATARRAYS© Microtiter plate-based microcavity array platform with 169 or 10 microcavities per well, respectively.

Other formats comprise 384 well plates with 18 or 60 microcavities per well, respectively, leading to 23.040 microcavities on the MCA384-plate.

For dynamic applications DYNARRAYS© microcavity arrays are inserted into a

bioreactor (fig. 3) that can house one or two chips with up to 1000 microcavities.

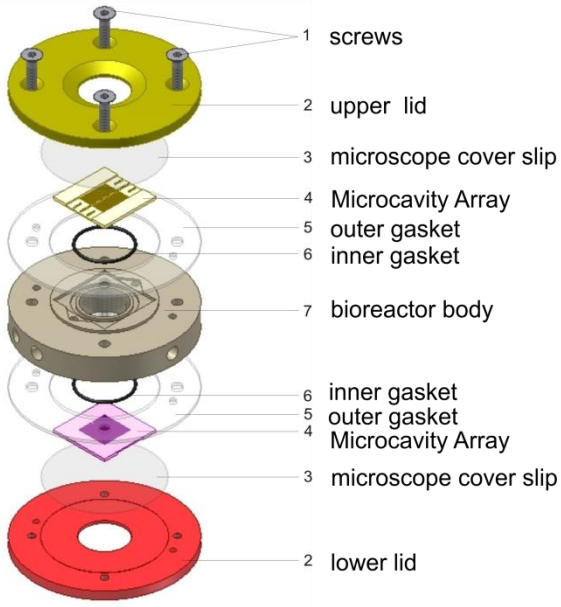

Fig. 3: Microbioreactor for the housing of one or two microcavity arrays.

The bioreactor can be operated in various modes depending on the experimental purpose. For a homogeneous supply of the tissue inside the microcavities with medium, the bioreactor is operated in perfusion mode (fig. 4) where the medium is entering the central compartment of the bioreactor and is forced to flow through the pores of microcavity arrays.

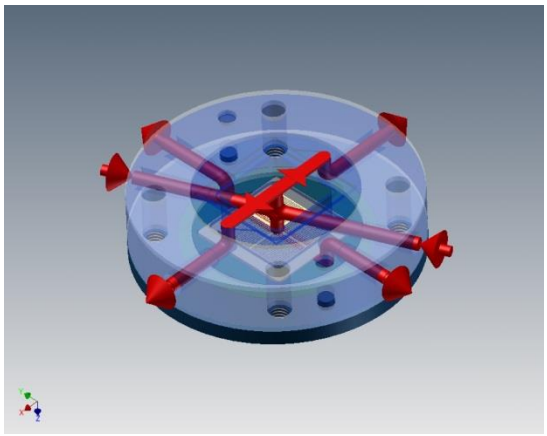

Fig. 4: Flow of medium in perfusion mode. Medium flow is perpendicular to the microcavity array surface.

Another operating condition is the superfusion mode. In contrast to the perfusion mode, in the superfusion the medium is flowing in parallel to the microcavity array surface (fig. 5).

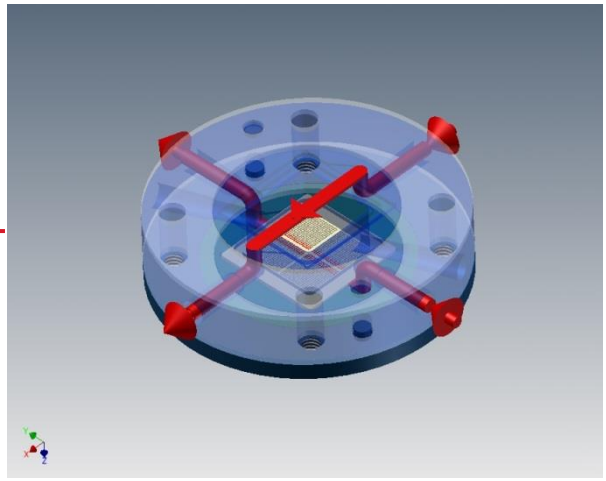

Fig. 5: Flow of medium in superfusion mode. Medium flow is in parallel to the microcavity array surface.

After the microcavity arrays have been mounted into the bioreactor, the system is connected to a roller pump and a medium reservoir that can be supplied with the appropriate gas mixture for cell culture applications (fig. 6).

With the latter setup, we have developed an artificial hematopoietic stem cell niche that was able to preserve the plasticity of hemtopoietic stem cells from cord blood after a cultivation period of 14 days in the system.

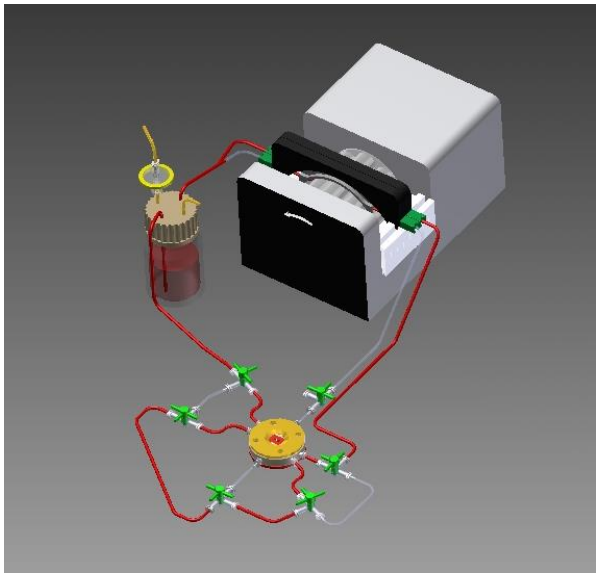

Fig. 6: Bioreactor setup with a closed circulation loop consisting of a medium reservoir, a roller pump and the bioreactor. The medium reservoir can be charged with gas according to standard cell culture conditions or gas mixtures leading to e.g. hypoxia.

Protocol:

1. Deaeration of the microcavity array:

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

- Using a forceps, gently dip the microcavity array in the solution for 10 seconds. Do this for each concentration and PBS, respectively.
- By using a pasteur pipette, dry the rims of the microcavity array and make sure not remove the fluid from the microcavities.
- Once this has been done, don't let the array 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 pipet 150 µl on top of the cavities. A droplet should form that is spanning exactly the microcavity array filed (fig. 7)
- Incubate at 4°C over night (4 h at least).
- Afterwards, wash three times with PBS using the technique explained under deaeration.

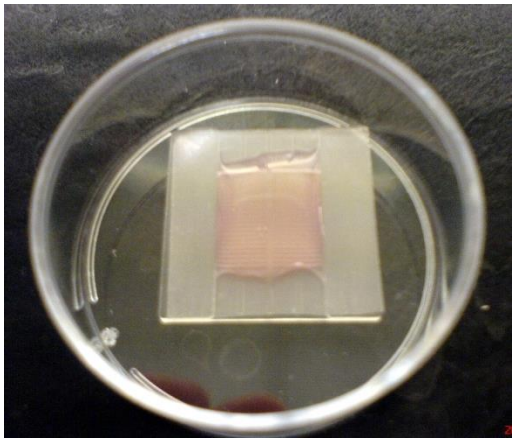

Fig. 7: Droplet formation on top of the DYNARRAY© microcavity array.

3. Inoculation of cells:

- For the hematopoietic niche model we co-cultured 300.000 human mesenchymal stromal cells and 200.000 human $CD34^+$ hematopoietic stem cells from cord blood.
- To inoculate the cells, combine the two populations in a volume of 150 µl medium and place the droplet on top of the microcavity array as described under collagen coating.
- After the inoculation, incubate at standard conditions for 2 h.

▫ Afterwards, mount the DYNARRAYS© into the bioreactor housing and set the pump to a pumping speed of 400 µl/min under superfusion conditions as depicted in fig. 5.

▫

Results

After 24h, the MSC form a stable network inside the microcavities with the HSC interspersed regularly. Figure displays the co-culture after a culture period of 21 days.

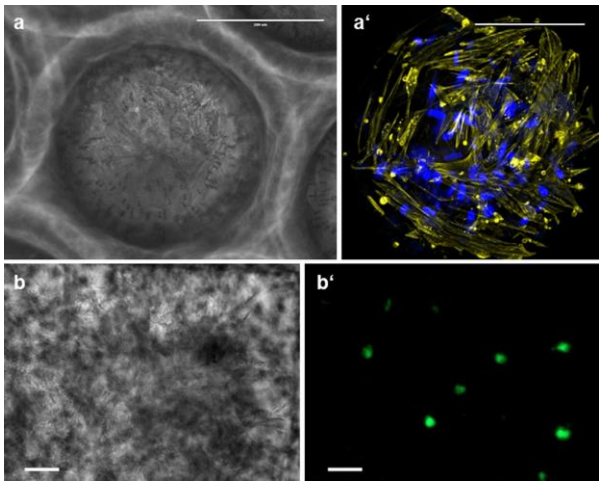

Fig. 8: Spatial distribution of MSCs and HSCs within the microcavity. a) Phase contrast and (a') CLSM image of the 3D-network of MSC and HSC after 21 days in co-culture. F-actin (yellow), cell nuclei (blue, DAPI staining). Scale bars: 200 µm. b) Live cell imaging with phase contrast and fluorescence staining (b') of HSCs marked with CellTracker Green in the microcavity to demonstrate the spatial distribution within the cell network. Scale bars 50 µm.

After the co-culture, the cells can be isolated from the microcavities and subjected to any type of downstream application such as RNA-isolation for RT^2-PCR or protein isolation for Western blotting.

Reference:

Wuchter P., Saffrich R., Giselbrecht S., Nies C., Lorig H., Kolb S., Ho A., Gottwald E.
Microcavity arrays as an in vitro model system of the bone marrow niche for hematopoietic stem cells. Cell Tissue Res. 2016; 364: 573-584, DOI 10.1007/S00441-015-2348-8.

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