3D CELL-BASED DEVICES FOR HTS/HCS

Solutions to develop organ in a well.
4Dcell customized solutions
IMPROVE DRUG DISCOVERY AND TOXICOLOGICAL ASSAYS

4Dcell philosophy is based on the conviction that the future of cell biology will rely on 2D and 3D cell culture technologies coming from the confluence of microfabrication, biophysics, chemistry and innovative biological knowledge.

4Dcell ambition is to promote a simple access to advanced biophysical technologies in order to make in vitro models more predictable.

4Dcell provides hardware solutions. Pharmaceuticals, CROs, cell providers and academics bring the cell model, 4Dcell develops the adapted device to reproduce the natural cell constraints.

That is why 4DCell team supports your experimental set up by providing our know-how, expertise and technical capabilities.

Interdisciplinary team expertise

- Biology (cell biology, molecular biology)
- Physics (biophysics, material sciences, optics, microfluidics)
- Mechanical engineering
- Chemistry (biochemistry, material chemistry)
- Bioinformatics
- Electronic engineering

Technical capabilities

- Cell biology
- Microfabrication
- Surface chemistry
- Photo-lithography
- Soft-lithography
- Mechanical design
- Computational simulation
- Prototyping

Examples of read-outs

- Adhesion
- Proliferation
- Cell cell interaction
- Differentiation
- Elasticity
- Mobility
- Permeability
- Shape
- Stability
- Contractility

Diseases models

- Immunology
- Oncology
- Cardiology
- Hepatology
- Dermatology
- Neurology

Product development process

- From proof of concept to industrialization
4Dcell technologies APPLICATIONS
NEW BREAKTHROUGH READ-OUTS FOR HTS/HCS

The 4Dcell technologies are a versatile solution for cell behaviour modeling with a myriad of applications. Among them, we highlight models for cancer, immune system, organs as heart and guts, circulating cells, wound healing, neuronal network, stem cells and genetic disorders as laminopathy.

Immune System
Sensing and migration in the tissues is the basis of the immune system functioning. 4Dcell technologies are the best tools to quantify immune activities such as cell migration, cell-cell interaction, nucleus plasticity, among others.

For instance, 4Dcell Microchannels can quantify leukocyte extravasation ability, with read-outs such as: cell speed, persistence and ability to pass through constricted spaces.

Cancer
Cell mechanics or cell organization are key parameters for cancer development and metastasis. 4Dcell enables us to better control these parameters and offer new read-outs to decipher cancer progression and invasiveness.

For instance, spindle assembly disorders of cancerous cells are easily assessed via 4Dcell Cell confinement technology.

Rare Diseases
Rare diseases are often linked with cell mechanics or cell organization disorders.

The 4Dcell technologies highlight alterations like these and one can measure molecular modifications more easily than with other models.

For instance, cell nucleus healing in laminopathy is evaluated using 4DCell Cell confinement by the observation of lamina reconstruction as a read out.

Organ Physiology
The regular physiology of cells in living organs is closely linked with its spatial and mechanical organization. 4Dcell offers simple and representative models highlighting organ specific read-outs.

For instance, 4Dcell Micropatterns induce striation of cardiomyocyte leading to standardized beating properties and internal organization.
3 TECHNOLOGIES FOR ORGAN IN A WELL

MICROCHANNELS  MICROPATTERNING  CELL CONFINEMENT

NOVEL SOLUTIONS FOR IN VITRO MODELS
**4Dcell MICROCHANNELS**

**CELL-CELL INTERACTION AND MIGRATION SOLUTIONS FOR HTS/HCS**

The 4Dcell Microchannels are a new generation of multiwell plates enabling more quantitative cell-cell interaction and cell migration assays than traditional designs.

Cells living in a specifically designed network of microchannels allows the quantification of cell activities such as cell migration, cell-cell interaction or nuclear plasticity.

The simplified migration, with restricted directionality, facilitates the automatic tracking of cells and the extraction of quantitative parameters to describe cell movement.

**APPLICATIONS**

**CANCER**
- Migration of metastatic cells
- Nuclear plasticity in metastasis
- DNA DSB repair (mechanically induced)
- Angiogenesis
- Immuno-oncology

**IMMUNOLOGY**
- Cell-cell interaction
- Migration of immune cells
- Antigen search
- Extravasation/diapedesis
- Chemotaxis/haptotaxis

**ORGAN PHYSIOLOGY**
- Migration of cancer cells
- Shear stress
- Angiogenesis
- Epithelium permeability/brain blood barrier
- Neural network

**RARE DISEASES**
- Cell nucleus integrity
- Nuclear plasticity
- Neural network

**FUNDAMENTAL RESEARCH**
- Cell adhesion
- All applications described above

**PRINCIPLE**

The structures are made of an innovative biocompatible elastomer, which allows gas and nutrients exchange with low absorption effect. Cells are loaded into a central chamber communicating with multiple microchannels. The surface of the channels is functionalized (with adhesive or repellent coating solution to promote or to prevent cell adhesion) allowing the control of the adhesive properties of the channels.

Microchannels are compatible with high resolution fluorescent and phase contrast microscopy and are therefore suitable to study localization of intracellular organelles and structures.
SUPPORTED CELL TYPES

All primary lineages and cell lines showing a migratory behavior: immune cells (dendritic cells, neutrophils, lymphocytes…), IPs, stem cells, tumoral cells,…

TECHNICAL SPECIFICATIONS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well dimension (mm)</td>
<td>6,4 mm diameter</td>
</tr>
<tr>
<td>Number of channels</td>
<td>Small and medium designs: average of 100 channels/access port / Large designs: from 20 to 2</td>
</tr>
<tr>
<td>Volume for microchannels loading</td>
<td>5 to 10 µl per access port</td>
</tr>
<tr>
<td>Materials</td>
<td>Flexdym; optical quality glass bottom for better imaging quality (170 µm±10 µm thickness, RI=1.525)</td>
</tr>
<tr>
<td>Read-out</td>
<td>Microscopy imaging, cell tracking, migratory behavior analysis, immunolabelling</td>
</tr>
<tr>
<td>Shelf life</td>
<td>12 months after date of production (at +4°C)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4DCELL Migration plate</th>
<th>Small size design</th>
</tr>
</thead>
<tbody>
<tr>
<td>4DCELL Migration plate</td>
<td>3, 4, 5, 6, 7, 8 µm width / 4 µm height</td>
</tr>
<tr>
<td>4DCELL Migration plate</td>
<td>Medium size design</td>
</tr>
<tr>
<td></td>
<td>10, 12, 14, 16, 18, 20 µm width / 10 µm height</td>
</tr>
<tr>
<td>4DCELL Migration plate</td>
<td>Large size design</td>
</tr>
<tr>
<td></td>
<td>50, 100, 200, 400 and 1000 µm / 25 µm height</td>
</tr>
<tr>
<td>4DCELL Migration plate</td>
<td>Small constrictions size</td>
</tr>
<tr>
<td></td>
<td>Channel: 5 µm width / 4µm height / 200 µm length</td>
</tr>
<tr>
<td></td>
<td>Constriction: from 4 to 1 µm width, 15 µm length</td>
</tr>
<tr>
<td>4DCELL Migration plate</td>
<td>Medium constrictions size</td>
</tr>
<tr>
<td></td>
<td>Channel: 8 µm width / 4 µm height / 200 µm length</td>
</tr>
<tr>
<td></td>
<td>Constriction: from 4 to 1 µm width, 15µm length</td>
</tr>
<tr>
<td>4DCELL Migration plate</td>
<td>Large constrictions size design</td>
</tr>
<tr>
<td></td>
<td>Channel: 12 µm width / 10 µm height / 200 µm length</td>
</tr>
<tr>
<td></td>
<td>Constriction: from 12 to 1 µm width, 5 µm length</td>
</tr>
</tbody>
</table>
4Dcell MICROPATTERNING
STANDARDIZATION SOLUTIONS FOR HTS/HCS

4Dcell micropatterned multi-well plates hold a perfectly organized culture substrate aiming for cell normalization. Evenly distributed on adhesive patterns, cells have a controlled geometry allowing a standardization of the assays.

**APPLICATIONS**

**CANCER**
- Migration of cancer cells (line patterns)
- Cell shape standardization

**ORGAN PHYSIOLOGY**
- Migration of somatic and cancer cells
- Cell shape standardization
- Cardiomyocyte beating properties
- Neural network

**RARE DISEASES**
- Cell nucleus integrity
- Nuclear plasticity
- Neural network

**FUNDAMENTAL RESEARCH**
- Cell shape standardization
- Standardized connectivity between neurons

**BIOMOLECULAR IN VITRO MODELS**
- Spatial autoorganization of macromolecules (at the microscale)

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**PRINCIPLE**

The 4Dcell micropatterning technology enables a 2D cell geometry control. A set of patterns is arranged on a glass bottom to receive cells. Using an antiadhesive agent and some ECM proteins such as fibronectin or collagen, cells can adhere to the shape imposed by the micropattern. Cells are going to embrace the geometry of this new environment.

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**FULLY COMPATIBLE WITH HIGH RESOLUTION IMAGING**

**STABLE MOLECULAR COATING**

PAcrAm™ TECHNOLOGY

**HOMOGENOUS SEEDING**

4DCELL HOMOGENIZER™ TECHNOLOGY

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**MULTIWELL PLATE**

from 9 to 96 wells

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**NOVEL SOLUTIONS FOR IN VITRO MODELS**
SUPPORTED CELL TYPES

Any adherent cells can be micropatterned in theory. Some cell types might need specific conditions (for instance, insect cell lines).

TECHNICAL SPECIFICATIONS

<table>
<thead>
<tr>
<th>Plate format</th>
<th>Standard SBS format (127.76 x 85.48 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of wells</td>
<td>1, 6, 12, 24, 48 and 96</td>
</tr>
<tr>
<td>Standard pattern shapes</td>
<td>Disk, line, rectangle, triangle, square, grid</td>
</tr>
<tr>
<td>Sizes of the standard patterns</td>
<td>10, 20, 30, 40, 50, 70 and 100 µm</td>
</tr>
<tr>
<td>Anti-adhesive agents</td>
<td>PLL-g-PEG, covalently bonded anti-fouling polymer</td>
</tr>
<tr>
<td>Read-out</td>
<td>Cell with a reproducible shape allowing quantification of cell organelles position, etc...</td>
</tr>
<tr>
<td>Alternative products</td>
<td>35 mm ø micropatterned dishes (MP-D), 24 mm ø micropatterned slides (MP-S)</td>
</tr>
</tbody>
</table>
**4Dcell CELL CONFINEMENT**

**FULL MICROENVIRONMENT CONTROL SOLUTIONS FOR HTS/HCS**

The 4Dcell confiner reproduces the natural cell environment. This is possible since we are able to control the mechanical and chemical properties surrounding cells as cell geometry, environment elasticity and surface chemistry.

### APPLICATIONS

**CANCER**
- Migration of metastatic cells
- Cell contractility in metastasis
- DNA DSB repair (mechanically induced)
- Genomic instability (cell division)
- Separated co-culture

**IMMUNOLOGY**
- Migration of immune cells
- Imaging of non-adhesive cells

**ORGAN PHYSIOLOGY**
- Migration of cancer cells
- Cell differentiation with stiffness control
- Wound healing
- Separated co-culture
- Cell compression response

**RARE DISEASES**
- Cell nucleus integrity

**AGING**
- Cell nucleus integrity
- Autophagy related diseases

**OBSERVATION OPTIMIZATION**
- Imaging of non-adhesive cells
- Planar imaging of organelles

**FUNDAMENTAL RESEARCH**
- Cell volume (cell cycle)
- Cell stretching response
- All applications described above

### PRINCIPLE

The 4Dcell confiner allows squeezing micrometer-sized cells or other biological samples between two parallel surfaces, with micrometer precision. The method for confinement is based on the application of a confining slide on the cell culture (or tissues, ...). The patented technology developed at 4Dcell ensures the uniformity of the confinement height. The confiner was developed to be used with commercially available multi-well plates, enabling confinement of multiple wells simultaneously.

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**3 TECHNOLOGIES OR ORGAN IN A WELL**

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**NOVEL SOLUTIONS FOR IN VITRO MODELS**
SUPPORTED BIOLOGICAL SAMPLES

- Cells (adherent and non-adherent)
- Uni/pluricellular organisms
- Tissues
- Organoids

TECHNICAL SPECIFICATIONS

<table>
<thead>
<tr>
<th>Plate dimension (mm)</th>
<th>Standard SBS format (127,76 x 85,48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of wells confined</td>
<td>1, 6, 12, 24, 48 and 96</td>
</tr>
<tr>
<td>Confinement height</td>
<td>1, 2, 3, ..., 20 µm</td>
</tr>
<tr>
<td>Materials</td>
<td>Confinement slides (optically transparent glass and PDMS)</td>
</tr>
<tr>
<td>Read-out</td>
<td>Cell imaging of non-adhesive cells, cell contractility, cell volume, nuclear plasticity, etc ...</td>
</tr>
<tr>
<td>Alternative product</td>
<td>4DCell dynamic confiner, confinement can be modulated with a pressure source</td>
</tr>
<tr>
<td>Integration with other 4Dcell products</td>
<td>The cell confiner can be used with the micropatterned slides and with the hydrogels</td>
</tr>
</tbody>
</table>