A Disposable Cell Culture Chip for Live Cell Imaging

by Ulf Rädler

Our understanding of intracellular processes has increased significantly over the past few decades. In contrast, the basic procedures of cell culture have not changed fundamentally since the turn of the century. However, optical methods have continually improved and pushed biological research to new frontiers. Phase contrast microscopy, differential interference contrast (DIC), confocal microscopy, two-photon microscopy, and laser microdissection have opened up new possibilities in biotechnological applications. In particular, fluorescence techniques have revolutionized biological imaging and advanced into quantitative tools to monitor molecular processes inside living cells. They are used in routine investigations for clinical diagnostics, molecular research, and high-throughput screening. Consequently, the demands for high-end optical cell culturing systems have increased drastically.

The µ-Slide, an easy-to-use, disposable cell culture chip for optical microscopy (Integrated BioDiagnostics [ibidi] GmbH, Munich, Germany), consists of a plastic carrier with a channel and a thin, optically high-quality bottom foil (*Figure 1*). The cells can be seeded into the channel and can be supplied with medium. The advantage of the chip is that it combines a cell culture dish with an optical observation chamber equivalent to the glass slide/coverslip setup that is required for high-resolution microscopy.

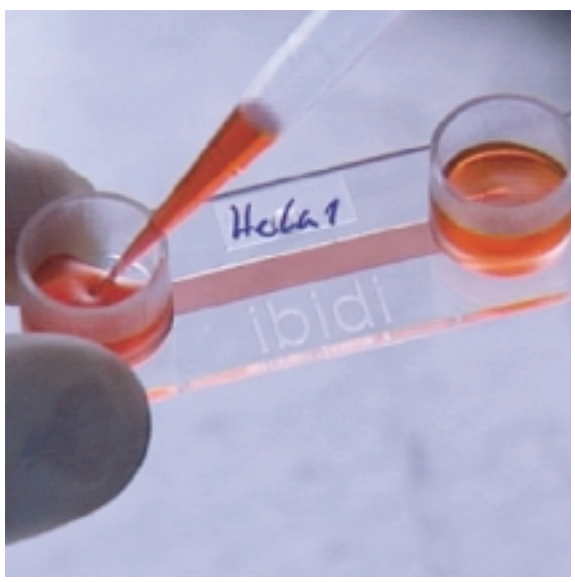

Figure 1 µ-Slide is loaded with medium to feed cells that are cultured in the 5-mm-wide channel between the two reservoirs.

Cell culture

The µ-Slide meets the demands of a cell culture system. It is constructed of plastic and is chemically robust, gas permeable, and compatible with glass. The slides are supplied in a sterile packaging and can easily be loaded with the cell suspension. When the slides are placed in an incubator, gas exchange allows cell growth inside the channel over long time periods. The small, 100-µL channel volume saves a considerable amount of cells and media. There is no need for washing and autoclaving since the labware is disposable.

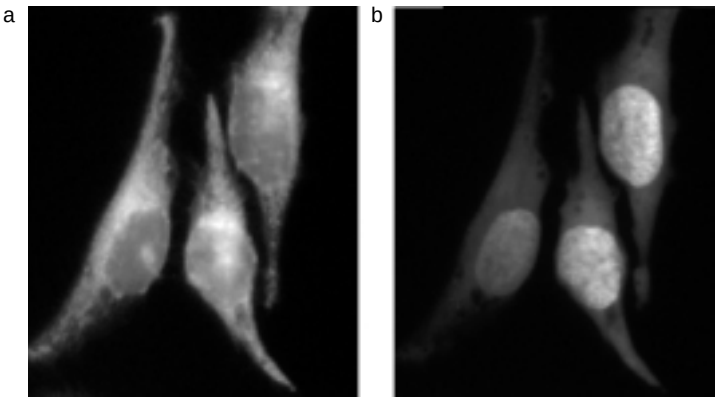

Figure 2 a and b) Fluorescence images of 3T3 fibroblasts stained in a µ-Slide.

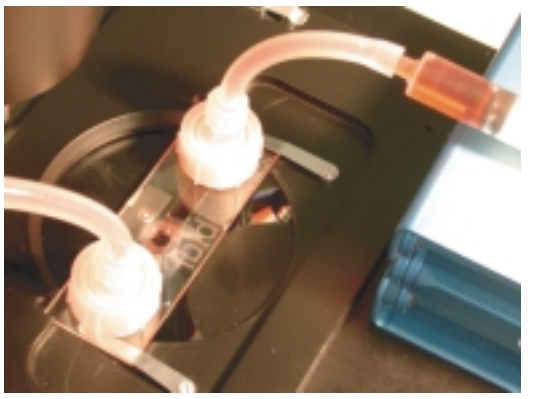

Figure 3 System for blood vessel simulation inside a µ-Slide channel.

Biological imaging

One of the problems with classical microscopy methods is the transfer of the cells onto a coverslip for optical analysis. Coverslips are fragile and can be difficult to handle. The cell culture µ-Slide overcomes this problem with a plastic membrane of very high optical quality that allows high-resolution microscopy inside of the cell chamber. Thus, many cell biology techniques such as immunofluorescence, fluorescence in situ hybridization, Ca^{2+} imaging, and gene transfection can be performed using a simple procedure. *Figure 2a* shows stained mitochondria of 3T3 cells; Figure 2*b* shows the stained DNA and RNA of the same cells. The DNA and RNA dyes (MitoFluor 589 and SYTO 16, Molecular Probes, Eugene, OR) were used in a concentration of 200 n*M*. Cells were incubated for 15 min after rinsing the µ-Slide channel with phosphate-buffered saline (PBS).

Flowthrough system

With the µ-Slide platform, optical tests can be designed that require multiple fluid changes. The channel can be conveniently filled from two reservoirs or can be attached to a pump (*Figure 3*). The devices can be used when only a small quantity of cells is available, as for primary cell lines or stem cell analysis.

Artificial capillaries

The µ-Slide geometry is also optimized for

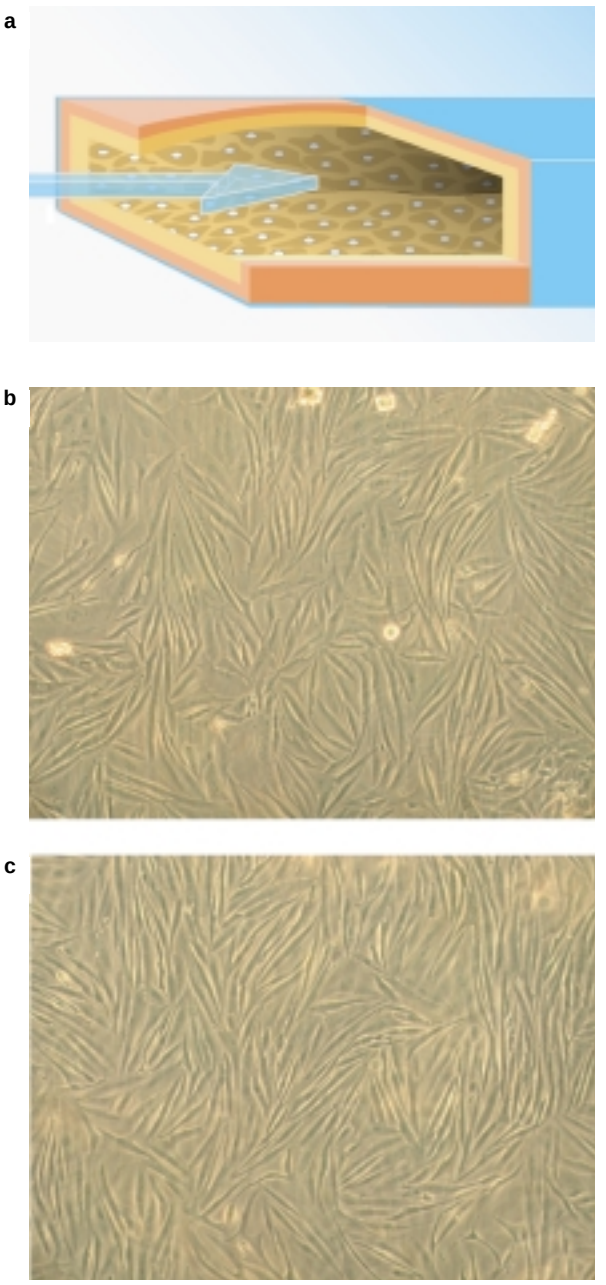

Figure 4 a) Schematic drawing of an artificial capillary µ-Slide with confluent epithelia cells. b) Confluent layer of B16 (mouse melanoma) cells growing in a µ-Slide I ibiCoat 1 treated channel (focus on the bottom surface). c) Same as (b), but focus on the top channel surface.

Figure 5 Use of µ-Slide for automated preclinical and pharmacokinetic studies.

other cell culture applications, for example, the simulation of vascular systems (see *Figure 4*). The channel can be completely coated with endothelial cells. Artificial capillaries provide a model system for interaction studies between a blood component and the epithelia vessel wall. In studies conducted at ibidi GmbH, human umbilical vein endothelial cells (HUVECs) were cultivated on different functionalized surfaces inside a perfusion channel under static conditions. The morphology and growth rates of HUVECS were characterized using phase contrast microscopy. HUVECs demonstrated very different stability under shear stress induced by perfusion through the channel. Tests of different biopolymer coatings were performed and showed a lower stability than physical treatments of the surfaces.

Automated microscopy

The use of molecular biochips and assays is widespread in biotechnological applications. Integrated biochips, which host living cells as detectors or sources for biomolecules, are now being used (see *Figure 5*). Microfluidic systems will serve to grow different kinds of cells in defined microenvironments, and communication between cells will be controlled by microfluidic channels. The in situ analysis of protein expression of living cells is important to drug discovery. An inevitable requirement for almost all cell systems is optical control. Hence, transparent plastic housings must be developed that are suitable for miniaturization and automated analysis.

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