sciFLEXARRAYER Application Note No. 08005

Drug screening and siRNA transfection using a "DropChip" cell array

In order to perform information-rich high-throughput screening, a "DropChip" microarray has been developed for multiplexed cell-based assays. With the arrayed cell culture nanoliter droplets, synergic effects of siRNA and cisplatin were analysed. With up to 100 cells per drop, cell behavior at the individual cell level could be analysed, using high-resolution fluorescence microscopy and automated image analysis. This novel cell array format could enable highly informative functional genomic studies and large scale in vitro toxicity testing.

Materials and methods

After characterization of DropChip functional parameters, this novel format was applied to direct multiple transfection. 100 nL U373 human glioblastoma cells (0.2 x 106 cells/mL) cultivated and treated with cisplatin (CDDP) on the 500 µm spots of the 400 positions "DropChip" slide (custom made by Memscap SA.) were directly transfected with 5 nL siRNA (0 to 100 nM final concentration) using the sciFLEXARRAYER. The cells were subsequently incubated for 5 days to allow gene silencing, then the chips were fixated and analysed using a microscope.

Results and discussion

The DropChip microarray, designed on a 2.5 x 2.5 cm2 chemically modified glass chip, creating a highly hydrophobic barrier surrounding the hydrophilic sites (Fig. 1), has three major advantages: the shape and volume of the drops are controlled by the characteristics of the glass slide, the liquid convection within each drop provides excellent conditions for cell-based screening, mixing cells and molecules in a homogeneous and continuous manner, and the number of cells per drop can be adjusted for each experiment, which is favourable for high-content automatic cell screening.

With the direct siRNA transfection and anticancer treatments of cancer cells using the DropChip, the combination of nucleic-acid and chemical-based assays was tested in a pharmacological study. We have evaluated how ERCC1, nucleotide excision repair protein 1, gene silencing enhanced the cytotoxic effect of an anti-cancer drug, cisplatin, in human glioblastoma cells.

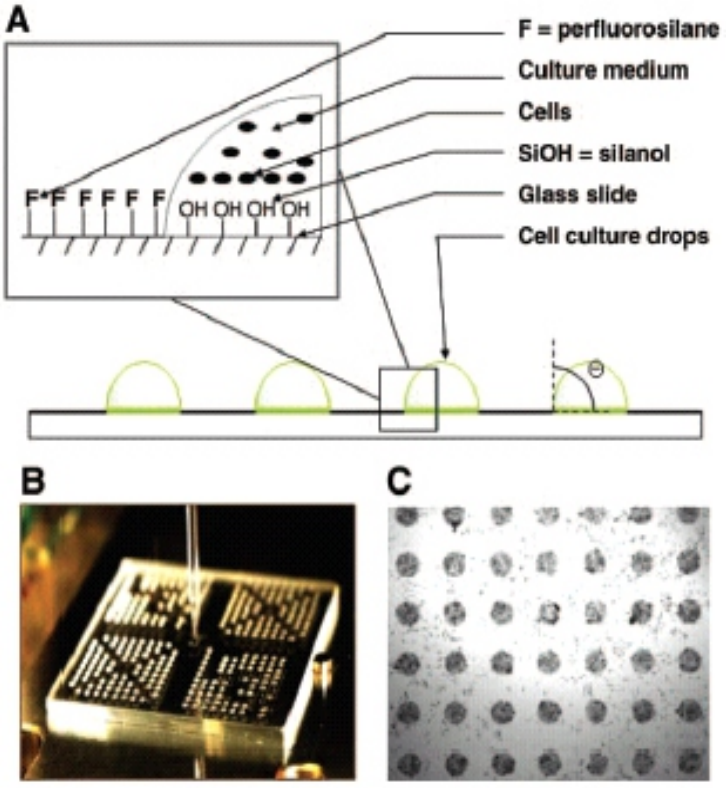

Fig. 1 The DropChip device, A: schematic representation, B: formation of 100 nL drops on 400 – 500 µm spots with the piezoelectric nozzle, C: after dipping the chip in a cell culture during 2 d, followed by two successive rinses with PBS, HeLa cells concentrated on the bare glass spot surface avoiding the hydrophobic areas of the chip. This showed the compatibility of the surface chemistry with the cell physiology.

In the absence of cisplatin, ethidium homodimer-2 did not stain the U373 cells, confirming that the siRNA does not exhibit any toxic effect at concentrations ranging from 5 to 100 nM. In contrast, in the presence of 5 µM cisplatin, the red signal in cells increased in correlation with the siRNA concentration (Fig. 2A). Fig. 2B illustrates a precise analysis of cytotoxicity by automated cell analysis coupled to the DropChip, reported as the distribution of the red signal within spots.

We have demonstrated that cell-based assays can be performed easily in nanoliter drops for the purpose of dose-response gene-silencing experiments.

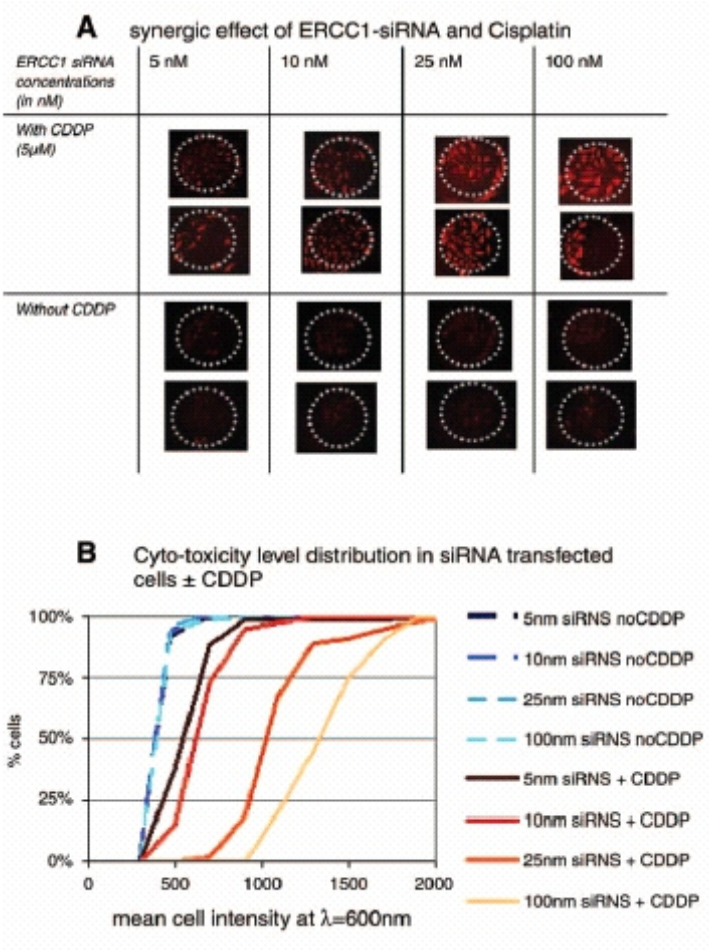

Fig. 2 ERCC1 gene silencing combined with cisplatin treatment on the DropChip device, A: Ethidium homodimer-2 signal capture in U373 cells (duplicate experiments), treated or untreated with 5 µM CDDP and transfected with various concentrations of the anti-ERCC1 siRNA, B: percentile distribution of the ethidium red level for each U373 cell treated with various concentrations of the siRNAs in the presence or absence of CDDP

Courtesy of Béatrice Schaack. An article on this topic has been published by Schaack, B. et al. (2005) A „DropChip" cell array for DNA and siRNA transfection combined with drug screening; NanoBiotechnology, Vol. 1, DOI:10.1385/Nano:1:2:183.

This work was performed at the Laboratoire Biopuces, CEA, Grenoble.

Feb 2008