sciFLEXARRAYER Application Note No. 08003

Multiplexed toxicity assays in Nanodrops

Limiting the extend of animal testing and complying with REACH policy objective and drug ADMETOX prediction requires the development of high-throughput and highly relevant *in vitro* toxicity assays. A new *in vitro* method for toxicity testing has been established, combining bioassay and morphometric endpoints.

Materials and methods

We generated a stress inducible fluorescent HepG2 model in which heat shock protein (*hsp*) promoters controlled enhanced-green fluorescent protein (EGFP) expression upon exposure to cadmium chloride ($CdCl_2$), sodium arsenate ($NaAsO_2$) and paraquat. After a 6 h incubation period with the toxicants, the chip was washed 3 times in PBS solution, then placed into a culture medium to allow EGFP expression. Incubation of the culture was resumed until day 4, then the slides were fixed, stained and analysed.

The miniaturized format of the Cell-on-Chip requires only minute amounts of media compared to current microwell formats. This feature is particularly relevant when cell availability is limited as in the case of rare differentiated cells and patient biopsies, the volume of the tested compound such as potentially toxic compounds needs to be reduced in order to reduce hazards to the manipulators, or the compounds are expensive products in such cases as candidate drugs and siRNAs.

In addition, the drop reactor is a wall-free system well suited for toxicity assays compared with even small-sized microwells since there is a high efficiency of gas exchange, continuous liquid swirling, no adsorption of chemicals on plastic walls, hence limited amounts of toxicants remain after washing, and unrestricted analysis of the whole assay in the absence of walls shadowing the liquid.

Results and discussion

We have developed a Cell-on-Chip device, where several hundreds of individual nanoliter drops arrayed on a small patterned glass substrate act like as many independent cell cultures. We have combined this device with IMSTAR (Paris, France) Pathfinder™ automated image capture and image analysis system to conduct high resolution image-based phenotype screening on multiple parameters obtained using three fluorescent markers. By this means, we cannot only analyse cell viability and fluorescence intensities, but also cell morphology, providing invaluable information on the behaviour of individual cells in the presence of a compound.

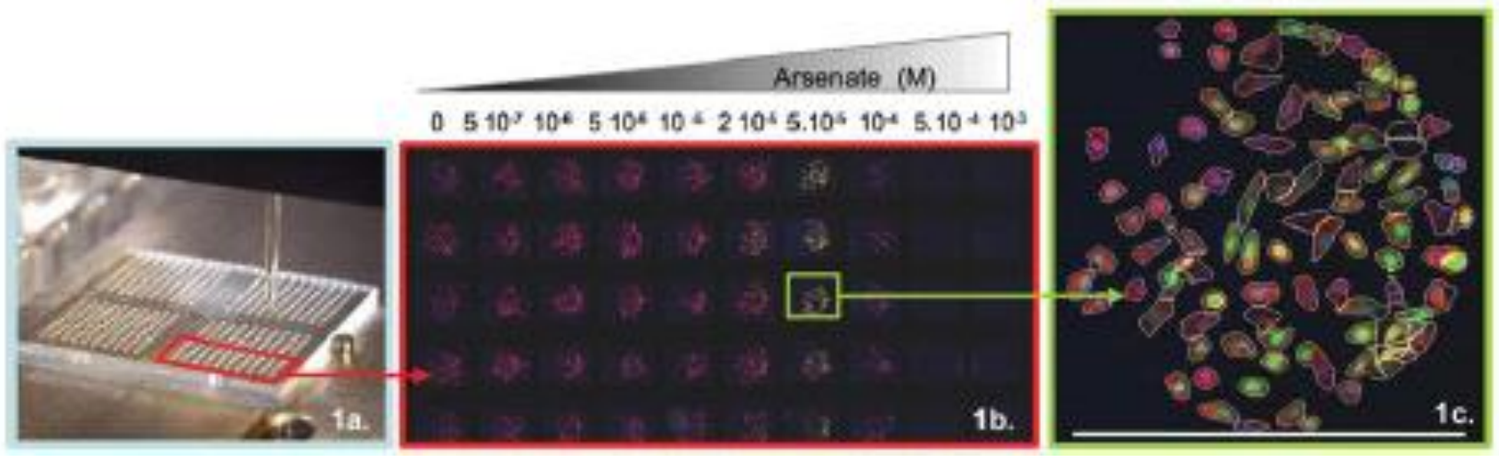

Fig. 1 **Multiplexed toxicity assay in drops**. The 'Cell-on-Chip device was used to obtain 400 independent HepG2 stress inducible fluorescent cell-based assay measurements using 2 *hsp* promoter containing clones and and 3 toxicants at ten different doses. The experiments were performed in quintuplicate measurements. **1a** Cell dispensing with the sciFLEXARRAYER. **1b** Zoom on the assembled mosaic of images corresponding to A10 clone after 6 h exposure to 10 doses of arsenate in quintuplicate (columns); the hsp induction is monitored by the green EGFP signal, cell nucleus is stained in blue and cell cytoplasm is stained in red. **1c** Heterogeneity in cell response is illustrated by an example of hsp response to 5 x 10^{-5} arsenate exposure. Scale bar represents 500 µm. Fully automated image capture with a 10 x objective followed by dedicated image analysis. All cells were individually segmented to extract information (signal intensity, morphology) on every single cell within each drop.

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We recorded cytotoxic effects on HepG2 cells cultivated in 100 nL drops (Fig. 1) exposed to $NaAsO_2$, $CdCl_2$ and paraquat. We especially determined IC50 values (Fig. 2) in agreement with published data. Individual cell identification via image-based screening allowed us to perform multiparametric analyses.

Using pre/sub lethal stress instead of cell mortality, we highlighted the high significance and the superior sensitivity of both stress promoter activation reporting and cell morphology parameters in measuring the cell response to a toxicant. These results demonstrate the first generation of high-throughput and high-content assays, capable of assessing chemical hazards *in vitro* within the REACH policy framework.

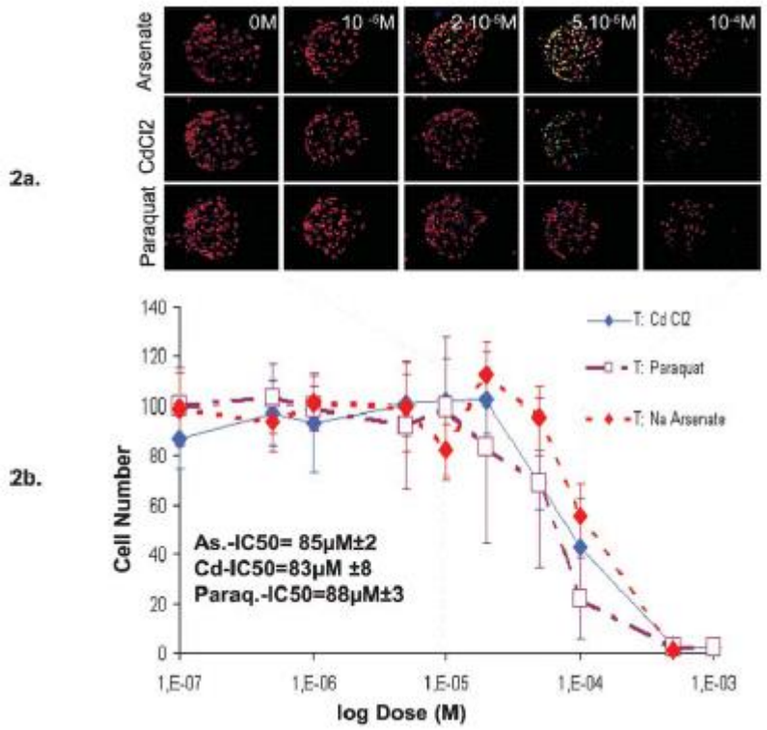

Fig. 2 **Toxicity measured as cell mortality**. Arsenate, cadmium and paraquat were tested in a HepG2 A10 stress inducible clone. **2a** A sample image among the quintuplicate experiments is shown for the control and four toxic concentrations around the maximum EGFP induction zone (orange). **2b** After cell detection and cell counting cell viability is plotted versus the log scale of toxic dose with 0 M control plotted as 10^{-7} M data point. The error bars correspond to the standard deviation of the 5 replicate independent experiments.

Courtesy of Frédéric Lemaire. An article on this topic has been published by Lemaire, F. et al. (2007) Toxicity assays in nanodrops combining bioassay and morphometric endpoints, PLoS ONE 2(1): e163.doi:10.1371/journal.pone.0000163.

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

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