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# Cells on chip for multiplex screening

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ABSTRACT

Microarray technology was developed in the early 1990s to measure the transcription levels of thousands of genes in parallel. The basic premise of high-density arraying has since been expanded to create cells microarrays. Cells on chip are powerful experimental tools for high-throughput and multiplex screening of samples or cellular functions. Miniaturization increases assay throughput while reducing both reagent consumption and cell population heterogeneity effect, making these systems attractive for a wide range of assays, from drug discovery to toxicology, stem cell research and therapy.

One form of cell microarrays, the transfected cell microarray, wherein plasmid DNA or siRNA, spotted on the surface of a substrate, is reverse-transfected locally into adherent cells, has become a standard tool for parallel cell-based analysis. With the advent of technologies, cells can also be directly spotted onto functionalized surfaces using robotic fluid-dispensing devices, or printed directly in bio-ink material.

We are providing herein an overview of the last developments in optical cell microarrays allowing high-throughput and high-content analysis.



## 1. Introduction

Microarray technology was developed for the analysis of gene expression (Schena et al., 1995), when short nucleotide probes were printed onto the surface of slides for testing their degree of hybridization to the investigated cell-derived cDNA. Since then, many other applications of this technique have been introduced and the types of probes expanded drastically to lead to the most complex ones: living cells. Indeed, since cells are the basic unit of a multicellular organism, understanding the complex effect of the tested molecules may require the use of living, intact cells as detecting agents instead of only one of its purified components.

Cell-based array technology permits simultaneous detection of several different activities located at the surfaces or inside cells, allowing the complex characterization of cells with an amount of information that is hardly assessed by any other technique. Furthermore, binding of cells to printed antibodies or ligands may induce their activation, and consequently the outcome of these interactions, such as phosphorylation, gene expression, secretion of various products, differentiation, proliferation and apoptosis of the cells are also measurable on arrays. Moreover, since cells can be transfected with printed vectors, over- or under-expression of selected genes is also achievable simultaneously, creating a nice tool for assessing the function of a given gene.

The high-throughput cell-based microarray technology enables testing the effect of external stimuli on a scale barely thinkable earlier. These microarrays are also of great importance for a variety of applications, including drug testing, toxicology and basic cell biology (Kawasaki, 2004; Rettig and Folch, 2005).

Cell-based arrays are built on two approaches. The single-cell microarray technique analyses cells individually, using microchamber array chips with thousands of cells situated in microwells (Taylor and Walt, 2000; Yamamura et al., 2005). The other approach explores the influence of numerous arrayed materials on several cells (Moeller et al., 2008).

Cell multiplexing is multiple cell lines on a single assay (or surface), cell arrays are not necessary multiplexed. The challenges in the fabrication of multiplexed cell assays are: (a) keeping cell alive after localization, (b) avoiding cell migration from one location to another, (c) getting a biochemical signal from each cell lines without cross-talking and (d) culture different lines in the same condition. In the present review, we are illustrating a few non-multiplexed cell microarrays which allow high parallelization of one cell line and one phenotype, then three different possibilities of multiplexed cell microarrays: (a) multiplexing on chip through in-situ transfection which allows multiple phenotypes with a unique cell line transfected, (b) multiplexing on chip through localized cell deposition which allows multiple cell lines and (c) other techniques that allows multiplex cell-based microarrays. This

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review is focused on optical methods but it is worth to mention that cell-based microarrays could be also developed based on other transduction techniques such as electrochemistry (Kelso et al., 2000; Sen et al., 2012; Takahashi et al., 2009).

## 2. One cell line, multiple phenotypes

To speed up the functional exploration of the human genome, there is a need for high-throughput technologies allowing transfection of thousands of nucleic acids in parallel and the simultaneous analysis of thousands of resulting phenotypes. In 2001, Ziauddin and Sabatini succeeded in scaling down high-throughput gene function analysis to the microarray level. Different cDNA expression plasmids were spotted onto slides using a microarray spotter. The dried slides were exposed to a transfection reagent, placed in a culture dish and covered with adherent mammalian cells in medium. This operation created microarrays of simultaneously transfected cell clusters with different plasmids in distinct and defined areas of a lawn of cells (Ziauddin and Sabatini, 2001). With this cell chip, they pave the way to the transfected cell microarray systems.

Based on the reverse transfection format, Baghdoyan et al. developed a cell microarray to simultaneously transfect thousands of different nucleic acid molecules, to analyse and quantify phenotypes resulting from either gain or loss gene function. Using a regular DNA arrayer, they were able to print and simultaneously transfect high numbers of nucleic acid. As the cell microarrays are also printed by the same robots as regular DNA arrays, massively parallel transfection of up to 5000 cell clusters per slide could be feasible for effective siRNA (Kumar et al., 2003) or shRNA probes for inhibition of target gene expression (Mousses et al., 2003).

The applications of current microarray-based transfection techniques were limited to cells that are easy to grow and transfect, such as HEK293T and COS-7 (Wu et al., 2002). These cells, while typically used as models, have limited relevance to physiological systems in the biomedical field, whereas relevant primary cells, such as hMSCs, are notoriously difficult to handle and transfect (Michiels et al., 2002). That's why Yoshikawa et al. realized a transfection microarray for hMSCs by using the property of fibronectin to increase the transfection efficiency of DNA/polymer complexes. The robustness and versatility of the developed technology was demonstrated through its use in the achievement of on-chip RNAi gene knockdown as well as generalization of the technique to a multitude of mammalian cells (HEK293, hMSC, HeLa, HepG2, NIH3T3) (Yoshikawa et al., 2004).

However, since existing methods of measuring transcription provide discrete measurements of a transcriptional response obtained from large populations of cells, they suffer from two major drawbacks. First, quantifying transcription dynamics using microarrays at multiple time-points is expensive when long processes are under study. Second, despite improvements in assay sensitivity, these approaches typically involve pooling mRNA from thousands of cells. The averaged response measured in this way is adequate for classifying different cell or tissue type, but it is not well-suited for studying processes with cell-to-cell variation, such as cell division, differentiation, or drug responsiveness. Recent developments in cell-based assays combined with advances in reporter technology allow to address these limitations, since expression levels can be repeatedly assayed in single-cells.

Rajan et al. described a high-throughput platform for measuring transcriptional changes in real time in single mammalian cells. They used reverse transfection microarrays to be able to transfect fluorescent reporter plasmids into 600 independent clusters of cells plated on a single microscope slide and imaged these clusters every 20 min. They used a fast-maturing, destabilized and nuclear-localized reporter (Venus-NLS-PEST fluorescent reporter (Nagoshi et al., 2004)) that is suitable for automated segmentation to accurately measure promoter activity in single-cells. They tested this platform with synthetic drug-inducible promoters that showed robust induction over 24 h. Automated segmentation and tracking of over 11 million cell images during this period revealed that cells display substantial heterogeneity in their responses to applied treatment, including a large proportion of transfected cells that do not respond at all (Rajan et al., 2011).

Though the cell microarrays have slowly evolved to become a more widely accepted screening technology, in many publications, the individual arrays have contained only a modest number of samples, and data from multiple small arrays have been combined for large-scale coverage due to technological limitations of the methods (Doil et al., 2009; Neumann et al., 2010). Rantala et al. described the optimization of a cell spot microarray (CSMA) method which provides a patterned array platform with spatially confined cell spots that allow simple production of cell microarrays with significantly increased sample coverage in microplate-sized array plates readily compatible with standard imaging instruments. To allow rapid adaptation of the technique, they optimized an application protocol of the CSMA for 85 cell types and applied the platform to functional genetics profiling of G-protein coupled receptor coding genes in cultured prostate cancer cells and non-malignant epithelial prostate cells, demonstrating the potential of the CSMA for context specific target discovery (Fig. 1A) (Rantala et al. 2011).

It is usually necessary to maintain a distance of 500 μm or more between micro-spots on a transfected cell microarrays (Erfle et al., 2007). For higher-density micro-spots, methodological breakthroughs are required to prevent the migration of cells and to limit the diffusion of spotted materials among the micro-spots in the array. The micropatterning of a glass substrate via generation of a hydrophobic or hydrophilic surface is very effective for the regulation of cell adhesion and prevention of the migration of cells among spots (Hook et al., 2009). Fujita et al. described the development of a super-dense transfected cell microarray. To create this microarray, they used an inkjet printer to spot a mixture of plasmid, extracellular matrix (ECM) protein, and other reagents for induction of reverse transfection on a glass substrate that had been previously grafted with polyethylene glycol (PEG). The microspots containing ECM were separated from one another by a hydrophobic barrier generated by PEG, which has proven to be extremely effective in preventing the migration of cells and the cross contamination of reagents among adjacent spots. The densest transfected cell microarray that they prepared had arrays with spots of 50 μm in diameter and 150 μm in pitch (Fig. 1B) (Fujita et al., 2013).

To improve reverse transcription efficiency and to prevent spot-to-spot diffusion, the distance between the different transfection clusters was adjusted and various glass material coatings have been used (Hodges et al., 2005; Peterbauer et al., 2006). However, these methods still have as limitation high cost coating material and complex experimental steps. In their study, Oh et al. used a polyethylene glycol diacrylate (PEGA) microwell to generate a spatially separated cell adhesion area and applied it to cell culture and reverse transfection platform for cell-based high-throughput screening. For the first time, olfactory receptors were expressed on the microwell platform using reverse transfection technique. The various olfactory receptors can be expressed simultaneously using this technique and the microwell spotted with olfactory receptors genes can be used as a high-throughput screening platform. The odorant response was detected via fluorescence analysis on the microwell using a cAMP response element (CRE) reporter assay (Oh et al., 2014).

Recently, Yamaguchi et al. developed a novel technique for

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