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Cell microarrays in drug discovery

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There is an increasing need for systematic cell-based assays in a high-throughput screening (HTS) format to analyze the phenotypic consequences of perturbing mammalian cells with drugs, genes, interfering RNA. Taking advantage of the recent progress in microtechnology, new cell microarrays are being developed and applied to a large range of issues in metazoan cells. This article compares different approaches and evaluates their potential use in the drug discovery process. Although still an emerging technology, cell microarrays hold great promise to optimize the efficiency:cost ratio in cell-based HTS.

Cell-based assays have become an integral part of drug screening in the pharmaceutical industry. These assays are extremely useful to evaluate potential drug targets by functionally characterizing their effect in cells, to assess specificity and efficacy of drug leads, to identify the targets for drugs of unknown mechanism of action or to perform ADME–Tox of potential drugs in *in vitro* studies.

There is also an increasing need for high throughput cell-based assays in the functional exploration of genomes. Indeed, to comprehend the role of a gene of unknown function in the cell, one would need to analyze phenotypes resulting from either gain or loss of that gene function in the cell.

Cell assays have been miniaturized by growing cells in 96-, 384- or 1536-well microtiter plates or by mixing cells with one-bead-one-compound chemical libraries. Several screens have been published recently using that format [1,2]. However, as chemical libraries and gene collections grow, one will need to further miniaturize cell assays to increase parallelism of cell analysis (Figure 1). To that end, cell microarrays provide an attractive solution. Because they can hold at least 5000–6000 spots on one slide, this enables a genome-wide screen on only a few slides [3,4], which would increase the throughput significantly. Also, cell microarrays offer many other advantages over microtiter plates. For example, because of extreme miniaturization, only small quantities of expensive reagents, scarce biological samples or rare cells are needed. Moreover, most cell microarrays are implemented on glass slides, which allow a larger range of detection methods to be used. Finally, and more importantly, cell microarrays could enable scientists to study biological questions that were not addressable before.

Because the technology is still in its infancy, some important limitations remain to be tackled (such as cell stress, efficacy of transfection, flexibility of detection, etc.) for which a profusion of technological solutions are currently being proposed. To date, two major types of cell microarrays have been described: positional cell microarrays, in which cells are arranged on a surface and given x and y coordinates, and batch or nonpositional cell microarrays, in which cells are encoded, phenotypes are assayed in batch and cells of interest are retrieved through decoding. In this review we will describe these different formats of cell microarrays, their applications and their potential use in the field of drug discovery.

Positional cell microarrays

To manufacture cell microarrays, cells can be printed directly onto a plane surface using different types of arrayers. Alternatively, different substrates (such as polymers, carbohydrates, antibodies, proteins from the extracellular matrix, etc.) can be arrayed; cells are then deposited onto the array and preferentially attach to the printed substrates. Whatever the chosen strategy, one generates positional cell microarrays, in which clusters of cells are physically separated and characterized by specific x and y coordinates.

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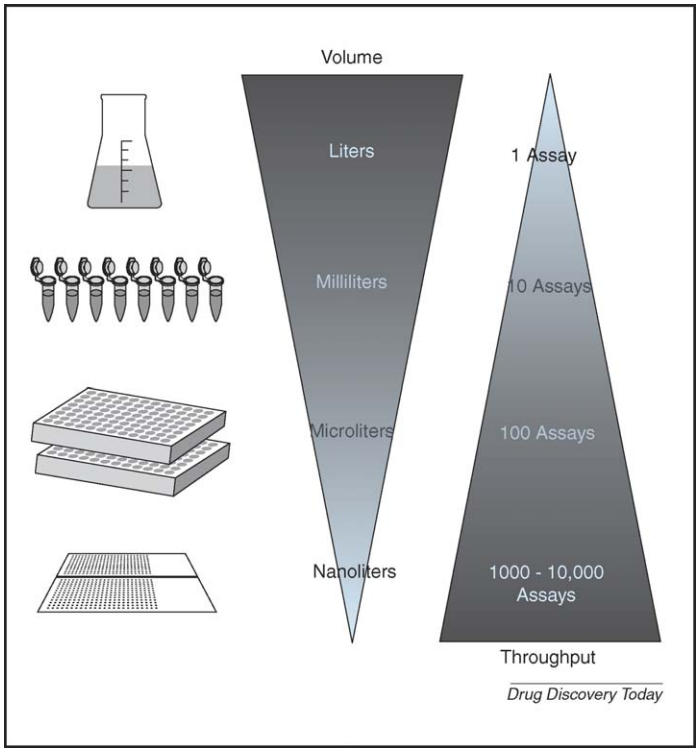

FIGURE 1

Continuous increase of throughput in screening. Improvements in laboratory techniques in the past decades have allowed a dramatic miniaturization of cell assays. As a direct result, parallelism was significantly increased. With cell microarrays, the simultaneous screen of thousands of compounds can be achieved using very small volumes of expensive reagents and small numbers of rare cells. This results in a reduction of cost along with an acceleration of high-throughput screening.

Arraying cells

The most obvious way to generate cell microarrays is to miniaturize microtiter plates. Thus, a 'nanotiter plate' has been developed containing 96 wells with a volume range from 100 to 1000 nl addressable by a piezonozzle. This microarray can be used in the same way as any 96-well plate in various cell assays, such as phenotypic characterization [5]. More complex arrays have been developed as well. An array containing several types of frozen cells has been proposed [6]. It is very similar to a tissue microarray and can be analyzed with antibodies, ligands, DNA or RNA probes. This array represents a low-cost solution for high-throughput qualitative screening of the binding of reagents to different cell types. However, this technology is challenging, and frozen cell microarrays are not easy to manufacture. Another way of generating a cell array is to print cells on a glass slide covered with cell-repulsive tri-ethoxyaminopropylsilane to inhibit their adhesion to the slide. A matrix of circular spots is left without repulsive agent allowing cells to specifically adhere in the spots and grow. These microarrays have been used in combination with laser scanning cytometry to measure nuclear DNA content, and with immunofluorescence to monitor the amounts of cyclin A and B1 at different stages of the cell cycle [7,8]. Ink-jet printers have been adapted to print cells such as Chinese hamster ovary cells or motorneuron cells without major loss in viability [9]. It is also possible to print fixed cells using a pin and ring arrayer, in which cells are captured within the ring and pushed onto the surface by the pin. This microarray of fixed cells has been used to screen antibody specificity against surface antigens [10].

An elegant way to array cells is using dielectrophoretic properties. When cells flow over an array of electrodes, they are trapped above one electrode when current is applied to it. Different cells can be separated using such an array of electrodes based on their different dielectric properties [11,12]. Cells have also been immobilized in hydrogel prepolymer solution by dielectrophoresis, and micro-resolution 3D cell patterns have been formed by photopatterning [13]. By displacing the current on the array of electrodes, individual cells can be moved from one position to another and generate a dynamic cell microarray [14,15].

A very promising approach would be to use microfluidic techniques to array cells. Quake and colleagues [16] recently developed a microfluidic bioreactor that enables the long-term culture of small populations of bacteria. They used a chip, which is composed of several independent reactors, each containing a few nanoliters applied to the reactors by pump and valves. Bacteria were genetically engineered with a 'population control' circuit that regulates cell density through a negative-feedback mechanism based on quorum sensing. Microfluidic control of growth medium supply was successfully applied to genetically engineered bacteria allowing the culture of a constant number of living cells over hundreds of hours [16]. Although not yet applied to eukaryotic cells, this format seems very promising and it is very likely that more cell microarrays based on microfluidic technology will appear in the near future. This will be of particular interest for suspension cells, because most cell microarrays to date only work with adherent cells.

However, direct cell arraying remains particularly challenging because most devices used for this purpose (such as piezonozzle, hollow pins, and ink jet devices) can have a deleterious effect on cells. Although cells can stay alive, it is possible that their physiology is affected by the arraying process, jeopardizing a meaningful interpretation of their phenotypes. So far, this issue has not been extensively evaluated and it might be an attractive alternative to generate an array of chemical substrates to which cells specifically attach. Using that format, cells are softly overlaid on substrates, it is therefore better for the overall condition of cells.

Substrate arraying to manufacture cell microarrays

Cell microarrays based on polymers

Recently, Anderson *et al.* [17] developed a microarray composed of different polymers to identify new synthetic biomaterial suitable for cell growth and proliferation. They printed 576 combinations of 25 different monomers on an epoxy-coated slide and irradiated the arrays with UV light to trigger polymerization. Human embryonic stem cells were grown on these arrays and selectively attached to some of these polymers. The substrates were also found to vary in their ability to promote cell growth and proliferation in the presence or absence of growth factors. Polymer cell microarrays could therefore turn into a convenient tool to rapidly identify synthetic material that supports attachment, proliferation and differentiation of any cell type. This could be very valuable because some cells remain particularly difficult to handle *in vitro*.

Cell microarrays based on glycans

Because carbohydrates and lectins have a major role in cell–cell interactions, cell signaling, immune response and development, glycan cell microarrays make an elegant way to screen the effects of various carbohydrates on cell fate. Nimrichter *et al.* [18] have analyzed $CD4^+$ T cells on an array composed of 45 different sugar moieties and demonstrated the selective binding of these cells to their complementary glycans.

Cell microarrays based on peptides

Peptide recognition is an essential feature of cell physiology, particularly in immunology. Falsey *et al.* [19] recently proposed direct covalent linkage of peptides to aminosilane slides derivatized by a glyoxylyl group. Different cells were immobilized on the peptide arrays and specific induction of cellular responses by the various peptides was then tested. As a proof of concept, the authors have verified that a peptide known to induce tyrosine phosphorylation was indeed inducing that response in the expected cell type. When the cell response depends on the quantity of peptide interacting with it, one can immobilize larger amounts of peptide by printing molecular scaffolds complexes onto plane surfaces. Using this technology, 60 different peptides have been screened for the ability to bind to Jurkat cells [20]. Chemolabil linkers have also been used to covalently couple compounds and peptides to maleimide-functionalized glass slides. Cells were incubated on the arrays, the linker was hydrolyzed and the fluorescent peptides, which were thereby released, were seen entering the cells [21]. The characterization of antigen-specific T-cell populations is crucial for understanding the immune system and its responses in health and disease. Two groups have been using peptide–MHC complexes printed on microarrays to detect rare T-cell epitopes and to simultaneously determine the functional outcome of the interaction of peptide–MHC with T cells [22,23].

Cell microarrays based on antibodies

Antibody-based cell microarrays have been designed to identify cell surface antigens in mixed cell populations. The concept of a microarray of antibodies had been proposed as early as 1983 to determine allotypes of human leukocyte antigens in blood cells [24]. More recently, this concept was applied to clinical studies of prostate cancer [25] and leukemia [26,27]. A good concordance was observed when data obtained with antibody-based cell microarrays were compared to results obtained by flow cytometry. Markers identified with antibody-based cell microarrays could be subsequently used to isolate a particular cell type, such as neural stem cells [28]. In this example, an array of antibodies raised against different neural cell surface proteins was produced. Primary neural cells isolated from rat fetuses were then seeded onto the array. Maturity of bound cells was further analyzed using specific antibodies of both mature and immature markers of neural cells.

Cell microarrays based on extracellular matrix

The extracellular matrix (ECM) includes various proteins and proteoglycans whose properties play a key role in the fate of cells. It is therefore extremely interesting and very valuable to systematically screen the effect of ECM components on cell behavior: cell differentiation and specialization into various lineages, cell adhesion, cell proliferation, cell division, and so on (Figure 2). Recently, Flaim *et al.* [29] developed an array containing 32 combinations of five well-characterized ECM proteins. They used these arrays to screen key liver function in primary hepatocytes and differentiation of embryonic stem cells. The results showed that, as expected, some of the combinations of ECM proteins were more efficient to promote the cellular phenotype. A similar approach has been developed to compare the adhesion of three different types of cells to 14 ECM proteins [30]. Finally, in a very elegant study, patterns of ECM proteins were printed onto slides. Growing cells followed exactly the shape of the pattern allowing study of cell division and cytokinesis in a restrained environment. Using this technology, the authors were able to show that the ECM, rather than cell morphology, controls the orientation of cell division and, consequently, the fate of daughter cells (Figure 2b) [31].

Cell microarrays based on membranes

More complex substrates can be used to manufacture cell microarrays. Artificial double-layer lipid membranes, so-called 'supported-membranes', have recently been used to study interaction between cells and to define components of the membrane *in vitro*. Supported membranes are produced by coating substrates (e.g. glass) with a phospholipid bilayer containing lipid anchored proteins. Lateral fluidity is conserved in supported membranes allowing them to behave like cellular membranes *in vivo*. Microarrays of supported membranes have been developed to screen different membrane compositions and their interactions with cells [32], and to characterize key molecules involved in cell adhesion [33].

Cell microarrays based on RNA interference

As more genomes are sequenced, we are now facing the challenge of rapidly deciphering the function of genes. Methods currently used by scientists to characterize gene function, such as knockout mice, are based upon loss of protein function and analysis of the resulting phenotypes to infer a potential role for the protein under scrutiny. In such approaches, RNA or DNA constructs that eliminate synthesis of gene products are introduced into the cell, and cellular modifications are analyzed. Such phenotypic studies give a good idea of the potential function of the gene product. However, these methods are time consuming and low throughput. Fortunately, RNA interference (RNAi), which is a natural process for sequence-specific posttranscriptional gene silencing initiated by double-stranded RNA, was recently discovered [34]. It was demonstrated that short (<30 nucleotides) double-stranded interfering RNA (siRNA) molecules, homologous to a target gene, could specifically inactivate gene function when introduced into mammalian cell. Thus, siRNA offers the possibility of high-throughput 'knock-down' studies for the analysis of thousands of genes of unknown function.

To accelerate the functional exploration of the human genome, one needs micro-technologies to transfect thousands of nucleic acids in parallel and simultaneously analyze thousands of resulting phenotypes. To that end, Ziauddin and Sabatini [35] have described a cheap and flexible cell-based microarray system using reverse transfection. In that format, mixtures of nucleic acids,

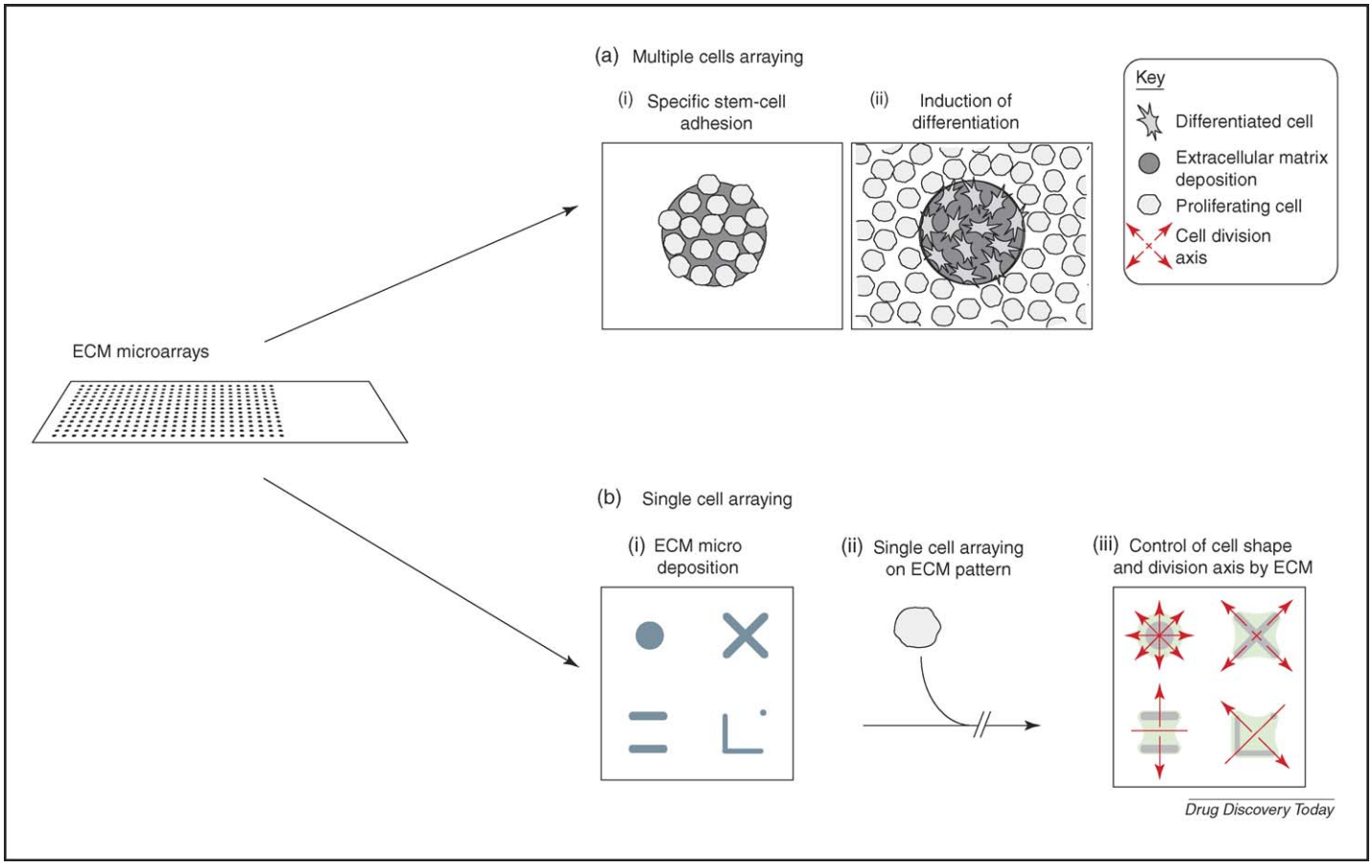

FIGURE 2

Applications of cell microarrays based on extracellular matrix. Extracellular matrix (ECM) arrays consist of a micro-deposition of ECM compounds onto a plane surface. They can be used in several applications: **(a)** arraying cells on ECM patterns can be used to analyze the role of ECM in, for example, (i) adhesion of stem cells or (ii) differentiation. **(b)** Patterns can be designed for single cell adhesion. As illustrated here, ECM deposition can be used to observe single cell adhesion, and control the shape of cells adhering and consequently the orientation of the division axis (shown with red arrows) [31]. ECM cell microarrays could be used with any drugs, cytokine or siRNAs screening, allowing studies of biological questions which were not addressable before.

polymer and transfection agent are arrayed on a glass slide. Then cells are seeded onto the array, and nucleic acids diffuse into cells, justifying the 'reverse transfection' denomination. To improve efficacy of transfection into adherent cells with spatial and temporal control, a micropatterned gold electrode has been used to perform parallel electroporation [36].

Several groups have extended the reverse transfection format to transfect thousands of different siRNA or short hairpin RNA (shRNA), generating separated clusters of transfected cells in which one given gene is specifically knocked down (Figure 3) [3,4,37]. This format was used to select from a collection of siRNA the most efficient one to silence one specific gene [38]. In the most recent applications, lentivirus-infected cell microarrays extend massively parallel extinction of genes to primary cells [39].

Several microarrays can be manufactured from a single source plate containing the siRNA solutions and stored for months before addition of cells (Figure 3c). These microarrays are very flexible and numerous phenotypes resulting from specific gene knock-down can be analyzed either in fixed cells by immunostaining or *in situ* hybridization, or in living cells using gene expression reporters, calcium fluxes with calcium-sensitive fluorophores [40] or labeled proteins. As collections of interfering RNA are getting larger, almost covering the entire human genome, more genome-wide screens using siRNA cell microarrays should appear in the near future [41].

Cell microarrays with electronic interfacing

Most cell microarrays have been developed to analyze cells. In some cases it could be interesting to stimulate cells – for example, with electric pulses. In an attempt to interface electronic devices with living cells, Fromherz and colleagues [42,43] designed a silicon chip with immobilized living neurons synaptically connected. Electric pulses generated from one transistor were transmitted to a single nerve cell, then to a neighboring cell through synaptic connections, and finally recovered in a second transistor.

Although technically complicated, this hybrid circuit of cells and noninvasive semiconductors opens the door to new applications in basic biology and drug discovery.

Batch cell microarrays

As the name implies, batch arrays are non-positional arrays and do not rely on x and y coordinates to deconvolute data. Instead each

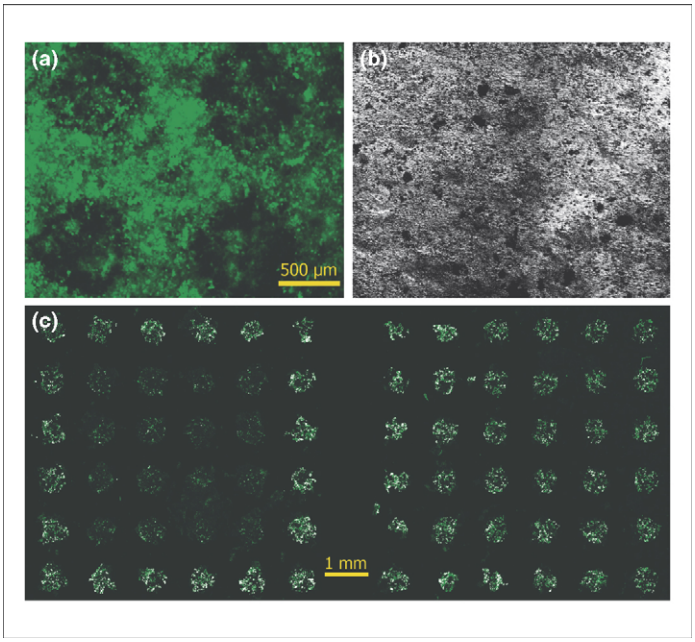

FIGURE 3

siRNA microarrays. (a) Endogenous gene silencing in HEK293T cells stably transduced with enhanced green fluorescent proteins (EGFP) on siRNA microarrays. EGFP-specific siRNA, together with transfection reagent and gelatin, were printed onto a glass slide and green fluorescence in cells was analyzed 72 h after reverse transfection. A focus on four spots of the cell array is displayed here, with characteristic dark areas corresponding to EGFP expression knock-down. **(b)** A picture of the same four spots in transmitted-light microscopy shows that the decrease in fluorescence results from a local knock-down of EGFP and not from an absence of cells. Both images were acquired using a fluorescence microscope. **(c)** Highly parallel gene silencing with EGFP-targeting siRNA co-transfected with an EGFP-encoding plasmid into HEK293T cells. Two blocks of either EGFP targeting siRNA or lamin A/C targeting siRNA were mixed with an EGFP-expressing vector and then printed on a cell microarray. Each block is composed of a surrounding square of 6×6 control spots (EGFP plasmid alone) and of a 4×4 filled square of sample spots: EGFP plasmid co-transfected with 0.60 μg of EGFP siRNA (left panel) or 0.60 μg of lamin A/C siRNA (right panel). Image acquisition was done after 48 h of cell culture on a conventional DNA microarray scanner. On the left panel, a specific knock-down of EGFP can be seen, whereas in the cells treated with lamin A/C siRNA, fluorescence levels remain identical to control spots (right panel). This microarray containing plasmids and siRNA was conserved for 1 year before cell deposition without any apparent loss of efficacy.

cell is associated with a different code. The exact nature of this code can be diverse, ranging from color to radio frequencies. Two main types of coding approaches have demonstrated feasibility so far.

The first technology uses encoded particles on which cells are grown. These particles are actually small cards (100 μm^2) that can be manufactured with different materials and which contain a barcode. The encoding strategy relies on colored bars, which are physically associated with cells without interfering with them. These cards are dispensed in 96-well plates and cells grow on them. The array is decoded and cells associated with each particle are analyzed using a charge-coupled device (CCD) imaging system. Using this system, up to nine cell lines have been assayed in parallel for their proliferative capacities [44].

Another coding system uses nanometer-sized semiconductor crystals, quantum dots, that can be incorporated into the cells [45,46]. Quantum dots fluoresce at different wavelengths according to their size. Combinations of five colors can generate up to 1023 codes [47]. Specific nanocrystals are associated with different cell types by internalization of the crystals into cells. Cells are mixed, dispensed into microtiter plates and assayed either by microscopy or flow cytometry (Figure 4). The association of the data with codes results in the deconvolution of multiplexed information to determine the response of each individual cell type. However, the complexity of this assay is limited because the emission spectra of the quantum dots overlap. Interestingly, the potential use of quantum dots is not restrained to adherent cells but can also be applied to suspension cells using flow cytometry. Finally, quantum dots allow the analysis of cell–cell interactions which would not be possible with color barcoded particles.

Using the same principle, several companies have recently proposed different coding technologies: fluorescent dye ratios from Luminex (http://www.luminexcorp.com); reflective barcodes from Surromed (http://www.surromed.com); and radio frequencies from Pharmaseq (http://www.pharmaseq.com). All these technologies are theoretically not limited to multiplexing, but there are many practical constraints that limit each of these approaches, such as manufacturing simplicity, decoding ability, coding combinatory. Surprisingly, only a few academic laboratories are involved in that type of research and although batch microarrays seem promising, only few applications have been published so far.

Potential applications of cell microarrays in drug screening

As we mentioned earlier, the most obvious application for cell microarrays is for screening compound libraries. Compared to microtiter plates, cell microarrays increase throughput while reducing the overall cost of screening by reducing the amount of expensive reagents and materials, the quantity of compounds and the number of cells used in each assay. Typically tens to a few hundreds cells could be analyzed in each feature on cell microarrays, which could be extremely valuable for rare cells. The main advantage of cell microarrays is the opportunity to measure parameters on hundreds of individual cells and average them, instead of measuring the parameters of a whole cell population. With the continuous improvement of instruments and software dedicated to image acquisition and analysis of cell microarrays, one should be able to monitor in each single cell of a population several key parameters simultaneously (e.g. nuclei staining, localization of a specific protein, cell cycle status, etc.). However the rapid acquisition and analysis of ten thousand features in parallel remains challenging.

An even more promising avenue would rely on cell microarray technologies to analyze the relationship between compound-induced effects and the result of gene overexpression or extinction. One could transfect cells with an expression vector to generate gain of function or, using interfering RNA, to obtain a loss of function, and then screen libraries of small molecules on these genetically engineered cells. Perfect examples for such screens are the G-protein-coupled receptor (GPCR) ligands. Indeed, as much as 30% of drugs available on the market today target GPCRs. Using

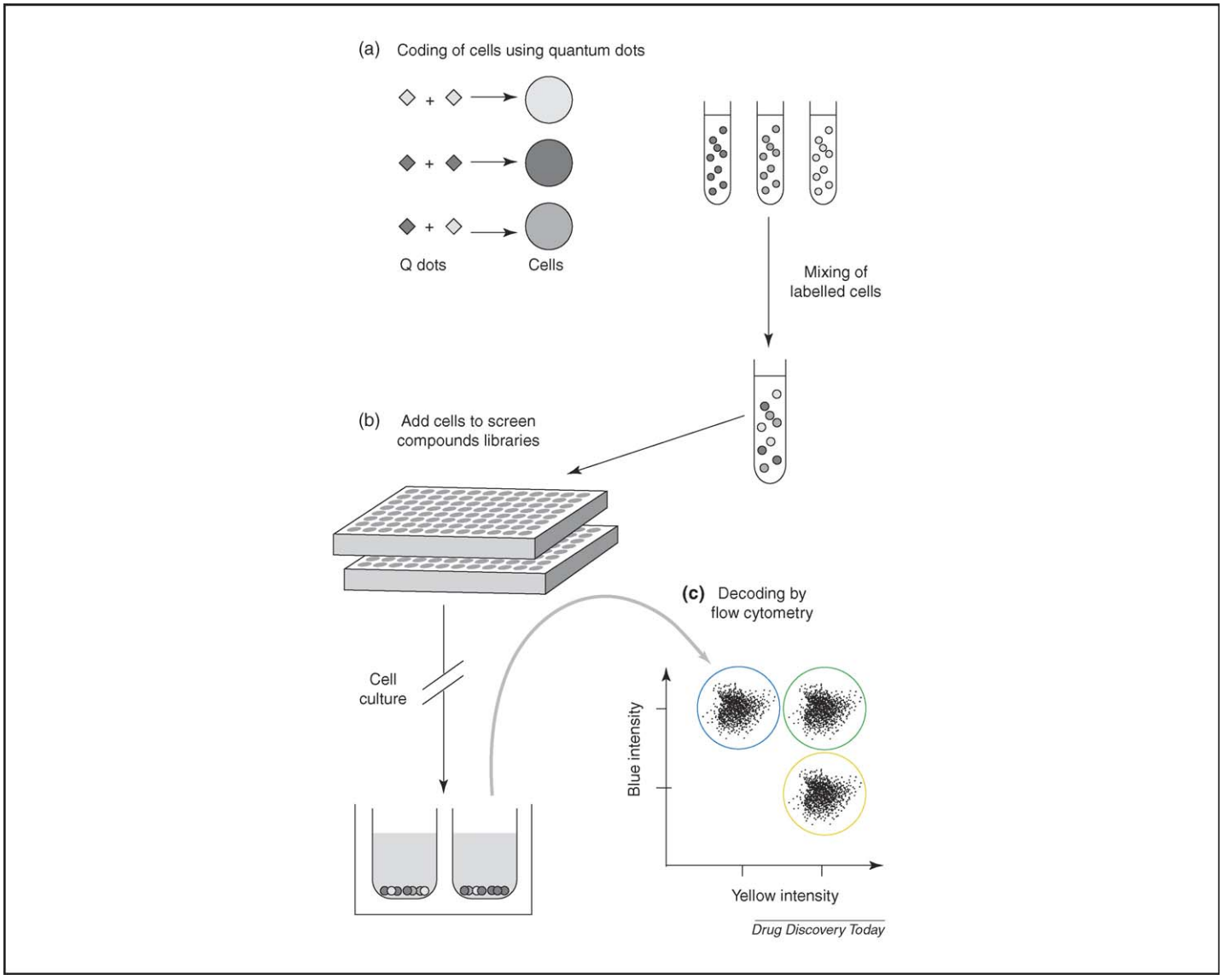

FIGURE 4

Quantum dots to generate batch cell microarrays. (a) Several cell types are fluorescently labeled by combining quantum dots with different emission wavelengths. Fluorescence-coded cells are then mixed, allowing a multiplexing of the subsequent screens. **(b)** The pool of cells is arrayed and cultured in a microtiter plate format along with compounds libraries to be tested (drug candidates, siRNAs, etc.). **(c)** The composition of the cell population in each well is decoded by flow-cytometry: the number of cells of each type is assessed using their quantum dots labels. This method is powerful because any further fluorescent endpoint can be studied simultaneously (Immunofluorescence, reporter assay, etc.).

cell microarrays, a chimera of GPCR–GFP can be overexpressed and subsequent fixation of a ligand can be analyzed by internalization of the receptor [48].

Another possibility is to screen anticancer drugs in the presence of different siRNA to analyze whether the loss of function of one gene sensitizes cells to anticancer agents and can trigger cancer cell death. The same approach could be used to screen compounds that could reverse phenotypes of cells harboring a mutation that mimics a genetic disease.

Eventually, the best format for the development of the type of assay that would associate a genetic defect with a small molecule screen would be nucleic acids printed at the bottom of a 96-well microtiter plate. Plate-based robots common to many facilities that specialize in high-throughput screening could then be used to screen molecules as usual. As a demonstration, recent work by Mishima *et al.* [40] illustrates this point. Thirty-six different GPCR constructs, containing a fluorescent reporter to monitor the activation of the receptor, were arrayed on the bottom of microtiter plates and cells were transfected. The screening of 25 agonists revealed 15 functional GPCR-agonists out of 900 possible combinations.

Conclusion

Cell microarrays could constitute new weapons of mass analysis in the arsenal of biologists and pharmacists. They could eventually be used in the pharmaceutical industry because of their ability to accelerate several key features of this industry, such as target identification, lead assessment and ADME–Tox studies [49]. Although numerous technological approaches exist today, it is very likely that a reference format, combining several advantages

of each approach, will emerge in the future. The combinatorial use of different substrates (e.g. ECM), genetic modification of cells (gain or loss of function) and screening of small molecules in cell microarray format will probably offer new avenues of investigation in basic cell functioning as well as new possibilities to evaluate drugs in the continuous fight against disease.

References

1 Abraham, V.C. *et al.* (2004) High content screening applied to large-scale cell biology. *Trends Biotechnol.* 22, 15–22
2 MacKeigan, J.P. *et al.* (2005) Sensitized RNAi screen of human kinases and phosphatases identifies new regulators of apoptosis and chemoresistance. *Nat. Cell Biol.* 7, 591–600
3 Baghdoyan, S. *et al.* (2004) Quantitative analysis of highly parallel transfection in cell microarrays. *Nucleic Acids Res.* 32, e77
4 Wheeler, D.B. *et al.* (2004) RNAi living-cell microarrays for loss-of-function screens in *Drosophila melanogaster* cells. *Nat Methods* 1, 127–132
5 Angenendt, P. *et al.* (2004) Cell-free protein expression and functional assay in nanowell chip format. *Anal. Chem.* 76, 1844–1849
6 Stephan, J.P. *et al.* (2002) Development of a frozen cell array as a high-throughput approach for cell-based analysis. *Am. J. Pathol.* 161, 787–797
7 Oode, K. *et al.* (2000) The development of a cell array and its combination with laser-scanning cytometry allows a high-throughput analysis of nuclear DNA content. *Am. J. Pathol.* 157, 723–728
8 Takita, M. *et al.* (2003) An analysis of changes in the expression of cyclins A and B1 by the cell array system during the cell cycle: comparison between cell synchronization methods. *Cytometry A* 55, 24–29
9 Xu, T. *et al.* (2005) Inkjet printing of viable mammalian cells. *Biomaterials* 26, 93–99
10 Schwenk, J.M. *et al.* (2002) Cell microarrays: an emerging technology for the characterization of antibodies. *Biotechniques* (Suppl), 54–61
11 Huang, Y. *et al.* (2002) Dielectrophoretic cell separation and gene expression profiling on microelectronic chip arrays. *Anal. Chem.* 74, 3362–3371
12 Gray, D.S. *et al.* (2004) Dielectrophoretic registration of living cells to a microelectrode array. *Biosens. Bioelectron.* 19, 1765–1774
13 Albrecht, D.R. *et al.* (2005) Photo- and electropatterning of hydrogel-encapsulated living cell arrays. *Lab Chip* 5, 111–118
14 Pethig, R. (1996) Dielectrophoresis: using inhomogeneous AC electrical fields to separate and manipulate cells. *Crit. Rev. Biotechnol.* 16, 331–348
15 Fuchs, A.B. *et al.* (2006) Electronic sorting and recovery of single live cells from microlitre sized samples. *Lab Chip* 6, 121–126
16 Balagadde, F.K. *et al.* (2005) Long-term monitoring of bacteria undergoing programmed population control in a microchemostat. *Science* 309, 137–140
17 Anderson, D.G. *et al.* (2004) Nanoliter-scale synthesis of arrayed biomaterials and application to human embryonic stem cells. *Nat. Biotechnol.* 22, 863–866
18 Nimrichter, L. *et al.* (2004) Intact cell adhesion to glycan microarrays. *Glycobiology* 14, 197–203
19 Falsey, J.R. *et al.* (2001) Peptide and small molecule microarray for high throughput cell adhesion and functional assays. *Bioconjug. Chem.* 12, 346–353
20 Xu, Q. *et al.* (2004) A novel approach to chemical microarray using ketone-modified macromolecular scaffolds: application in micro cell-adhesion assay. *Mol. Divers.* 8, 301–310
21 Hoff, A. *et al.* (2004) Chemolabile cellular microarrays for screening small molecules and peptides. *Mol. Divers.* 8, 311–320
22 Soen, Y. *et al.* (2003) detection and characterization of cellular immune responses using peptide-MHC microarrays. *PLoS Biol.* 1, e65
23 Stone, J.D. *et al.* (2005) HLA-restricted epitope identification and detection of functional T cells responses by using MHC-peptide and costimulatory microarrays. *Proc. Natl. Acad. Sci. U. S. A.* 102, 3744–3749
24 Chang, T.W. (1983) Binding of cells to matrixes of distinct antibodies coated on solid surface. *J. Immunol. Methods* 65, 217–223
25 Liu, A.Y. (2000) Differential expression of cell surface molecules in prostate cancer cells. *Cancer Res.* 60, 3429–3434
26 Belov, L. *et al.* (2001) Immunophenotyping of leukemias using a cluster of differentiation antibody microarray. *Cancer Res.* 61, 4483–4489
27 Belov, L. *et al.* (2003) Identification of repertoires of surface antigens on leukemias using an antibody microarray. *Proteomics* 3, 2147–2154
28 Ko, I.K. *et al.* (2005) Antibody microarray for correlating cell phenotype with surface marker. *Biomaterials* 26, 687–696
29 Flaim, C.J. *et al.* (2005) An extracellular matrix microarray for probing cellular differentiation. *Nat Methods* 2, 119–125
30 Angres, B. *et al.* (2004) Multiple Substrate Array, MSA™: miniaturized screening of extracellular matrix-dependent cell behavior. *Eur. Cell. Mater.* 7, 40
31 Thery, M. *et al.* (2005) The extracellular matrix guides the orientation of the cell division axis. *Nat. Cell Biol.* 7, 947–953
32 Hovis, J.S. and Boxer, S.G. (2001) Patterning and Composition Arrays of Supported Lipid Bilayers by Microcontact Printing. *Langmuir* 17, 3400–3405
33 Groves, J.T. *et al.* (2001) Control of Cell Adhesion and Growth with Micropatterned Supported Lipid Membranes. *Langmuir* 17, 5129–5133
34 Fire, A. *et al.* (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391, 806–811
35 Ziauddin, J. and Sabatini, D.M. (2001) Microarrays of cells expressing defined cDNAs. *Nature* 411, 107–110
36 Yamauchi, F. *et al.* (2004) Micropatterned, self-assembled monolayers for fabrication of transfected cell microarrays. *Biochim. Biophys. Acta* 1672, 138–147
37 Mousses, S. *et al.* (2003) RNAi microarray analysis in cultured mammalian cells. *Genome Res.* 13, 2341–2347
38 Kumar, R. *et al.* (2003) High-throughput selection of effective RNAi probes for gene silencing. *Genome Res.* 13, 2333–2340
39 Bailey, S.N. *et al.* (2006) Microarrays of lentiviruses for gene function screens in immortalized and primary cells. *Nat Methods* 3, 117–122
40 Mishina, Y.M. *et al.* (2004) Multiplex GPCR assay in reverse transfection cell microarrays. *J. Biomol. Screen.* 9, 196–207
41 Wheeler, D.B. *et al.* (2005) Cell microarrays and RNA interference chip away at gene function. *Nat. Genet.* 37 (Suppl), S25–S30
42 Braun, D. and Fromherz, P. (2004) Imaging neuronal seal resistance on silicon chip using fluorescent voltage-sensitive dye. *Biophys. J.* 87, 1351–1359
43 Zeck, G. and Fromherz, P. (2001) Noninvasive neuroelectronic interfacing with synaptically connected snail neurons immobilized on a semiconductor chip. *Proc. Natl. Acad. Sci. U. S. A.* 98, 10457–10462
44 Beske, O. *et al.* (2004) A novel encoded particle technology that enables simultaneous interrogation of multiple cell types. *J. Biomol. Screen.* 9, 173–185
45 Watson, A. *et al.* (2003) Lighting up cells with quantum dots. *Biotechniques* 34, 296–303
46 Smith, A.M. *et al.* (2004) Quantum dot nanocrystals for *in vivo* molecular and cellular imaging. *Photochem. Photobiol.* 80, 377–385
47 Mattheakis, L.C. *et al.* (2004) Optical coding of mammalian cells using semiconductor quantum dots. *Anal. Biochem.* 327, 200–208
48 Ghosh, R.N. *et al.* (2005) Quantitative cell-based high-content screening for vasopressin receptor agonists using transfluor technology. *J. Biomol. Screen.* 10, 476–484
49 Angres, B. (2005) Cell microarrays. *Expert Rev. Mol. Diagn.* 5, 769–779