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High-throughput cellular microarray platforms: applications in drug discovery, toxicology and stem cell research

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Abstract

Cellular microarrays are powerful experimental tools for high-throughput screening of large numbers of test samples. Miniaturization increases assay throughput while reducing reagent consumption and the number of cells required, making these systems attractive for a wide range of assays in drug discovery, toxicology, stem cell research and potentially therapy. Here, we provide an overview of the emerging technologies that can be used to generate cellular microarrays, and we highlight recent significant advances in the field. This emerging and multidisciplinary approach offers new opportunities for the design and control of stem cells in tissue engineering and cellular therapies and promises to expedite drug discovery in the biotechnology and pharmaceutical industries.

Introduction

The past decade has produced remarkable developments in biomedicine. In the pharmaceutical industry, rational use of combinatorial synthesis together with increased access to natural product sources has enabled the rapid synthesis and identification of new compounds with clinical potential [1]. These advances have led to a growing interest in the potential of cell therapies and drug discovery, along with a dramatic increase in the number of screenable drug candidates available to the pharmaceutical industry [2]. The development of *in vitro* high-throughput screening methods (see Glossary) for evaluating the effects of new growth factors and culture conditions in cell models might assist in the rapid and cost-effective development of novel drugs and also advance understanding of the conditions that selectively control cell fate. Finally, stem cells offer a unique source of cell types and, because of their unique properties (e.g. self-renewal capacity and the ability to differentiate

into specialized cell types), are beginning to be exploited as alternative sources of mature cells for cell therapies [3] and drug discovery [4,5].

Cell-based assays offer the opportunity to perform screens of chemical libraries for molecules that modulate a broad range of biological events. A classical approach is to use antibody binding to track cell-fate decisions using known markers [6], but the cost of high-throughput phenotypic cell-based screening using conventional well-plate platforms can be prohibitive. Moreover, these techniques often lack the ability to provide quantitative information on cell function. In this context, microscale technologies are emerging as powerful tools for tissue engineering and biological studies [7], as well as for drug discovery [8]. In particular, microarrays can provide more information from smaller sample volumes and enable the incorporation of low-cost high-throughput assays in the drug discovery process [9]. With the advent of robotic spotting technology and microfabrication, it is now possible to distribute nanoliter volumes of different chemicals, biomolecules and cells in a spatially addressable footprint [10–13]. Therefore, cell-based microarrays are especially well-suited for high-throughput screening of large numbers of very small samples [14,15].

This review focuses on the potential of high-throughput cell-based microarrays to analyze the consequences of perturbing cells with drugs, genes and other molecular cues, such as extracellular matrix (ECM) proteins, biopolymers or RNA interference (RNAi). The range of applications is remarkably broad and includes protein therapeutic and drug candidate evaluation, enzyme activity and inhibition assays and toxicity screening. Moreover, as we shall demonstrate, this growing field promises to impact the design and control of stem cells for regenerative medicine and biological studies.

Cellular microarrays: design and fabrication

A cellular microarray consists of a solid support wherein small volumes of different biomolecules and cells can be displayed in defined locations, allowing the multiplexed interrogation of living cells and the analysis of cellular responses (e.g. changes in phenotype) [16]. A broad range of different molecules (small molecules, polymers, antibodies, other proteins, etc.) can be arrayed using robotic spotting technology or soft lithography [17–20], and the strategy of choice in the conception of these platforms is related to the type of application and problem under study. For example, surfaces on which cells interact are important for maintaining cellular functions, and the features of these surfaces often act as signals that influence cellular behavior [21,22]. Microarrays can be used to dissect the crucial features of polymer–cell interactions and to unravel key features of specific cellular responses using robotic dispensing systems [23]. Soft lithography using elastomeric materials has also been used to generate micro-bioreactor arrays for high-throughput experiments using human embryonic stem (ES) cells [24] and patterned surfaces for the growth of neural stem cells (NSCs) [25]. Polydimethylsiloxane (PDMS), a biocompatible silicone rubber, has been used to generate substrates for the culture of single cells in micro-well arrays with 3D cell shape control [26], and micropatterning of hydrogels, such as hyaluronic acid or agarose, can also serve as an alternative means of generating arrays of cells for high-throughput screening applications [27,28].

Cells can also be directly spotted onto functionalized glass surfaces using robotic fluid-dispensing devices. With this strategy it is possible to generate patterns of cells encapsulated in 3D hydrogel matrices (e.g. collagen or alginate), which support cell growth at the micro-scale. One example advanced by Lee and colleagues [29] involves the use of glass slides that are spin-coated with a polymeric material (poly[styrene-*co*-maleic anhydride], PS-MA) to increase the hydrophobicity of the surface while providing reactive functional groups to attach a hydrogel matrix. A mixture of poly-L-lysine (PLL) and barium chloride (BaCl₂) is first spotted onto glass slides coated with PS-MA. The positively charged PLL serves as a substrate to bind Ba²⁺ ions and assist the attachment of the negatively charged alginate. The Ba²⁺ ions also cause essentially immediate alginate gelation to give rise to 3D cell-containing matrix spots possessing volumes as low as 20 nL (Figure 1a).

Microfluidic devices that enable parallel analysis in a high-throughput fashion have also been produced using soft lithography techniques [30]. An increasing demand for fully automated and quantitative cell culture technology has driven the development of microfluidic chip-based arrays [31,32]. In one example, the continuous perfusion of medium inside parallel culture chambers allowed the evaluation of how a range of transient stimulation schedules influenced the proliferation, differentiation and motility of human mesenchymal stem cells [32]. Microfluidic cell culture arrays, therefore, offer an attractive platform with a wide range of applications in high-throughput cell-based screening and quantitative cell biology, and some recent applications are summarized in Table 1.

Cellular microarrays in drug discovery

High-throughput cell-based screening assays are routinely performed in the pharmaceutical industry and utilize 96- or 384-well microtiter plates with 2D cell monolayer cultures [33,34]. However, this multiwell plate format suffers from several limitations, including the inefficient removal of reagents from the wells and the consequent difficulty of washing out chemicals from the cell monolayer [35]. Furthermore, high-throughput cell-based screening using such microtiter-plate platforms is expensive because relatively large amounts of high-cost reagents and cells are needed, particularly when 96-well plates are used. Miniaturization of cell-based assays in a microarray format, which would also increase parallelism of cell analysis, offers an attractive solution. Moreover, as we will describe in detail below, this technology can be used to evaluate the effects of drug leads in cells, to identify targets for drugs of unknown mechanism and to study the *in vitro* toxicology of drug, agricultural and cosmetic candidates.

Cellular microarrays for high-throughput toxicology studies

The number of approvals of new drugs per year by the regulatory agencies has not increased significantly in the past decade, especially when compared with the growing investment in pharmaceutical research and development. Although there are numerous causes for this low productivity, including the complexity of biological systems as related to drug targets within the cell [36], one particularly troubling cause is an unacceptably high rate of toxicity among drug candidates in the late clinical stages. Miniaturized cell-based assays with high-throughput capability can be used to identify potentially toxic compounds much earlier in

the drug development process, thereby removing these compounds from further consideration and saving significant financial resources.

Lee and colleagues developed miniaturized, high-throughput systems that mimic the effects of human liver metabolism and simultaneously evaluate cytotoxicity of small molecules and their metabolites [29,37]. These methods combine enzyme catalysis with cell-based screening on a microscale biochip platform. Test compounds are added to the chip (called the MetaChip ['Metabolizing Enzyme Toxicology Assay Chip']), which contains ~2000 combinations of human cytochrome P450 (CYP450) isoforms, and are then stamped onto a complementary 3D cellular microarray (the DataChip ['Data Analysis Toxicology Assay Chip']) for high-throughput screening of test compounds and their CYP450-generated metabolites [29]. This methodology provided IC₅₀ values for nine compounds and their metabolites. The values were generated from the individual cytochrome P450 isoforms CYP1A2, CYP2D6 and CYP3A4 and a mixture of these three P450s designed to emulate the human liver (Figure 1b). Despite near-2000-fold miniaturization, similar results were obtained when compared with conventional 96-well plate assays, demonstrating that scale reduction of this order does not influence cytotoxicity response. In addition to these systems, microfluidic sensor cell-based chips have also been proposed to detect cytotoxic reagents in a sensitive and high-throughput fashion [38]. By using microfluidic cell culture systems in combination with sensor cells (e.g. cells transfected with reporter plasmids encoding green fluorescent protein [GFP]), the authors were able to demonstrate that cytotoxic reagents can be quantitatively detected in a rapid, sensitive and dose-responsive manner. Overall, these strategies exemplify the potential of microscale high-throughput screening approaches for toxicity studies.

Establishing stem cells and their committed progenitors as an innovative platform for toxicity studies in drug screening might be the next logical step in developing unique *in vitro* models for predicting the toxicity of drug candidates and chemicals in humans (reviewed in [39]). Therefore, the development of miniaturized stem cell-based assays represents a promising and innovative strategy for early efficiency in toxicity screening, allowing an improved selection of lead candidates and the reduction of adverse outcomes in later stages of drug development.

Cellular microarrays for small molecule screening

The use of microscale technologies to engineer the cellular environment for drug development has already shown some promise [40] and represents an emerging opportunity for small molecule screening. One of the first systems developed for screening small molecules in mammalian cells consisted of 200- μ m-diameter discs composed of a biodegradable poly-(D),(L)-lactide/glycolide copolymer in which each compound was impregnated [41]. Cells were seeded on top of these discs and compounds slowly diffused out, affecting proximal cells. A similar example of this strategy was demonstrated for the identification of G-protein-coupled receptor (GPCR) agonists [42]. Human embryonic kidney cells expressing a human dopamine receptor and a chimeric G protein, together with a fluorescent calcium indicator (Fluo-4), were incorporated into an agarose sheet and then placed on top of a polyester sheet, which contained over 8500 spotted test compounds that

were able to diffuse into the gel. Binding of the test compounds to the dopamine receptor increased calcium ion levels, thereby resulting in binding to the reporter Fluo-4 and enhanced fluorescence.

The aforementioned systems suffer from region-to-region contamination caused by lateral diffusion of the spotted test compounds. An alternative format is a 3D cell-based array, in which cells are printed onto a solid surface, as described above. One example of this approach is the development of an in-cell, on-chip immunofluorescence technique [43]. This design was used to demonstrate the small molecule activation and dysregulation of hypoxia-inducible factor 1 α (Hif-1 α), a transcription factor expressed under hypoxic cellular conditions and related to a series of pathologies, including several types of cancer. Levels of the Hif-1 α subunit in human pancreatic tumor cells were measured on-chip [43]. Cells were cultured either under normoxic (control) conditions that suppress Hif-1 α expression or under induced hypoxia, where Hif-1 α expression is upregulated. In both cases, the expected expression levels of Hif-1 α were observed. This technique allowed the rapid assessment of effects of inhibitors on protein expression levels. For example, different concentrations of the Hif-1 α inhibitor 2-methoxyoestradiol (2ME2) were added to reduce the levels of the target protein in the microarray and thereby provide dose–response information. Thus, rapid identification of small molecule inhibitors of Hif-1 α could be achieved in future developments. Such an approach could conceivably be performed using different cell types and protein targets, as well as a range of small molecule inhibitors, activators or modifiers.

Cellular microarrays can also be used with genetically modified cells to mimic a particular genetic disease or condition. For example, lentivirus-infected cell-based microarrays have been created for the high-throughput screening of gene function [44]. To provide proof-of-concept, Bailey and colleagues [44] spotted arrays with lentiviruses encoding short interfering RNA (siRNA) or DNA. Cells seeded on top of this pattern became infected, creating a living array of stably transduced cell clusters. Thousands of distinct parallel infections on a single glass slide were performed, including the transfection of primary cells (human fibroblasts and mouse dendritic cells) with GFP-expressing lentiviruses. In the future, screening drugs in the presence of different siRNAs might be used to assess whether the loss of function of one particular gene sensitizes cells to specific agents that can trigger different cell responses. This approach has obvious applications in cancer research and developmental biology [45].

Cellular microarrays in stem cell research

Owing to their unique characteristics (e.g. unlimited self-renewal capacity and potential to generate fully differentiated, mature cells [46]), stem cells are at the forefront of potential regenerative medicine therapies and might also provide an unlimited source of highly uniform cell types for pharmaceutical applications, especially when compared with genetically abnormal transformed or tumor cell lines [3,47]. Nevertheless, several major challenges hinder the transition from fundamental science to functional technologies, including the need to identify the signals and conditions that regulate and influence cell function and the application of this information towards the design of stem cell bioprocesses and therapies [48]. Conventional cell culture methods are unable to meet the demands for

increased throughput; therefore, the ability to track stem cell fate decisions and quantify specific stem cell markers on microarray platforms has the potential to increase our understanding of the cellular mechanisms involved. This fundamental information will then facilitate the development of high-throughput cell-based screening devices for fast and effective identification of small molecules and other signals that can be used to direct cellular responses. Applications include automated, high-throughput methods for synthesizing and screening combinatorial libraries of biomaterials, as well as methods for creating and screening local microenvironments of soluble factors, such as small molecules, siRNAs and other signaling molecules. The effects of these signals on stem cell fate can then be evaluated and the resulting information translated into improved bioprocesses for cell-based therapies or drug discovery programs.

Cellular microarrays for exploring the stem cell microenvironment

Signals emanating from the stem cell microenvironment, or niche, are crucial in regulating stem cell fate. These signals include physical cues (e.g. matrix elasticity [49], cell–cell and cell–ECM interactions) and soluble factors (e.g. growth factors and small molecules), and fate can also be determined by the 3D architecture that supports cell growth and differentiation [50] (Figure 2). Small-scale, high-throughput microarray systems provide optimal environments for unveiling complex interactions.

The functional immobilization of signaling molecules on a surface has been shown to direct stem cell fate [51]. Consequently, ECM microarray platforms were developed to facilitate screening of ECM molecules and their effects on stem cells in a combinatorial and parallel fashion. With the aid of robotic spotting devices, specific ECM components, such as collagen I, collagen III, collagen IV, laminin and fibronectin, were immobilized on hydrogel surfaces and different combinations of these molecules were screened [52]. The commitment of mouse ES cells towards an early hepatic fate was evaluated, leading to the identification of several combinations of ECM components that synergistically impact ES cell differentiation and hepatocyte function. This platform was further advanced to probe experimentally the interactions between ECM components and soluble growth factors on stem cell fate [53], resulting in the analysis of 1200 simultaneous experimental endpoints on 240 unique signaling microenvironments. These studies were consistent with those previously reported in the literature, which resulted in the discovery of hitherto unknown and less-intuitive interactions between growth factors and ECM molecules. Similar work has been conducted using human ES cells and has led to the identification of novel peptidic surfaces that support human ES cell growth and self-renewal [54].

In addition to ES cells, robotic spotting techniques can also be used to study the effects of signaling molecules and growth factors in adult stem cell populations. For example, combinations of ECM molecules, growth factors and other signaling proteins were spotted onto arrays to evaluate their effects on the proliferation and differentiation of human adult neural precursor cells [55]. Quantitative single-cell analysis revealed significant effects of some of these signals on the extent and direction of differentiation into neuronal or glial fate. Similarly, the spatial patterning of bone morphogenetic protein 2 (BMP-2) on fibrin-coated surfaces was used to study the osteogenic or myogenic differentiation of muscle-derived

stem cells using a single chip [56], thus demonstrating the utility of this approach in the study of complex cell–ECM interactions.

Synthetic polymers can also be arranged in a site-specific, combinatorial fashion using photo-assisted patterning. High-throughput, miniaturized arrays for the rapid synthesis and screening of combinatorial libraries of photopolymerizable biomaterials were used to identify biomaterial compositions that influence human ES cell attachment, growth and differentiation [57]. Over 1700 cell–biomaterial interactions were screened, revealing several unexpected cell–polymer interactions, including materials that support high levels of growth and differentiation into epithelial cells. It is also possible to immobilize both growth factors and synthetic matrices together in the same array [58]. In this case, substrates can be loaded with growth factors known to have neurotrophic activities to expand undifferentiated NSCs or to induce differentiation of NSCs into specific lineages. Once again, this array-based method was able to identify favorable composite materials for the growth and differentiation of stem cell populations. Overall, the analysis of different combinations of biomaterials and growth factors in a microarray platform can provide valuable insights into the mechanisms regulating stem cell fate and can provide important information for the development and design of scaffolds for cell-based therapies.

Microwell and microfluidic arrays in stem cell research

Soft lithography can be used to fabricate arrays of micro-wells with defined dimensions, allowing the size of cellular aggregates to be controlled (Figure 3). For example, the use of poly(ethylene glycol) (PEG) microwells as templates for directing the formation of embryoid bodies (EBs) offers an attractive solution for the control of certain parameters, such as size, shape and homogeneity of these aggregates, which are used for the expansion of human ES cells [59,60]. Despite promising results, this technology has been limited by difficulties in obtaining reproducible cultures of homogeneous EBs. However, improvements in material composition of the microwells, cell seeding procedures and retrieval of cellular aggregates from microwells have allowed the controlled generation of EBs for differentiation studies and high-throughput stem cell experimentation [61].

The combination of hydrogel matrix technology with microscale engineering has also been used to develop a microwell array platform on which the fate of NSCs and neurosphere formation can be followed, starting from a single founding cell [62]. Compared with conventional neurosphere culture methods on plastic dishes, the viability of single NSCs was increased twofold using this platform, and an effective confinement of single proliferating cells to the microwells led to the formation of neurospheres of controlled size. In addition, microwell microarrays can be used for parallel and quantitative analysis of single cells, so the study of clonal stem cells can be conducted more efficiently with microwells than with time-consuming microtiter-well-plate formats. Clonal microarrays can be constructed by seeding a population of cells at clonal density on micropatterned surfaces that have been generated using soft lithographic microfabrication techniques. Clones of interest can be isolated after they have been assayed in parallel for various cellular processes and functions, including proliferation, signal transduction and differentiation [63].

Microfluidics also presents the potential to influence stem cell research, particularly for high-throughput analysis of signals that affect stem cell fate. Microfluidic channels are normally formed through the casting of PDMS over a pre-fabricated mold. Using this method, a microfluidic device was developed to analyze ES cell cultures in parallel [64]. A logarithmic range of flow rates within channels was tested, and flow rate was shown to significantly influence colony formation. In another example, a micro-bioreactor array, containing twelve independent micro-bioreactors perfused with culture medium, was fabricated using soft lithography. This system supported the cultivation of cells, either attached to substrates or encapsulated in hydrogels, at variable levels of hydrodynamic shear, and automated image analysis detected the expression of cell differentiation markers [24]. Various conditions and configurations were validated for different cell types, including a mouse myoblast cell line, primary rat cardiac myocytes and human ES cells. In another example, a microfluidic approach was used to analyze gene expression of human ES cells at the single-cell level [65]. Total mRNA from individual single-cells could be extracted and cDNA synthesized on a single device with a high efficiency of generating cDNA from mRNA, thereby making it possible to profile single-cell gene expression on a large-scale.

High-throughput DNA and RNA systems for stem cell studies

High-throughput DNA and siRNA systems have also been developed, creating an efficient screen for the effect of genetic control elements on stem cell behavior. In an effort to study ES cell function, a subtractive library approach was used to identify the multiple genes (e.g. *zinc finger protein 42* [*Zfp42*], also known as *Rex-1*) involved in the regulation of expression of the master embryonic transcription factor octamer 4 (Oct-4) and of self-renewal [66]. This study indicated that RNAi screening is a feasible and efficient approach to identify genes involved in stem cell self-renewal and differentiation.

Microscale methodologies enable higher throughput and require less reagent than traditional microtiter well-plates. In one example demonstrating these advantages, human mesenchymal stem cells were cultivated on-chip and transfected with previously arrayed plasmid DNA with high efficiency and spatial resolution. The ECM protein fibronectin was found to be an essential factor for the efficient on-chip transfection of these cells [67]. Furthermore, the delivery of both siRNA and plasmid DNA could be performed in parallel, and a dose-response gene knockdown was observed. Concomitant with the development of small-molecule platforms, high-throughput RNAi screens are similarly emerging as important tools in cell and developmental biology, particularly in stem cell research [67].

Conclusions and future trends

The miniaturization of cell-based assays promises to have a profound impact on high-throughput screening of compounds by minimizing the consumption of reagents and cells. To achieve routine adoption of high-throughput cellular microscale platforms, future development will most certainly need to focus on automated, high-throughput methods for the study of cellular microenvironments and growth conditions in 3D environments [68]. In this regard, array-based formats have been developed that have proved useful as enhanced-

throughput platforms for 3D culture of various cell types [69,70], and much effort is now being directed towards cell culture models that better reflect *in vivo* function.

Cell–cell and cell–ECM interactions are important factors that control cell behavior, and the 3D architecture of the cellular niche has been shown to improve the quality of information obtained from *in vitro* assays [71]. Incorporating biosensing elements alongside the cell culture has been used for local detection of secreted cellular products, which further enhances the utility of cellular microarrays. This concept was demonstrated using hepatocytes and their secretion of liver proteins (albumin and α 1-antitrypsin) as a model system [72]. Liver-specific proteins secreted by hepatocytes were captured on antibody domains immobilized in the immediate vicinity of the cells, detected with an immunofluorescence assay and quantified using a microarray scanner. By using these tools, among others, to create more *in vivo*-like cultures and more sensitive detection methods, cell-based drug discovery and target validation is likely to be improved, and the ability of *in vitro* assays to predict the *in vivo* response will become more reliable. Such improvements are expected to lead to increased adoption and greater importance of high-throughput cell-based assays throughout biotechnology.

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Glossary

Biosensing element

an element, typically an antibody, placed alongside the cell spots to enable local detection of secreted cellular products, for example proteins

Cellular microarray

analytical platform consisting of a solid support, or chip, where small volumes of different biomolecules and cells can be displayed in defined locations, allowing the multiplexed interrogation of living cells and the analysis of cellular responses (e.g. changes in phenotype or secreted factors)

Embryoid body

cellular aggregate of embryonic stem cells that can be obtained *in vitro*. Upon aggregation, differentiation starts and the cells begin to recapitulate, at least in part, embryonic development. Cells of the three embryonic germ layers can be obtained from an embryoid body

High-throughput screening

a method of scientific experimentation that is particularly used in drug discovery and that is also relevant to the fields of biology and chemistry. High-throughput screening allows rapid analysis of thousands of chemical, biochemical, genetic or pharmacological tests in parallel. This process allows the rapid identification of active compounds, antibodies or genes that modulate a particular biological process

Microfabrication

generic term used to describe the process of fabrication of small structures, typically of micrometer sizes. The first microfabrication methods were used to fabricate semiconductor devices in integrated circuits. Advances have led to the application of microfabrication methods to other fields, such as biotechnology, where these methods are being re-used, adapted or extended

Microfluidic cell culture array

microfluidic device designed to allow the culture of cells in separate chambers with continuous perfusion of medium. This layout allows parallel screening of different culture conditions. Also referred to as a micro-bioreactor array

Neurosphere

cellular aggregate of spherical shape that is generated by neural stem cells *in vitro*

Robotic spotting

automated method of spotting different materials, biomolecules or cells onto solid surfaces

Soft lithography

refers to a group of techniques for microfabricating or replicating structures using soft materials. The term 'soft' denotes the use of elastomeric materials, most notably PDMS. Soft lithography is commonly used to construct features measured on the micrometer scale

Stem cell

a cell that has the ability to self-renew through mitotic cell division and to differentiate into a broad range of specialized cell types. Typically, stem cells are grouped into embryonic stem cells, which are isolated from the inner cell mass of the blastocyst in the developing embryo, and adult stem cells, which are found in adult tissues such as the bone marrow or adipose tissue

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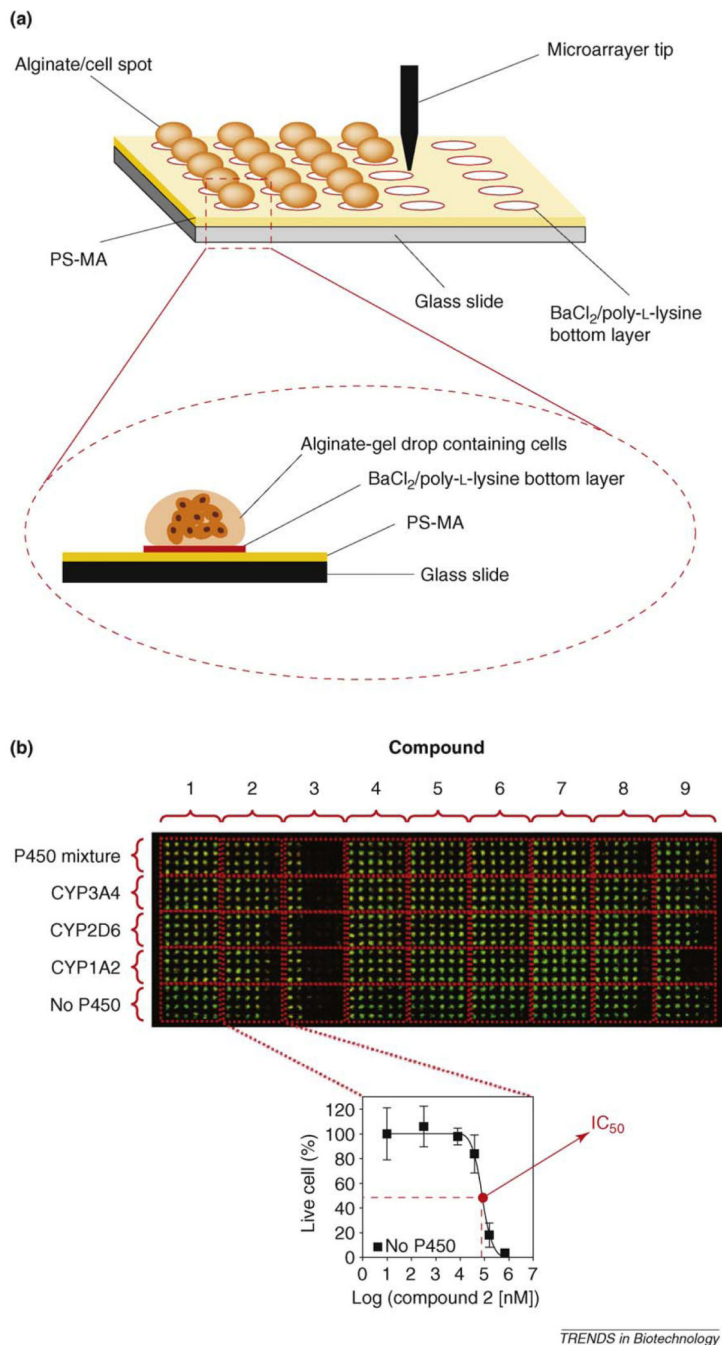


Figure 1. Schematic illustration of a high-throughput 3D cellular microarray platform. **(a)** Preparation of a 3D cellular microarray. Cells are directly spotted on functionalized glass slides using a microarray spotter. Cell encapsulation is the result of alginate gelation mediated by the presence of Ba²⁺ ions (see insert). Positively charged poly-L-lysine promotes attachment of the negatively charged polysaccharide constituent of alginate upon gelation, keeping each spot in its location. PS-MA: poly(styrene-*co*-maleic anhydride). **(b)** Use of a cellular microarray for high-throughput toxicology assays. The platform shown in (a) can be used for

direct testing of compound toxicity or for evaluating toxicity of P450-generated metabolites, as illustrated in the shown array. Dose–response curves are obtained from fluorescent staining of live cells, and IC50 values can be determined for each compound or for P450-generated metabolites (adapted, with permission, from Ref. [29]).

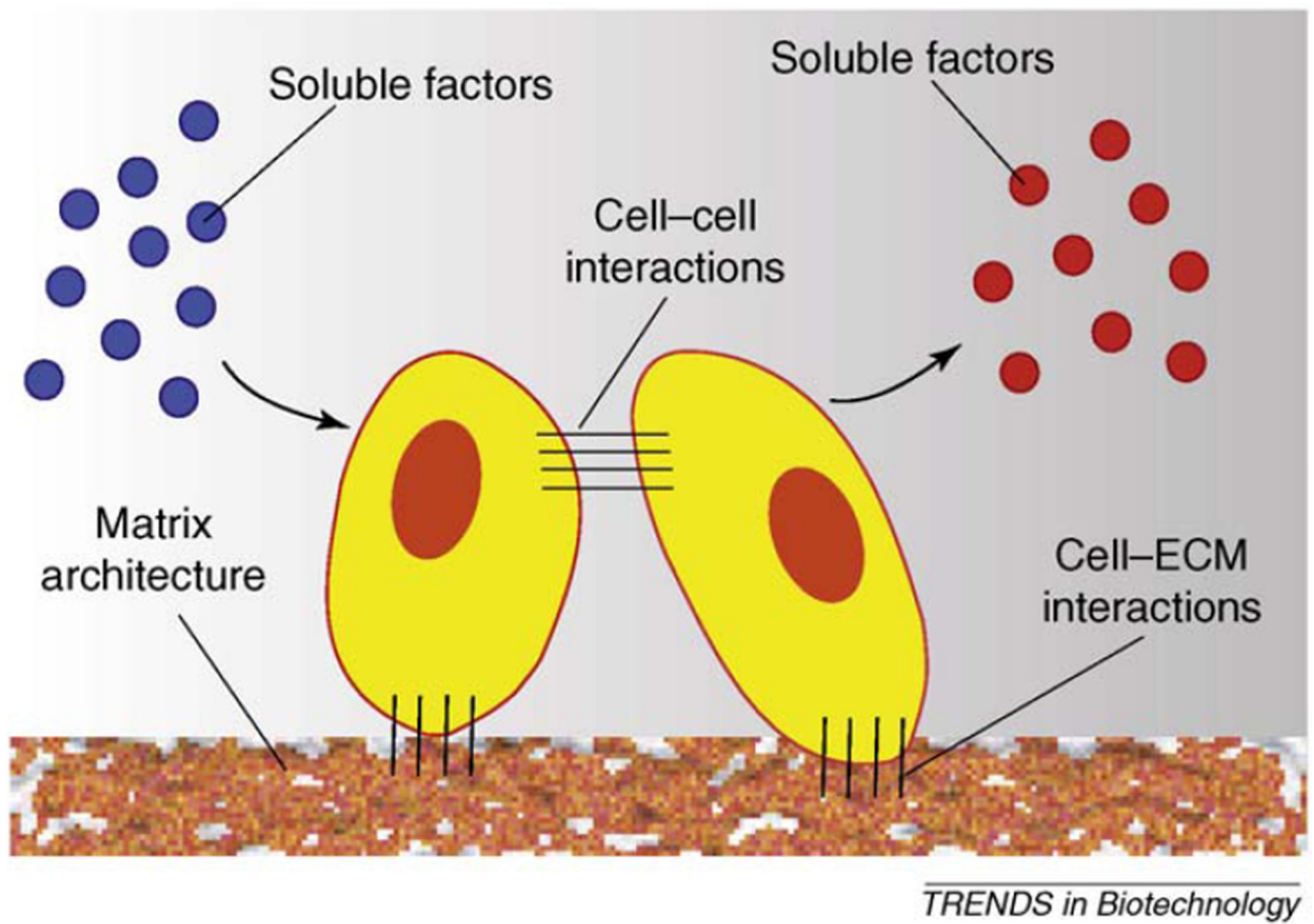
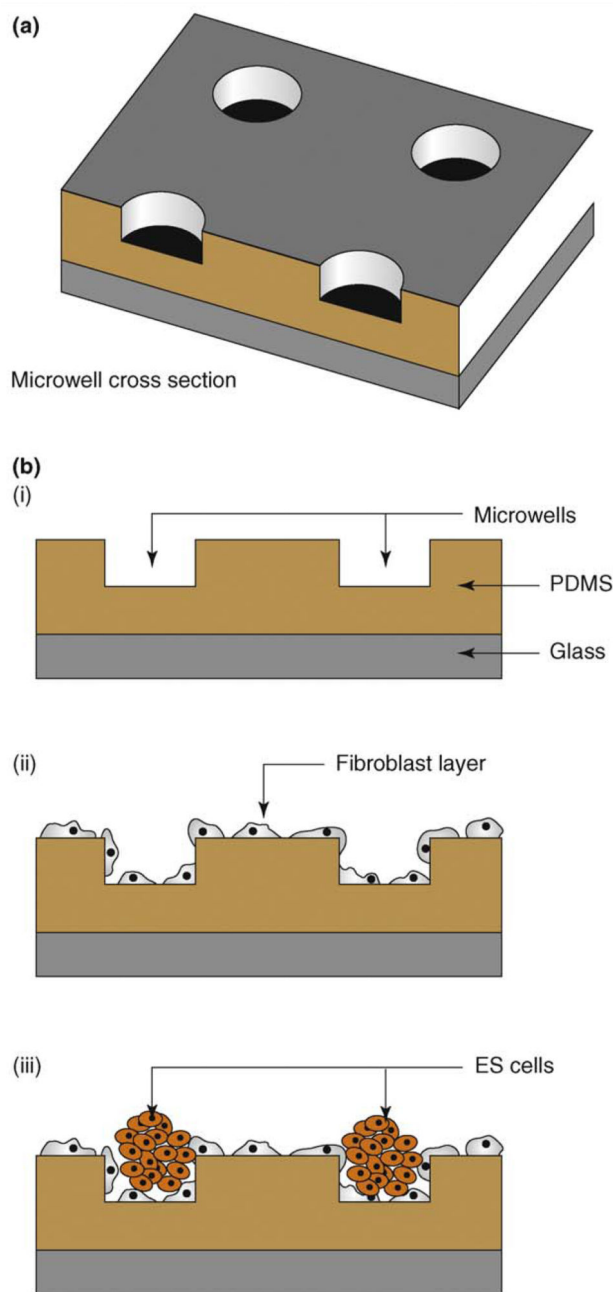


Figure 2. Factors affecting stem cell fate in the stem cell niche. These include physical cues, such as matrix elasticity and topography, cell–cell and cell–ECM interactions and soluble factors (e.g. growth factors and small molecules) that synergistically support cell growth and differentiation.



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Figure 3. Schematic representation of a microwell platform for controlling the size of cellular aggregates. (a) Cross section of a microwell platform. (b) the process of creating cellular aggregates. (i) PDMS is cured on a silicon master to produce microwell-patterned surfaces. (ii) Surfaces are treated with fibronectin and seeded with mouse fibroblasts, which grow into a monolayer. (iii) The confluent monolayer is then inactivated and human ES cells are

seeded inside the microwells, where they form cellular aggregates Adapted, with permission, from Ref. [59].

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Table 1

High-throughput cell-based screening methods in drug discovery and stem cell biology

Method for array fabrication	Application	Refs
Robotic spotting	Cellular microarrays for high-throughput toxicology assays	[29,37]
	Cellular microarrays for high-throughput compound screening	[41–43]
	Transfection microarrays for gene function screening	[44,67]
	Peptide and small-molecule microarrays for high-throughput cell-adhesion assays	[12]
	Cellular microarrays for the identification and characterization of multiple antigen-specific populations of T cells	[17]
	Biomaterial microarrays for microscale testing of biomaterial interactions with stem cells	[23,57]
	Extracellular matrix microarrays for probing embryonic stem cell fate	[52,53]
	Cellular microarrays of signaling microenvironments for adult stem cell differentiation	[55,56]
Photo-assisted and soft-lithography approaches	Microfluidic cell culture arrays for cytotoxicity determination and high-throughput cell-based assays	[31,38]
	Miniaturized, multiwell culture of human liver cells for drug development	[40]
	Protein surface arrays for modulation of T cell responses and stem cell growth and differentiation	[10,18,19,25,54,58]
	Microwell arrays for single-cell analysis and stem cell culture	[11,26,59,61–63]
	Microfluidic cell culture arrays for stem cell research	[24,32,64,65]