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Direct assembling methodologies for high-throughput bioscreening

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Abstract

Over the last few decades, high-throughput (HT) bioscreening, a technique that allows rapid screening of biochemical compound libraries against biological targets, has been widely used in drug discovery, stem cell research, development of new biomaterials, and genomics research. To achieve these ambitions, scaffold-free (or direct) assembly of biological entities of interest has become critical. Appropriate assembling methodologies are required to build an efficient HT bioscreening platform. The development of contact and non-contact assembling systems as a practical solution has been driven by a variety of essential attributes of the bioscreening system, such as miniaturization, high throughput, and high precision. The present article reviews recent progress on these assembling technologies utilized for the construction of HT bioscreening platforms.

Keywords

Assembling technologies; Bioengineering; Bioscreening; High throughput; Methods

1 Introduction

Recently, the screening of specific targets of interest as a versatile and effective tool has gained great interest in a diversity of biomedical applications. Bioscreening refers to the technique that entails the screening of biochemical/compound libraries for their activities against biological targets. With the increasing number of available compounds and molecular targets, miniaturization and high throughput (HT) have been becoming two important requirements for modern bioscreening and scaffold-free systems. The field of HT screening, which first appeared in the early 1990s, has been seen not only as an improvement to conventional screening systems, but also as an adaptation to various biomedical needs to lead to exciting discoveries [1]. In terms of the throughput, HT

screening is generally defined by the ability to screen the compounds to be tested at a rate of 10 000–100 000 per day, and ultra-HT screening (uHT) by screening in excess of 100 000 per day [1].

The HT and uHT scaffold-free bioscreening processes have been used for a wide range of applications, including but are not limited to:

1. *Drug discovery.* With the model of animal or bacterial cellular organisms screened along with chemical libraries, the combinatorial and multiparallel procedures act as a key element to expedite the drug discovery process for rapid identification of active ingredients for disease treatment. The sharp increase of therapeutic compounds and biological targets has led to rapid development of HT bioscreening. Patterning cell microarrays at HT have demonstrated great potential on screening drug candidates for efficacy and toxicity [2]. Studies have demonstrated the effectiveness of in vitro microarray fabrication for drug-screening applications [3–6]. Development of HT bioscreening techniques will enable companies to increase the probability of finding novel compounds of significant therapeutic and commercial value.
2. *Diagnosis* Routine clinical diagnosis can be improved by the implementation of HT bioscreening systems. With HT screening, there is the possibility of gaining more information using a single assay. Taking advantage of rapid combinatorial approaches, small volumes of the patient's samples (including saliva, blood, and urine) can be screened for more targets, thus resulting in less sample usage and more valuable information for an accurate disease diagnosis in the field of medical, environmental and food analysis. The use of HT technologies (inkjet) has already been reported for cancer diagnosis. Utilizing gold nanoparticle arrays and chemical detection of the cancer biomarker interleukin-6 (IL-6) in serum [7] provides appropriate detection limits, sensitivity and linear dynamic range for clinical settings. Moreover, immunochemical sensing devices have been demonstrated to be useful for pathogen detection in a paperfluidic and inkjet dispensing-based approach [8].
3. *Stem cells research* The combinatorial study of stem cells and their niche factors is an emerging area using HT screening technologies. For instance, using a robotic spotter, Flaim et al. [9, 10] have successfully identified the ideal combinations of extracellular matrices that can synergistically impact both hepatocyte function and embryonic stem cell differentiation. Anderson et al. [11] have developed an HT platform for rapid, nanoliter-scale synthesis of biomaterials and description of their relations with stem cells. Utilizing this new platform they have characterized over 1700 human embryonic stem cells/material interactions.
4. *Genetics* Advances in the genomics also demand the development of HT/uHT bioscreening technologies. For instance, applications such as DNA sequencing, gene expression and gene regulation analysis require the construction of high-density libraries for rapid analysis of genomic information.

Some fundamental attributes of the HT bioscreening platforms include the capability to manage small volumes of samples and reagents, less analytical time, operational adaptability, and easy automation and integration of functional components. To fill these requirements, appropriate assembling technologies for such HT and uHT screening platforms become critical. Over the last few decades, various methodologies for assay miniaturization and lab automation have been developed to achieve HT and uHT screening. These technologies play a vital role in modern drug discovery and other biomedical

applications. The present paper reviews these current and emerging technologies that have proven to be practical for HT bioscreening platforms.

2 Methodologies used to assemble HT screening platforms

Based on the general working principle, the available technologies for assembling HT screening platforms can be classified into two major categories: contact- and non-contact-based methodologies. Contact methods are technologies that establish direct contact with the reservoir and the deposition site, and non-contact methods are droplet-based. Such technologies have a dispensing volume ranging from attoliters to microliters. As depicted in Fig. 1, the contact-based methods include extrusion, pinned, micro-contact printing, magnetic assisted method, and dip-pen nanolithography (DPN)/atomic force microscopy (AFM)-assisted method. As shown in Fig. 2, the non-contact-based methods include nozzle-based inkjet printing (thermal, piezo-electric), laser inkjet printing, and electrostatic methods.

2.1 Contact-based methods

2.1.1 Extrusion, pinned and micro-contact printing—Combined with the well-plate technologies, extrusion-based methods are widely used to build the HT screening platforms. The extrusion method is based on robotic-controlled pressure-based pipettes to transport material. The implementation of 384- and 1536-well plates has been applied as one of the routine approaches for HT screening. Moreover, biological assays in 3456-well plates (total assay volume 1–2 mL) have been reported [12]. These new systems with a large number of wells offer a relatively higher throughput (up to 400 000 compounds/day) [12]; however, the expensive robotic systems usually required for dispensing the large number of the wells increase the cost of its utilization and development. During the last decade, many research groups have been working with alternative more economical extrusion systems [13–15], but, with regards to throughput, improvement over well-plate methodology is still very slow. Researchers are investigating other technologies that can exceed the currently used HT methods and downsize the volume significantly, for instance, to the range of attoliters in the case of AFM [16]. Some other emerging systems such as BioLP™ laser-based technology have also been developed for HT bioscreening [17]. We briefly review these methods in the following sections.

Pinned printing is one of the oldest material transferring technologies. Spots can be constructed utilizing pins loaded with the suitable solute and solvent. Pinned contact printing has the advantages of ease of transfer and the lack of small orifices in the system that can become blocked. The resulting spot size is determined by the size and shape of the pin used. This technique was first developed to fabricate an acrylate microarray on a poly(2-hydroxyethyl methacrylate) (pHEMA)-coated slide by Anderson et al. [11] using on-slide UV photo-initiated polymerization. High-precision contact-printing robots are also required to achieve the HT and high density level of screening. For instance, a pin-based robotic system (ORCA system) has been developed to deliver protein samples onto slides, yielding spots of ~150–200 μm in diameter in a high-density manner (1600 spots/ cm^2) [18]. More sophisticated robotic systems are under development to achieve higher throughput and density.

Micro-contact printing is extensively used to transport materials to surfaces [19], and has been used to create patterns of cell-adhesive proteins on solid surfaces using an elastomeric stamp primarily made from polydimethylsiloxane (PDMS) [20]. Due to the elastomeric nature of the PDMS, the stamp can be used to pattern non-planar substrates [21]. This technique has proved to be a powerful tool for creating micro-fabricated cellular patterns for stem cell research. By applying this direct contact methodology, specific cell-adhesive

molecules are coated onto the bottom of a PDMS stamp and then transferred to the substrate surfaces. The unfilled areas could be designed to repel stem cell adhesion either directly [22] or by further treatment [20]. As a result, the stem cells can only grow on the areas predefined by the micro-contact printing. This method offers a high resolution on the scale of 2–500 μm [21], allowing for the production of single-cell patterns. Its similarity with the pin contact technique arises from touching the source and the destination plates. However, with the micro-contact printing method uses a die instead pin, which enables the construction of more complicated arrays.

2.1.2 DPN/AFM-assisted method—DPN was first developed by Mirkin and coworkers [23–25]. It is a scanning probe microscopy-based nanofabrication technique that uniquely combines direct-write soft-matter compatibility with the high resolution and registry of AFM. As a result, it is a powerful tool for depositing soft and hard materials, in the form of stable and functional architectures, on a variety of surfaces [23]. This methodology might be considered as part of the pin or pen-like technology since it has the same working principle, nonetheless at smaller pin or tip dimensions. However, it is worth listing this technique separately due to its unique and promising characteristics. The major advantage is the miniaturized volume that this method can provide, down to the range of the attoliters. This mask-less dispensing system can operate under ambient environment and permit the direct creation of droplet arrays comprising single liquid droplets with volumes down to a few attoliters and sub-micrometric spacing [16].

Over the last 12 years, the DPN method has developed rapidly. Recently, Hong et al. [26] have applied this method to successfully transfer fragile molecular inks coated on an AFM tip to a solid substrate, at nanometer resolution (feature sizes as small as 15 nm). However, the regular DPN has a relatively low transportation speed. To address the issue of low throughput, two different approaches have been developed. The first applies special tips individually actuated by piezo-electric, thermoelectric, or electrostatic mechanisms, and the second utilizes a large numbers of passive tips [23]. Espinosa and coworkers [27–29] have developed a novel microfluidic AFM probe called the nanofountain probe (NFP), consisting of a hollow tip, integrated microchannels and an on-chip reservoir. This new probe possesses sub-100-nm patterning capability. When an ink solution is fed into the reservoir, it is driven by capillary action through the microchannel to the tip to form a liquid-air interface around the tip core. Molecules are transferred by diffusion from the interface to a substrate and a water meniscus is formed by capillary condensation [25–27].

2.1.3 Magnetic cell patterning—Another important technology for creating rapid cell arrays involves the “at-a-distance” physical manipulation of the cell through magnetism. This technique might be considered as a non-contact-based method but due to its inherent cell invasive nanoparticles we locate them in this section. This method uses biocompatible nanoparticles/beads embedded into the cells to achieve various arrays as a promising approach for HT applications. Basically, this patterning technique consist of a two-step process involving: (i) labeling the desired biological entity with magnetic material, and (ii) separating out these magnetic-embedded entities via a magnetic separation device. Recent progress has been made in the manipulation of biological entities, e.g., the isolation of T cells from human blood [30]. In addition, microdevices employing magnetic beads have been developed for immunoassays [31–33], droplet-based DNA purification [34], and active fluid mixing [35].

Initially, magnetic separation was restricted to slow accumulation rates [36]. However, alternative methods have emerged to overcome such limitations. In fluid-base magnetic manipulation, a HT method without any obstructions being placed in the column uses special field gradient systems, such as a quadrupolar arrangement that creates a magnetic

gradient radially outwards from the center of the flow column [33]. Further efforts have been put into building HT devices. Bu et al. [37] have reported a system based on an array of small neodymium-ferrous-boron (Nd-Fe-B) permanent magnets along with integrated permalloy elements, demonstrating improvement in the capture-efficiency rate of the magnetic beads, suggesting the capability of the technology to be utilized in HT sample preparation. Compared with other contact-based methods (e.g., micro-contact printing), recent studies have demonstrated that this method is a simple and rapid cell patterning technique, using magnetite nanoparticles and magnetic force. It is capable of allocating cells on arbitrary surfaces. Successful fabrication of patterned lines of single cells, where the number of cells seeded can be adjusted, and complex cell patterns (curved, parallel, or crossing patterns) have been realized [38]. Once the cell pattern is achieved it is important to maintain this pattern. Kamihira's group has labeled target cells with magnetite nanoparticles encapsulated in cationic liposomes (magnetite cationic liposomes, MCLs). Moreover, to promote cell attachment, an Arg-Gly-Asp (RGD) motif-containing peptide was coupled to the phospholipid of MCLs (RGD-MCLs). This resulted in improved cell proliferation [39]. More recently, the efficiency of the magnetic cell patterning have improved by using polyethylene glycol (PEG)-modified magnetite particles (PEG-Mags). As shown in Fig. 3, an array-patterned magnet was used for patterning of PEG-Mags on the surface of a tissue culture dish. The resultant substrate surface contained two different regions: the PEG-Mag surface to act as a cell-resistant region and the native substrate surface that promotes cell adhesion. When human keratinocyte HaCaT cells were seeded onto the PEG-Mag-patterned surface, cells adhered only to the native substrate surface, resulting in predesigned cell patterns on the tissue culture dish [40].

Although a variety of biocompatible magnetic nanoparticles have been investigated, concerns about cellular toxicity of the magnetic nanoparticles/beads and their magnetic effect on cellular functionality remain [41, 42]. Magnetic nanoparticles can participate in three well-recognized types of toxicity: chemical, biological, and physical [43]. The metals iron and cobalt, and their salts, especially oxides, from magnetic particles act chemically as inorganic toxins. Biologically, the potential transformation of cells into cancer cells due to the release of toxic particle coatings interacting with chromosomal DNA inside the cell represents a biological hazard. Physically, magnetic nanoparticles produce toxic effects while flowing through an artery (abrasion) or when being subjected to an alternating magnetic field (heat production). Moreover, the tendency of downsizing magnetic particles directly influences the toxicity effect since nanoparticles seems more toxic than microparticles [44].

2.2 Non-contact-based methods

HT bioscreening platforms can also be formed using techniques based on non-contact mechanisms. In general, the non-contact approaches are best suited for the production of vast arrays of varying materials, where different physical/mechanical mechanisms are used for the delivery of molecules.

2.2.1 Nozzle-based methods—The main nozzle-based technologies include thermal and piezo-electric inkjet printing methods [21]. These technologies use cartridges comprising various sets of micrometer-sized nozzles (orifice). Each nozzle has either a heater or piezo-electric element that ruptures the surface tension of the dispensing substance, creating droplets allocated onto a computer-assisted target. These methods are carried out in a non-contact manner, and can dispense tiny drops (in the range of picoliter in volume) onto a surface, but might require a dry substrate to ensure fidelity of printed patterns. Attractive features for inkjet technology include additive operation, the ability to process different materials (such as fragile organics or biological materials), flexible fluidic drawing/

patterning through a computer-controlled system, large-area printing, and cost effectiveness [45]. However, it loses control once the liquid has left the confinement of the ejection nozzle, consequently suffering from issues related to drying and spreading of the ink on the surface, and provides a relatively lower resolution (no smaller than 20–30 mm) [26]. In addition, another drawback is that only fluids with relatively low viscosity and low cell density can be printed [46].

Based on the method by which the inkjet droplets are formed, inkjet technology primarily includes the thermal and piezo-electric printing. Our group has pioneered the adaptation of the off-the-shelf thermal inkjet printer for biomedical applications [47, 48]. Inkjet printing technology has proven useful for creating viable cellular patterns with a recorded resolution of 350 μm at HT rate [21]. Recently, our group has evaluated the bioscreening capability of the thermal printing method in a cellular model [49], which evaluated *Escherichia coli* cells in the presence of different antibiotics. In this study, we focused on the development of thermal printing-based deposition method for drug screening by simultaneously placing therapeutic drugs and cells, at very small amounts (a few picoliters), onto target sites to fabricate cell/drug screening chips. In particular, using a modified Hewlett-Packard (HP) 5360 CD printer, droplets of GFP-expressing *E. coli* were deposited on an agar-coated coverslip chip under a small volumetric constraint (~ 180 pL) for each colony dot. Figure 4A shows a representative phase-contrast image of the printed *E. coli* spots. Figure 4B shows representative fluorescence and filtered images of the live cells on the printing spots. The viability and function of the printed cells (with no antibiotics) was recorded, and 98% viability and DNA functionality was maintained. Along with the bacteria, different antibiotics were patterned in such a way that the growth of the bacteria was evaluated in the presence of various antibiotics through a fluorescent live/dead assay. This technology has been reported as an HT process, printing 250 000 droplets/ s (or 18 000 000 compounds/ day) [47]. Due to the reduced volume, this method will increase the effectiveness of emerging drug screening processes by minimizing the resources required. The method promises to be a practical and economic bioscreening platform for future drug screening of new biochemicals.

Piezo-electric inkjet printing is another important method for rapid deposition of biomolecules. Recent work has demonstrated the drug screening capabilities of the piezo-electric inkjet printing technology using a Dimatix Materials Printer (DMP-2800, Fujifilm). This methodology, coupled with a simple and generalized detection method, was proposed for satisfying the requirements of rapid, low-cost, miniaturized (down to a single spot), and HT screening by easily spotting entire chemical libraries onto solid-supported biological targets. The main advantages of the piezo-electric system include the picoliter-scale volume (1–10 pL), high density, and HT (10 spots/s). More recently, Arrabito et al. [50] have applied this technology to drug screening applications. In their study, a model of substrate/ inhibitor (*D*-glucose/*D*-glucal) along with a simple colorimetric screening method was utilized to prove the bioscreening capabilities of this piezo-electric-based inkjet technology. Although this technology shows a promise with such capabilities for bioscreening, further studies are required to prove its applicability for larger applications of HT screening.

2.2.2 Laser-based deposition—Laser inkjet technology has gained attention because of its obvious advantages, such as precise delivery, HT, single-cell resolution, and ease of sterilization. In particular, since the laser printing is nozzle free, solutions with higher viscosity and higher cell density can be printed compared to thermal or piezo-electric inkjet technology. This system utilizes a pulsed laser beam through different layers to propel materials (e.g., cells) onto a receiving substrate. These layers usually include an adhesive laser transparent print ribbon that holds thin layers of biological materials on both sides (for further detail refer to the comprehensive review [51]).

Laser-induced forward transfer (LIFT), also called laser printing, was developed to transfer small amounts of various materials (including living cells) in 2-D or 3-D patterns. LIFT has previously been used for printing single to tens of cells simultaneously without any observable damage to pheno- and genotype [17]. Laser jet printing has successfully been used to generate cellular patterns either by printing cell-adhesive molecules for subsequent cell seeding or by directly depositing cells onto substrates with predesigned patterns in the form of a 2-D structure [52–56]. Barron et al. [57] and Othon et al. [58] transferred single cells, in a 3-D structure, using a layer-by-layer technique. The cells survived the transfer procedure with a viability rate of $98 \pm 1\%$ for skin cells and $90 \pm 10\%$ for hMSC, respectively. This study demonstrates that LIFT is a suitable technique for computer-controlled positioning of different, unharmed, cell types [46]. Regarding the resolution and volume of the droplets dispensed; droplet diameters as small as $10 \mu\text{m}$ have been reported and a corresponding volume of $\sim 12 \text{ fL}$ (femtoliters) per droplet can be obtained [59]. However, the major concern for this technology is the potential laser damage to the living cells, with their genetic material, when exposed to the laser at relatively high dosage.

2.2.3 Electrostatic-based dielectrophoresis—Recently, dielectrophoretic forces have been used to micro-pattern biological entities from DNA to cells [60–64]. The motion of suspended particles relative to that of the solvent resulting from polarization forces produced by an inhomogeneous electric field is defined as “dielectrophoresis (DEP)” [65]. Figure 5 depicts a regular DEP construction process for precise patterning of cells. Initially, the general use of DEP forces for patterning cells to fixed locations has not been extensively pursued [66], in part because DEP forces are inherently transient and disappear when the field is removed [67]. In addition, when DEP forces are weak, forces such as inter-particle attraction may dominate, resulting in particle aggregation instead of patterning [68]. Since living cells appear to tolerate high electric fields for relatively short times [69–71], the long-term biological experimentation requires a method to stabilize cell position in the absence of DEP forces. Recent strategies for restraining cell migration after active DEP localization include the incorporation of cell-adhesive proteins [72], or etched microwells [73]. Alternatively, encapsulation of patterned cells within hydrogel biomaterials [74] can further enable incorporation of cells that are non-adherent or require 3-D microenvironments to maintain tissue-specific functions [75–78]. Recent studies using DEP have reinforced the organization of 3-D architectures [60] to positively alter cellular responses. Rapid formation of cell clusters were recorded in 1–3 min. Moreover, this method has high reproducibility and high resolution and has been proposed as a platform for evaluating multicellular processes in regards to tissue architecture.

2.2.4 Molecular vapor deposition—The molecular vapor deposition (MVD) technique (MVD™ is a trademark of Applied MicroStructures, Inc.) was originally developed for the fabrication of micro-electromechanical systems (MEMS) [79]. This methodology comprises injectors that disperse the substance being dispensed into molecular vapor. Subsequently, these particles are directed to a designated position by electrical forces to give a patterned substrate inside a vacuum environment (see Fig. 2 for simplified scheme; for further details refer to [80]), resulting in the formation of high-resolution and high-density patterns in a repeatable fashion. The MVD technique has recently been used in the field of biomedical engineering. Jing et al. [79, 81] have demonstrated that this new technology is useful for cell assembly and patterning, and the immortalized mouse hypothalamic (GT1–7) neurons and mouse (MC3T3) osteoblast cells have been successfully patterned and maintained for at least 14 days in vitro. Furthermore, this technology is characterized by attractive features including: (i) single-cell resolution, (ii) easy alignment of the patterns with the pre-existing patterns on the substrate, (iii) easy formation of nanoscale patterns (depending on the exposure equipment), (iv) long shelf life of the substrate pattern prior to cell culturing, (v)

compatibility with conventional, inverted, optical microscopes for simple visualization of patterns formed on a glass wafer, and (vi) the ability to support patterned cell (osteoblast) networks for at least 2 weeks. Even though its throughput rate was not reported, its unique characteristics make this a promising technology for bioscreening applications.

3 Comparison of these assembling technologies for HT bioscreening platforms

The ideal conditions for an appropriate HT bioscreening platform include the ability to manipulate cells and biochemical agents at low volume, maintenance of cellular environments close to physiological conditions, high resolution and density capabilities, operational flexibility (such as easy automation and facile integration of functional components), low fabrication cost, size of printable molecules, and speed towards reduction of analysis times and project expenditure. Methodologies discussed above have their respective advantages and disadvantages, which are summarized in the Table 1. The development of HT bioscreening technologies can be adapted to different modalities depending of the area, the investment, and the technical requirements for the HT platform. For instance, areas and circumstances with low-resource settings would expect to apply relatively economic technologies without compromising the throughput and the speed.

4 Future perspectives

Much research has been conducted into developing the technologies for assembling HT screening and scaffold-free platforms. Discussed here are some of the more mature technologies with more relevant applications used in the on-going research into developing HT bioscreening methodologies. However, the door remains open for emerging technologies to line up with the ones described here.

There are several challenges associated with technologies for direct assembling of HT screening platforms. For drug screening, one of the key challenges consists of efficient loading of drug candidates on top of cell arrays [2], some other challenges included, but are not limited to: cross-contamination restricting spot density, construction of 3-D arrays that better mimic in vivo conditions, rapid collection of the data screened, and adaptability of the processes for different applications.

The development of contact- and non-contact-based methodologies for HT bioscreening and scaffold-free systems is expected to continue. Although each method has its own limitations to overcome (see Table 1), in general these methods are expected to move towards rapid automation, increase of the number of the assays, and the improvement of the screening quality. Although some of the technologies described here have being investigated as actual options for HT screening platforms (such as the case of inkjet methods), there are some others (e.g., AFM) that may need further development to fully establish their assembling capability for HT bioscreening platforms used in rapid drug discovery, diagnosis, and genetic medicine. At present, the bioscreening industry is inclined to use the contact mechanisms due to the vast amount of previous research as well as many commercially available products focusing on the contact technologies. However, the advantages, such as lower volume, higher-throughput and higher density, offered by the non-contact technologies are attracting further development and promise a wide range of applications for the industry in the future. Therefore, it is foreseeable that these technologies will be fully validated (e.g., with Federal Drug Administration approval) and prove their convenience in profitable applications.

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Abbreviations

AFM	atomic force microscopy
DEP	dielectrophoresis
DPN	dip-pen nanolithography
HT	high-throughput
PEG-Mags	PEG-modified magnetite particles
uHT	ultra-HT

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Figure 1. Schematic of the contact-based assembling methodologies for the HT bioscreening platforms. **(A)** Pressure-based methods (extrusion), **(B)** pinned/micro-contact printing- based methods, including a fluorescence micrograph of an array of HEK293K cell clusters expressing eGFP. Scale bar 700 μ m (reproduced with permission from [82], Nature Publishing Group). **(C)** Magnetic nanoparticles/beads-based method. **(D)** DPN, including an anti-p24 IgG protein nanoarray and topography trace of absorbed anti-p24 on mercaptohexadecanoic acid (reproduced with permission from [23], Nature Publishing Group).

Figure 2.

Schematic of the non-contact-based assembling methodologies for the HT bioscreening platforms. **(A)** Nozzle (orifice)-based method. **(B)** Laser-based inkjet technology, including an optical microscopy image of BSA-containing microarray with droplets of 10 μm in diameter. Scale bar 100 μm (reproduced with permission from [59], Elsevier). **(C)** Electrostatic based array formation, including a microarray of embedded fibroblast clusters. Scale bar 100 μm (reproduced with permission from [60], Nature Publishing Group). **(D)** Molecular vapor deposition illustration.

Figure 3.

Magnetic patterning of HaCaT cells using PEG-Mags. (A) Procedure for magnetic cell patterning using PEG-Mags. (B) A 96-well magnet plate was placed under the tissue culture dish, and PEG-Mags were added to the dish. Subsequently, HaCaT cells were seeded into the dish. After a 24-h culture, the magnet plate was removed, and the dish was washed with PBS to remove PEG-Mags on the dish. (C) Photograph of the tissue culture dish after the addition of PEG-Mags. (D) Crystal violet staining after cell seeding. (E) Representative photomicrograph of a border of the monolayer of cells in (D) (reproduced with permission from [40], Wiley)

Figure 4. Representative image of the thermal inkjet fabricated bioscreening spots of *E. coli* suspension. (A) Left spot, ~180 pL deposited dot, and right spot, ~500 pL deposited dot. Scale 4×100 . (B) Point size 3 printed. Left, fluorescence image showing viable cells; center, regular filtered image; right, modified picture to quantify cells on single spot (~23.5 cells/spot on average). Scale 10×100 .

Figure 5.

DEP fabrication method. (A) Cells in prepolymer solution are introduced into the transparent chamber (1) and localize via DEP forces to micropatterned gaps in the 1.8- μm -thick dielectric layer upon application of the alternative current chamber bias (2). Next UV light exposure polymerizes the hydrogel (3), embedding cells in a stable microorganization. The DEP all patterning (DCP) hydrogel can then be removed and cultured (4) or incorporated into multilayer constructs by expanding the chamber height and repeating steps 1–3; each layer may contain distinct cell organization, cell type and hydrogel formation (5, 6). (B) Electric field strength is high above circular gaps in the dielectric layer arranged in a hexagonal array (100 μm spacing), as shown by finite element modeling (CFD Research Corp.) (C) Microarray of embedded fibroblast clusters. Scale bar 100 μm (reproduced with permission [60], Nature Publishing Group).

Table 1

Comparison of the different assembling technologies used for HT bioscreening platforms

Technology	Advantages	Disadvantages	Resolution/density	Typical applications
<i>Contact methods</i>				
Pinned/micro-contact printing	High density and high precision Easy transfer Less blockages	Need of many pins to meet HT Mechanical stresses due to the physical contact	150–200 μm /16 spots/ mm^2 [18]	Materials transportation Array construction [11]
Extrusion	Controlled volume Validated technology for decades	Expensive robotic and infrastructure required	2–500 μm [21]/~30 k spots/ mm^2 (hiScan by Illumina®)	Materials transportation Cell/biomaterials 3-D assembly [14] Sequencing
Magnetic	At-a-distance manipulation High throughput Complex patterns formation	Magnetic nanoparticles may have cytotoxicity Substrate building is time consuming (need to fabricate the die-based substrate)	130 μm /25 spots/ mm^2 [38]	Immunoassays [24–26], droplet-based DNA purification [27], active fluid mixing [28]
DPN-AFM	Attoliter scale volume control Nano-resolution and high density	Restricted detection limits Lack of massive multiplexing capabilities [23]	15–100 nm [23, 26]/1M spots/ mm^2 [23]	Materials transportation Material characterization (e.g., phase separation) [23] Single virus particle control Antibody screening for HIV-1 [83]
<i>Non-contact mechanisms</i>				
Inkjet	Low investment HT Fair precision Picoliter scale volume control Additive operation	Obstruction and blockage Drying and spreading of the ink Limited to low-viscosity materials	15–50 μm /64 spots per mm^2 [50]	Drug screening, biosensing, environmental monitoring, tissue engineering [50]
Laser based	Printing of high-viscosity materials Ultra HT High resolution and density Additive operation	Laser source might have adverse effect on cellular genetic material	1–3 μm [51]/100 spots/ mm^2 [59]	Tissue engineering, cell selection studies, cell-based biosensors, stem cell engineering [51]
Dielectros-phoresis	High precision and high density	Slow throughput DEP forces are inherently transient Substrate building is time consuming (need to fabricate the die-based substrate)	~20 μm / 100 spots/ mm^2 [60]	Cell/biomaterials 3-D assembly, HT arrays, manipulation of biological particles (DNA, viruses, cells) [60, 67]
Molecular volume deposition	Single-cell resolution Complex patterns formation	Unreported throughput Substrate building is time consuming (need to fabricate the die-based substrate)	5–10 μm /100 spots/ mm^2 [72]	Cell-based sensors [84, 85], neurobiology, tissue engineering, patterning on chips (protein, cells) [79]