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High-Throughput Approaches for Screening and Analysis of Cell Behaviors

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Abstract

The rapid development of new biomaterials and techniques to modify them challenge our capability to characterize them using conventional methods. In response, numerous high-throughput (HT) strategies are being developed to analyze biomaterials and their interactions with cells using combinatorial approaches. Moreover, these systematic analyses have the power to uncover effects of delivered soluble bioactive molecules on cell responses. In this review, we describe the recent developments in HT approaches that help identify microenvironments allowing reproducible control over cellular behaviors and highlight HT screening of biochemical libraries for gene delivery, drug discovery, and toxicological studies. We also discuss HT techniques for the

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analyses of cell secreted biomolecules and provide perspectives on the future utility of HT approaches in biomedical engineering.

Keywords

High-throughput system; Cell-biomaterial interactions; Cellular microenvironments; Biomolecule delivery; Biomaterial screening; High-throughput biosensor

1. Introduction

The last decade has seen the rise of an unprecedented wealth of biomaterials and techniques to modify them in efforts to mimic the complicated microenvironments of native tissues. The developed biomaterials have been widely utilized in the tissue engineering field to try to create artificial organs and functional tissue constructs for both therapeutic applications and basic biological studies [1]. For advanced tissue engineering, comprehensive and insightful screening platforms are desired to engineer new biomaterials that recapitulate complex microenvironments present during development, homeostasis, and healing [2–4]. In particular, researchers have had a great interest in investigating the effects of the chemical and physical property of biomaterials, delivered bioactive molecules, or other microenvironmental stimuli on cellular function and fate. However, conventional approaches are not capable of efficiently screening the vast number of potential microenvironmental parameter combinations.

In recent years, researchers have developed high-throughput (HT) approaches that enable the analysis of hundreds to thousands of interactions among biomaterials, biomolecules, and cells on the same platform for drug screening, toxicology, and investigation of cellular responses [5, 6]. Compared to conventional approaches, HT strategies require small amounts of input biomaterials and cells, expedite analysis procedures, and enable combinatorial testing of multiple factors on a single platform.

Here, we review the most recent advances in HT approaches for microenvironment modulation, delivery of bioactive molecules and analysis of resulting cellular responses. We will group the HT platforms into three categories with their specific applications (Fig. 1). First, 2-dimensional (2D) and 3-dimensional (3D) HT approaches for the investigation of cell-biomaterial interactions and corresponding diverse cellular responses will be presented. Second, HT delivery of bioactive molecules of genes, proteins and drugs to cells and screening their responses after the delivery of molecules will be introduced. Lastly, HT analysis of gene expression and cellular production of carbohydrates, enzymes and proteins will be reviewed. Current challenges of HT platforms will be briefly highlighted along with future perspectives.

2. High-throughput microarray strategies for controlling cellular behaviors

Generally, cellular microenvironmental signals can be categorized into biochemical and physical cues [11]. Soluble growth factors, the biochemical composition of ECM, and cell-secreted proteins are examples of biochemical cues. Physical signals include substrate

mechanical properties, substrate topological patterns and applied mechanical/electrical stimuli. These microenvironment signals can interact to affect cellular behaviors including apoptosis, proliferation, migration, and cell differentiation depending on the combination and spatiotemporal presentation of microenvironmental signaling elements [12–18]. In this section, we introduce HT approaches to investigate the complex combinatorial effects of 2D and 3D microenvironments on cell behavior.

2.1. 2D cell-biomaterial interactions

Recent developments in biomaterials have led to promising technologies for disease diagnostics and therapeutic applications such as tissue engineering. For instance, biocompatible advanced biomaterials have been applied to stem cell-based therapies with the ability to control cell differentiation [21–23]. Since conventional approaches are inefficient to discover the optimal biomaterial composition for a specific application from a large number of candidates, HT approaches that can investigate the effects of a biomaterial's chemical and physical properties on cell adhesion, proliferation, differentiation and interactions with other cells in 2D culture systems have been utilized [24, 25].

On 2D cell culture substrates, cells interact with the material surface through only where they anchored, and their apical side is exposed to cell culture media. Although cells on 2D substrates do not sufficiently mimic physiological conditions in 3D native tissues, simplification of complex 3D cellular interactions into 2D models has been pursued as a way to initially unveil cellular behaviors in response to their chemical and physical microenvironments. 2D HT screening platforms combined with microarray fabrication technologies provide a facile way to generate a myriad of biomaterial combinations having different physical and biochemical properties that affect cell behaviors in diverse ways.

Stem cells have become attractive targets for regenerative medicine and the development of disease models due to their self-renewal ability and pluripotent properties [26–28]. Since microenvironmental cues including stiffness and ECM composition are known to affect stem cell function and differentiation [11, 29–31], screening interactions between biomaterials and stem cells is a valuable tool to predict stem cell fate. Flaim et al. studied the influence of soluble growth factors and ECM components of biomaterials on stem cells, using a combinatorial HT platform [32, 33]. Various mixtures of collagen types I, II, IV, laminin and fibronectin were spotted by using a commercial DNA spotting machine. Primary rat hepatocytes and mouse embryonic stem cells (ESCs) were seeded and cultured onto the microarrays for 48 hours in cell culture media containing different growth factors to monitor the cellular function of hepatocytes and stem cell differentiation toward an early hepatic fate. Using the developed 2D HT platform, they identified optimal ECM combinations that promote hepatocyte function and differentiation. Similarly, Mei et al. established a HT screening platform to examine the behavior of human ESCs in response to a library of copolymer microarrays composed of 496 different acrylate-based-monomers spotted on poly(2-hydroxyethyl methacrylate)-coated glass slides [34]. The effects of biomaterial properties, surface topography, surface chemistry, surface wettability and elastic modulus on cell behavior were investigated by using the developed HT platform (Fig. 2a). It was observed that polymer composition with high acrylate content allowed moderate wettability

and bioactive molecule absorption, which promoted colony-formation of the human ESCs (Fig. 2b). Gobaa et al. developed a HT screening platform using polyethylene glycol (PEG) hydrogel microwell arrays with tunable stiffness (shear moduli of 1–50 kPa) to study the effects of cell-biomaterial interactions on cell differentiation of mesenchymal stem cell (MSCs) and neural stem cells (NSCs) [35]. To form hydrogel microwell arrays coated with different ECM compositions, a microfabricated silicon stamp was utilized (Fig. 2c). Different ECM compositions were dropped onto the tips of silicon stamp posts using a DNA spotter with solid pins. Then, the ECM molecules on silicon stamp tips were directly contact printed onto a thin and partially cross-linked PEG hydrogel layer by applying pressure. The microfabricated stamp tips were then removed after fully curing the PEG hydrogel, resulting in the formation of microwell arrays with different ECM compositions. By using different concentrations of PEG, the stiffness of the hydrogel could be precisely controlled. Consequently, PEG hydrogel microwell arrays having different stiffnesses and ECM compositions could be obtained. Adherent MSCs or nonadherent NSCs were trapped in the generated microwells with isolated artificial niches via gravitational sedimentation to screen stem cell behaviors. The HT platform was used to demonstrate that stem cell fate was correlated to cell density, elastic modulus and ECM protein concentration (Fig. 2d, e).

The 2D HT platforms utilizing micro-contact printing enabled the fabrication of isolated microenvironments with different ECM components. Recently, wettability patterning techniques have received attention as they easily allow 2D patterning of cells and biomaterials not only for the investigation of 2D cell-biomaterial interactions but also for the analysis of cell-cell crosstalk via cell-secreted biomolecules [35–42]. Efremov et al. fabricated a microarray of hydrophilic spots surrounded by superhydrophobic borders to investigate the cell-cell interactions between adjacent different cell types in a HT manner [43]. The patterning of highly hydrophilic and superhydrophobic regions enabled the spatial placement of distinct cell types while maintaining physical separation. This co-culture microarray platform could be used for HT monitoring and analyzing crosstalk between distinct, separated cell population types in close proximity but without direct contact through cell-secreted molecules. Similarly, Popova et al. recently developed a miniaturized HT platform with wettability patterns to make the platform compatible with conventional fluorescence microscopy imaging technologies (Fig. 3a) [44]. Various cell types including HEK 293, HeLa, and A549 were seeded onto the hydrophilic patterned region to investigate cellular behaviors (Fig. 3b). Cell inoculation density could be controlled by varying the size of the superhydrophilic pattern and the volume of cell suspension droplets. Wettability patterns on the platform allowed a facile method to change media without pipetting. Cell culture media could be entirely changed by pouring fresh media onto the platform. The fresh media would wash away the old media that was located on the hydrophilic patterned areas. 2D cellular behaviors with delivered biomolecules could then be investigated using conventional fluorescence microscopy imaging methods. Recent findings suggest that the geometric features of cell adhered micropatterns also regulate cellular behaviors. Lee et al. found that peripheral geometry of micropatterned hydrogels regulated the activation of cancer stem cells (CSCs), which might help to investigate cancer metastasis [45]. They observed that micropatterns played a crucial role for a population of CSCs, which increased tumorigenicity. CSC markers were increased when the shapes of microhydrogels became

complicated, mainly due to the localized convex and concave features at the periphery of patterns. They used complicated photo-lithography and soft-lithography techniques to fabricate microhydrogel patterns. However, wettability patterning technique can provide more facile way to design 2D HT platforms to study the effects of biomaterial geometric features on cellular behaviors as microarrays of individual hydrogels with complex shapes can be obtained without a complex fabrication process [46].

Understanding of cell-biomaterial adhesive interactions is crucial to design novel biomaterials that match application specific requirements. For some implantable biomedical devices, surface biofouling should be prevented as the adsorption of cell-adhesive proteins onto artificial grafts may cause complications such as thrombosis [49]. In contrast, cell adhesion to biomaterials through, for example, adsorption of serum proteins, is crucial for cell survival for tissue engineering applications [50, 51]. Taylor et al. developed a HT platform to study protein adsorption to biomaterials [52]. Synthetic polymer libraries consisting of 576 polymers with different chemical compositions were printed on a glass slide. The adsorption of protein to each polymer was evaluated using fluorescently labeled fibronectin. This platform allows the rapid screening of synthetic biomaterial coatings that may enhance cell adhesion. Similarly, a synthetic biomaterial microarray platform was developed to screen cell interactions with surface-bound bioactive proteins [53]. Heterobifunctional linkers having thiol- and alkene-functional groups were arrayed onto a glass substrate. A PEG hydrogel layer was coated onto the glass substrate and peeled off to transfer patterned linker arrays. The transferred linker arrays can be quickly and reliably modified with bioactive molecules, such as peptides via thiol-ene chemistry. Consequently, peptides with a functional group that can react with thiol-ene chemistry could be directly attached to the PEG hydrogel's surface. The thiol-ene chemistry allows robust covalent coupling of a variety of bioactive molecules to the PEG hydrogel with either minimal or no modification of the binding molecules. Orner et al. developed a HT micro-patterned peptide array to study selective cell adhesion on biomaterials [54]. To investigate the cell-specific binding peptides, self-assembled monolayers were patterned on a gold substrate, followed by the immobilization of peptide ligands. It was found that the cell adhesion of neuroblastoma cells and fibroblasts was highly depended on the type of patterned peptides. This 2D HT approach is helpful for identifying the adhesion preference of different cells to cell-binding peptides, which could be utilized for implantable biomedical devices that require adhesion of specific cell populations.

As evidenced by the aforementioned examples, monitoring cell behaviors on 2D HT platforms can be used as an effective tool to screen cellular behaviors on biomaterials (Table 1), and results from these studies may be applied to applications where the engineering of the interface between cells and biomaterials is important.

2.2. 3D cell-biomaterial interactions

2D culture platforms do not entirely mimic the complex 3D physiological microenvironments of tissues in the body, which may be necessary to permit better regulation of cell function. For instance, it was recently observed that an engineered biomimetic 3D cell culture system not only promoted pluripotency of mouse ESCs but also

accelerated somatic cell reprogramming of fibroblasts to induced pluripotent stem cells better than a 2D culture conditions [55]. These different cell behaviors in 2D and 3D are mainly attributed to the way of interactions between cells and their surrounding microenvironments. In 2D cell culture systems, cell-cell and cell-ECM interactions via receptors and integrins of cells are limited as partial part of cells are anchored on a flat surface and cell-cell contacts are existed only at the shared periphery. On the other hand, in 3D culture systems, cells interact with their cellular microenvironments similar to the native tissue, results in different behaviors and functionality of cell [56, 57]. Thus, design and development of tissue-like 3D microenvironments are crucial to understand cell-cell and cell-tissue interactions properly [58, 59].

To resemble native 3D microenvironments, hydrogels have been actively studied for the last several decades because of their biocompatibility and ease of material modification to mimic native ECMs [60, 61]. A myriad of hydrogels with different chemical and physical properties have been developed to modulate cellular behaviors in 3D. Although it has been proven that engineered 3D microenvironments of hydrogels can synergistically affect cellular behaviors, conventional approaches are inefficient to screen and characterize the vast number of material combinations. Therefore, 3D HT platforms that can screen cellular behaviors in hydrogels have been considered as a promising technology for the development of new biomaterials for tissue engineering. Dolatshahi-Pirouz et al. developed a microarray of 3D cell-laden hydrogels to systematically study hMSC differentiation in response to multiple combinations of ECM proteins and growth factors [39]. For the screening of microenvironmental factors, cell-laden gelatin methacryloyl (GelMA) hydrogels containing combinations of ECM proteins (i.e., laminin, fibronectin, and osteocalcin) were microspotted and exposed to osteogenic bone morphogenic proteins (i.e., BMP-2 and BMP-5) with or without osteogenic induction media (Fig. 4a). Using this system, material usage could be decreased 1,000-fold compared to conventional assays. This approach identified optimal compositions of multiple combinations of ECM and growth factors that promoted alkaline phosphatase expression and osteogenic differentiation. Similarly, Ranga et al. printed ESC laden-PEG hydrogels to investigate cellular behaviors of ESCs encapsulated in 3D PEG hydrogels (Fig. 4b) [62]. Various microenvironmental factors, such as hydrogel stiffness and matrix-metalloproteinase (MMP) sensitivity, cell density, ECM proteins, cell-cell interactions, and soluble biomolecules were comprehensively screened using the developed HT platform. Systematic analysis demonstrated that pluripotency of mouse ESCs was highly dependent on specific soluble biomolecules (i.e., leukemia inhibitory factor, BMP-4, and fibroblast growth factor-4) and hydrogel stiffness.

Although the discussed hydrogel microarrays offer HT analysis of cellular behaviors in multiple microenvironments, they did not incorporate several *in vivo* environmental cues such as electrical and mechanical stimuli. Electrical stimulation can affect proliferation, alignment [63], maturation [64] and stem cell differentiation [65] of cardiac and neuronal tissues. In addition, Jin et al. recently found that biphasic electrical stimulation could significantly enhance *in vitro* and *in vivo* direct cell conversion efficiency of fibroblasts to functional neuronal cells, which means electrical stimulation could affect cellular behaviors in diverse ways [66]. It has been shown that the differentiation of MSCs into cells that form the load bearing tissues such as bone and cartilage is influenced by mechanical stimulation

[67–69]. Despite the significance of electrical and mechanical stimuli on regulating cellular behaviors, systematic HT investigations into these environmental stimuli have remained scarce. Moraes et al. developed a HT microarray capable of applying cyclic compressive strain onto 3D hydrogels [70]. Using this platform, the compression across 3D PEG hydrogels could be manipulated from 0 to 26 % (Fig. 5a). To simulate individual cell deformations under the applied compression, finite element model simulations were carried out. The simulation results showed that nuclear and cellular deformation of mouse MSCs was not linearly correlated to the applied compressive strain in 3D hydrogels because of the different stiffnesses of the hydrogel and cells (Fig. 5b). Recently, Liu et al. developed a microfabricated array platform that enables dynamic stretching of 3D cell-encapsulated biomaterials to identify the relationships between mechanical stretching and cellular responses [71]. Similarly, Li et al. fabricated magnetically actuated cell-laden hydrogel arrays for HT screening of fibroblast and myoblast behavior, including cell spreading, proliferation, and differentiation, under different static strains in 3D [72]. An advanced HT biomechanical stimulator system with strain sensors was developed to measure the applied strain in real-time (Fig. 5c) [73]. Carbon nanotube-based strain sensors were fabricated onto stretchable elastomeric membranes. When compression was applied to the membrane, membrane deflections were effectively detected by the resistivity changes of carbon nanotubes, although there was an asymmetry of measured strain between loading and unloading pressure in the biomechanical stimulator (Fig. 5d).

Although the current 3D HT biomechanical stimulation platforms could screen cell behaviors under different compressive strain regimes (Table 1), HT screening platform for the investigation of potential combinatorial effects of delivered bioactive molecules, ECM compositions, and mechanical/electrical stimuli have not been developed. The development of integrated HT platforms that can screen complex biochemical and physical microenvironments simultaneously would be valuable as they may provide insights into the interactions of these stimuli in directing cellular behavior.

2.3. 3D cell microtissues for cell behavior control

Hydrogels with tunable material properties can resemble native tissue's 3D microenvironments. Nonetheless, realization of the ideal hydrogel-based 3D cell culture system is not simple as there are many other complex signaling pathways and dynamic cell-ECM interactions, which are only partially understood, that can affect cell behavior. Consequently, 3D cell microtissues, such as cell spheroids, have received great attention in attempts to create more *in vivo*-like 3D model system [74–76]. It has been investigated that 3D spheroid systems often promote increased growth factor secretion, proliferation, and differentiation compared to 2D culture platforms, which can allow enhanced therapeutic efficacy [77–83]. Traditionally, spinner flask and hanging-drop cultures have been predominantly used to develop 3D spheroids. The spinner flask method enables generation of a large number of spheroids at a time, however, the size distribution of spheroids is often not consistent, which can result in different cell behaviors between the spheroids. Although more uniform spheroids can be obtained from hanging-drop culture or other static pellet culture approaches, the low-throughput nature of these methods limits the number of experimental conditions that can be examined at one time [84, 85]. Thus, high-throughput

production of 3D spheroids with uniform size and functionality is highly desired for potential advanced cell-based therapies.

Hydrophobic microwells can be used to obtain uniform 3D cell spheroids in a HT manner [86, 87]. Babur et al. reported the HT manufacturing of 3D cell spheroids using microwells to study the differentiation of human MSCs (hMSCs) (Fig. 6a) [88]. The HT microwell permitted the formation 12,000 hMSC spheroids at the same time, and the osteogenic and chondrogenic differentiation of 3D hMSC spheroids was investigated by varying the cell culture media composition and duration during a 14 day-culture period. It was observed that osteogenic or chondrogenic differentiation in the spheroids was induced after 8 days of culture in osteogenic media and chondrogenic media, respectively, and differentiation pathway could be altered by changing differentiation induction media. This HT microwell approach could produce sufficient cell spheroids for the construction of micro-tissues. Osteochondral tissue with defined distinctive bone and cartilage regions could be fabricated via co-culture of pre-differentiated bone-like and cartilage-like spheroids (Fig. 6a). The engineered tissue constructs could be used as osteochondral models for potential therapeutic applications and *in vitro* drug screening platforms. Similarly, a hydrogel-based HT microwell platform has been applied to rapidly form multicellular hMSC spheroids with controllable size and to sustain the delivery of growth factors from the microwells to increase their therapeutic efficacy [89]. Bone morphogenetic protein 2 (BMP-2) containing oxidized methacrylated alginate-PEG hydrogels were used to fabricate microwells. The sustained release of BMP-2 from the microwell resulted in spheroid mineralization within the microwells.

Numerous microfluidic systems have also been developed as new 3D cell spheroid culture platforms for both basic biological studies and drug discovery. Microfluidic cell culture systems can mimic various important features of native biological microenvironments that are challenging to replicate with conventional cell culture platforms [90–92]. HT microwell approaches combined with microfluidics provide a facile way to manipulate spheroid chemical microenvironments by flowing cell culture media with soluble biomolecules, such as growth factors and nutrients during cell culture (Table 1) [93]. Occhetta et al. designed an HT microwell-integrated microfluidic platform that allowed for the culture of hMSC spheroids under continuous flow perfusion [94]. Using this platform, uniform 3D cell spheroids were obtained within 3 hours, and the behavior of hMSCs in the spheroids was studied in response to delivery of various bioactive molecules. The proliferation of cells in the hMSC spheroids in the HT microfluidic platform was significantly increased compare to traditional static pellet culture. Chondrogenic differentiation of 3D hMSC spheroids was studied by varying the concentration and combination of supplied growth factors. Similarly, Frey et al. also developed a HT screening platform to investigate 3D cell spheroid behaviors in response to delivered soluble biomolecules (Fig. 6b) [95]. The platform was composed of semispherical shaped non-cell adherent microwells, integrated with a microfluidic chemical gradient generator. Due to the slow media flow and semispherical shape of the microwells, cells settled to the bottom of the microwells and formed 3D cell spheroids. The chemical gradient generator permitted flow of cell culture media with precisely controlled biomolecule concentrations. Thus, the system enabled spatiotemporal presentation of biochemical microenvironments to the 3D cell spheroids. Microfluidic systems integrated

with 3D microwells could be used as a universal platform for investigation of developmental processes involving stem cells in response to dynamic microenvironments and for various drug screening applications.

3. High-throughput delivery of bioactive molecules

Biological systems can also be modulated by delivered soluble biochemicals such as drugs, hormones, cytokines, RNAs, and growth factors. These biochemical deliveries can be applied in various aspects of advanced tissue engineering and therapeutic strategies [96, 97]. Changes in gene expression and subsequent alterations in protein activation by the delivered biochemical molecules result in distinct cell behaviors. Therefore, understanding how these various biomolecules in different combinations and concentrations interact with specific biosystems is of significant importance. HT strategies allow us to screen the efficiency of delivery materials, delivered molecule, and delivery methods, and the cell responses to the delivered biomolecules (Table 1).

3.1. Gene and protein delivery

Gene delivery enables changes of gene expression in cells, which allows control over cell behavior. Exogenous nucleic acid-based viral vectors or polymer-based non-viral vectors have been utilized to deliver nucleic acid into cells. Viral vectors have been widely utilized for gene delivery because of their high efficiency [98], however, safety issues related to immunogenicity and tumorigenicity are critical concerns for clinical use. Recently, Xu et al. reported clinical trials utilized viral vectors, and malignancy of the transfected cells was found after the gene therapy due to the predilection of the virus integration to oncogenic areas of transfected cells [99]. Moreover, most of the outcomes haven't shown any successful result, although more than hundreds of trials have been underway in many countries [100, 101]. As alternative gene delivery carriers, non-viral vectors have been considered for the safe delivery of nucleic acids, but the gene delivery efficiency issue is remained to be improved in order to ensure successful applications. The efficiency of non-viral gene delivery is varied according to the composition, size, and concentration of gene delivering materials and delivery methods. HT assays have been actively introduced to effectively evaluate and improve non-viral gene delivery efficiency [102–105].

Rantala et al. established an HT cell spot microarray (CSMA) system for small interfering RNA (siRNA) factor delivery (Fig. 7a) [105]. This system permitted rapid measurement of gene delivery efficiency to cells and subsequent changes in gene expression in an HT manner. The CSMA system was prepared by printing various matrix-siRNA compositions and then used to test gene delivery to 92 adherent cell types (Fig. 7b). For the gene transfection, the researchers utilized commercially available transfection reagents (Fugene HD) to test utilization of the CSMA system for disparate RNAi analyses. Fujita et al. also developed a microarray transfection system by spotting mixtures of plasmid DNA or siRNA with ECM proteins on a PEG-coated substrate (Fig. 7c) [103]. This PEG layer was used to inhibit cell migration and cross contamination among the adjacent substrates. In order to increase cell adhesion and transfection efficiency, various concentrations of type I collagen or fibronectin were applied to the substrate and tested. Certain concentrations of fibronectin

enhanced transfection efficiency compared to type I collagen (Fig. 7d). However, the type I collagen coated groups displayed relatively higher cell adhesion compared to fibronectin groups. The application of PEG and ECM proteins to the microarray transfection platform yielded a higher number of array spots per area as well as higher gene transfection efficiencies compared to previous systems.

HT methods can also be used to synthesize large libraries of lipid-like materials, polymers or nanoparticles to find the optimal structure and composition for gene delivery methodologies. Anderson et al. presented an automated polymer synthesizing system for developing non-viral gene delivery carriers [102]. By utilizing a fluid-handling robot and a multi-channel micropipette, the automated system efficiently mixed different types of monomers, allowing for 2,350 polymer formation reactions in a day. DNA incorporation into the polymer carrier was also performed by the automated microarray at various DNA to polymer ratios. Polymer carrier efficiencies were determined by delivering luciferase-expressing plasmids into monkey kidney fibroblast cell-lines (COS-7) and measuring luminescence using a luminometer.

HT systems have also been developed for efficient delivery and profiling of proteins. Oliveira et al. reported a chip-based protein assay for profiling the controlled release of proteins from polymer matrices [106]. To improve on conventional release kinetic monitoring systems, researchers created a superhydrophobic microarray platform and evaluated the release of protein from polymeric matrices. Specifically, the chip had arrays of superhydrophobic spots and fluorescein isothiocyanate-labeled bovine serum albumin (BSA-FITC) loaded alginate gels located at the centers of the superhydrophobic spots. A phosphate-buffered saline (PBS) droplet was then applied to cover each alginate hydrogel. The BSA-FITC released from the alginate into the PBS was tracked and quantified with a fluorescence imaging system. The obtained data was comparable to that of conventional protein release profile assays.

HT gene delivery system enabled the simultaneous screening of various combinations of genes and gene delivery carrier conditions. More approaches are expected to be developed to increase transfection efficiency of nonviral gene delivery system. The development of HT platforms that enable the simultaneous screening of gene delivery conditions will provide comprehensive information to find more advanced transfection systems. Compared to the other types of HT delivery systems, HT protein delivery platform has not been broadly applied for the protein delivery and profiling systems. Regarding the tissue engineering approaches, most of protein release profiling are achieved in a long-term manner to present the released protein in a sustained way. Current HT devices are not suited for the screening of protein release profiles from the functional biomaterials for longer terms, which may be the main reason of the less frequent application of HT devices to protein release profiling systems. Moreover, not many HT devices have been broadly presented for in situ protein detection system apart from those detecting BSA. Future HT devices need to be capable of a wider range of detectable proteins in situ, and the devices should also enable the monitoring of protein release for longer terms. Based on the proof of concept studies, HT protein delivery platform could be developed as a novel candidate that can be utilized for

investigating protein efficiencies as well as protein profiling systems to evaluate protein delivery systems.

3.2. Drug delivery and screening

Efficiently screening the toxicity and efficacy of potential drug candidates remains a significant challenge for early phase clinical trials [107]. Currently, drug development is limited by poor prediction, relatively long analysis time, and the high cost of conventional drug screening methodologies. In this respect, HT strategies could provide more efficient methodologies for screening potential drug candidates under various conditions while mimicking complex body system prior to clinical trials [108, 109].

Typically, HT microarrays for drug screening have been implemented by patterning candidate drugs using a printing robot. Kusi-Appiah et al. fabricated a microarray system by using a dip-pen nanolithography technique that enables micropatterning of drug-containing phospholipid multilayers on a chip to deliver small molecule drugs [110]. For drug screening, cells were cultured directly on the phospholipid patterns. The authors showed that drugs could be delivered to the cells directly over the patterns and there was no cross-contamination between adjacent patterns despite the lack of physical barriers among the arrays. Due to the sub-cellular dimensions of patterns, multiple drugs with specific doses could be delivered to cells, which can be potentially used as an advanced drug discovery platform. Similarly, Bailey et al. fabricated microarrayed poly-(D),(L)-lactide/glycolide copolymer (PLGA) layers with various drug compounds onto Ni-chelated glass slides [111]. The drug compounds slowly diffused from the microarrays, which affected the cells on the microarrays. This system did not use any restrictive walls or wells, so only one compound was allowed in a single chip for the detection and screening.

To inhibit cross-contamination of cells on drug screening devices, each spot of the microarray can be isolated using a sandwich microarray technique. In this technique, cells are seeded into individual microwells, fabricated on a glass slide, and various libraries of drugs are printed on another glass slide. By overlapping the two slides, drugs can be individually delivered to microarrayed cells without cross-contamination. Lee et al. developed a toxicological assay named MetaChip, which combined a metabolite-generating component with cell screening capabilities [112]. The system screened potential cytotoxic metabolites that could be derived from drugs. Following the treatment on cells, cytotoxicity was quantified using live/dead assays with fluorescent scanners. A similar 'sandwiching' HT microarray system was also presented for testing cytotoxicity of chemical drug libraries on cells (Fig. 8a) [113]. Wu et al. fabricated two parts to comprise the device: photo-crosslinkable polyethylene glycol diacrylate (PEGDA) microwell patterned glass and PDMS posts for the chemical compound delivery. Different drugs could be delivered to each isolated cell-containing PEGDA microwells by sandwiching the PEGDA microwells and drug printed PDMS. To validate the drug delivery property in a developed HT microarray system, green fluorescence intensities of live/dead images were evaluated. Three-hundred and twenty potential anti-tumor agents were spotted on the PDMS posts and delivered to MCF-7 human breast cancer cells in an HT microarray. After exposure to the chemical compounds, cell-containing microwells were observed under fluorescence microscopy to

determine the respective toxicity of each candidate compound. Similarly, Kwon et al. analyzed drug delivery by loading chemical drugs into PEGDA hydrogels (Fig. 8b) [114]. Each desired screening compound was printed with PEGDA complex and apoptosis of MCF-7 breast cancer cells following the sustained release of chemical compounds was investigated. Such HT systems can provide rapid screening of candidate drugs for improved drug usage and therapeutic applications. However, the drug screenings were mostly demonstrated by live/dead assay, which will only allow for the quantitation of cell death of the screened drugs. In addition to the toxicological analysis, other applicable analysis to evaluate metabolism, protein expression, and other cellular behavior in HT devices should be developed for the advancement of this field.

4. High-throughput approaches for biomolecular analysis

Accurate detection and analysis of biological molecules are crucial to develop therapeutic modalities as quantitative information of biomolecules is intimately related to the functionality of a biological system [115, 116]. However, most conventional biomolecular sensors and analytical tools do not allow for the efficient detection and rapid evaluation of numerous biomarkers [117, 118]. HT techniques provide an opportunity to overcome this current limitation [119, 120]. In this section, we introduce advances in the field of HT platforms for sensing of bioactive molecules, particularly on techniques to detect genetic, carbohydrate and protein biomarkers (Table 1).

4.1. Gene analysis

Gene expression analysis is an essential tool to understand and identify biological systems [121]. Since the development of the DNA microarray technique in the 1990s, there have been significant efforts to rapidly and robotically print specific complementary DNA (cDNA) to probe for genetic information [122]. In result, the oligonucleotide microarray of 70-gene prognosis profile (MammaPrint) was developed and commercialized that can be utilized for predicting distant metastasis of cancer [123]. Moreover, quantitative real-time polymerase chain reaction (qRT-PCR) is currently regarded as a ‘gold standard’ to monitor changes in gene expression at the RNA level due to its sensitivity, dynamic range and reproducibility [124]. To increase the number of genes that can be examined in a single assay, HT microarray techniques have been applied to qRT-PCR.

Shao et al. developed a multiplex amplification chip with an oligo microarray read out (MACRO) system [125]. This oligomer microchip contained 91 probes to monitor genetically modified organisms. Specifically-designed primer pairs with 5'-tags were printed on individual microwells and sample mixtures were loaded into the microwells for amplification on an *in situ* thermal cycler. Then, the initial PCR products were collected and used as templates for the next round of PCR. The next round of gene amplification was achieved with a pair of 5' Cy5 tagged universal primers. The fluorescent-labeled final products were analyzed by an oligo microarray. The specificity of the MACRO system was fairly consistent with those of conventional qRT-PCR.

As an advanced *in situ* RNA sequencing, droplet-based single cell transcription platforms have also been developed. For example, Klein et al. fabricated a droplet-based RNA

sequencing system (Fig. 9) [126] where nanoliter-sized droplets with cells, lysis buffer, reverse-transcription mix and barcoded primer-encapsulated hydrogels were generated using an oil/water emulsion. After the droplet creation process, UV light application released primers from the hydrogels, which resulted in barcoded cDNA production. Each droplet was broken after the cDNA production step and the cDNAs were amplified for sequencing and analysis. As another droplet-based sequencing system, microparticles were utilized to deliver barcoded primers during the transcription and amplification processes [127]. Cells were lysed within the droplets and mRNAs from the cells were bound to the barcoded primers conjugated to microparticles. The barcodes for labeling different types of cells consisted of 12 bases resulting in 4^{12} (16,777,216) different possible sequences. Due to the presence of cell barcodes, the amplification results can be organized and differentially classified by cell type.

The introduction of microarray and microfluidic techniques to qRT-PCR systems demonstrated high performance of gene analysis in a single test and could enhance the accuracy of the gene monitoring. Incorporating the cell capture, cell lysis, reverse transcription in a chip resulted in very handy and fast analysis method, but it could be challenging to monitor samples with low concentration of DNA contents as these systems do not include steps for checking DNA concentration or preconcentration of DNA. Regardless, the platforms have the potential to enable rapid and cost-efficient genetic diagnosis using non-invasive genetic biomarkers of various types of cancers [128, 129] and other diseases [130, 131].

4.2. Carbohydrate and enzyme analysis

Carbohydrates, also known as mono- or polysaccharides, play a pivotal role in various biological processes and living organisms. For example, glycoconjugates and glycans are largely deposited on the surface or inside of cells [132, 133] and regulate the important processes such as cell trafficking, adhesion, signaling and immune responses [134–136]. Moreover, interactions between glycans and proteins are of imperative importance in a myriad of physical processes including tumor metastasis [137], leukocyte recruitment to inflammatory sites [138], and immune system responses against infections of toxin, bacteria, and virus [135]. In this respect, the better understanding of glycan-protein interactions will enable significant development of therapeutic drugs and diagnostic tools. However, it is very challenging to discover the ligands of carbohydrate binding because each carbohydrate receptor has morphological specificity for differential reactions [139, 140]. These ligand-receptor specific interactions are typically found in ‘self’ and ‘nonself’ immune recognition events maintaining homeostasis and a plethora of other biological phenomena [141, 142]. HT technologies could be practical for screening carbohydrate-ligand binding interactions and detecting biomarkers for identification of diseases, physical condition, and recovery after therapeutic interventions.

Wang et al. microarrayed ~20,000 spots of microbial polysaccharides on a single glass slide using a high-precision robot, ultimately designed for carbohydrate-mediated molecular recognition and anti-infection responses [133]. Microbial polysaccharides were successfully immobilized on nitrocellulose-coated glass slides without any chemical conjugation. The

array included various structural types of glycoconjugates and showed stable and sensitive detection results. Carbohydrates covalently linked on device supports were also utilized for screening arrays [143]. Thiol-modified carbohydrate substrates were printed on maleimide-derivatized glass slides, and the carbohydrate-immobilized chip enabled analysis of the specific recognition of mannose and glucose moieties. In addition, bacteria detection carbohydrate microarrays were similarly fabricated [144], which has potential applications in the diagnosis of microbial or viral diseases.

Microfluidic techniques can be applied as rapid diagnostic tools. Sheng et al. fabricated a microfluidic device to measure glucose concentration in blood and urine electrochemically to monitor diabetes [145]. The device consisted of a tunable microreactor with localization of functionalized magnetic nanoparticles within the microchannel, and it could measure glucose concentration by applying an external magnetic field. Hou et al. improved the glucose detecting microfluidic device by employing heated colorimetric reaction and spectrophotometric detection [146]. As a cancer diagnostic tool, Cao et al. developed a microfluidic platform to detect cell- surface glycan alterations with potential applications in the analysis of cancer metastasis [147]. The researchers used lectin-functionalized electrodes to target the glycans and let them selectively bind to the electrodes in the microchannel, which evaluated the target glycan quantity after drug exposure.

Recently, the integration of enzyme assays and HT technology have attracted interests. The conventional enzyme assays are designed to visualize the enzyme-mediated transformational reactions with colorimetric or fluorescent dyes, and HT enzyme analyses have been suggested as integration of microarray and chromogenic or fluorogenic substrates on chips. Wahler et al. developed an enzyme fingerprint chip to assay a variety of enzymatic activities [148]. Various fluorogenic and chromogenic substrates were applied to the microarray that can assay a variety of enzymatic activities of esterases, lipases, proteases, peptidases, phosphatases, and epoxide hydrolases. The assay results were visually recognized with a two-color scale from the measurements of chromogenic and fluorogenic substrates showing differential stereoselectivity and activity. In addition, Houseman et al. applied a peptide chip to be utilized in activity assays of nonreceptor tyrosine kinase c-Src [149]. The peptide microarrays were then prepared for profiling and inhibitor screening of kinases.

The HT screening platforms demonstrated that the platforms can save the analysis time. However, we still do not have sufficient libraries of diagnostic markers that can be incorporated into HT platforms to investigate the status of disease regarding the broad application as a diagnostic tool. Moreover, the successful application of enzyme assay platforms is strongly related to the expansion of substrate libraries that can demonstrate specific enzyme activities. For the development of advanced HT platform, implementations of expanding screening libraries into HT devices are required. The future developments will continue to find novel chemistries and more automated systems to streamline the analysis of HT systems. The incorporation of HT systems into carbohydrate and enzyme assays will require collaborative efforts of experts from various fields including chemistry, biochemistry, material science, and biology.

4.3. Immunoassay for protein detection and quantification

Immunoassays can detect and quantify cell-secreted proteins [150, 151]. Antibody-antigen interactions are most widely used in immunoassays [152]. For example, the interactions can be used for disease diagnostics by detecting cell-secreted biomarkers. However, conventional immunoassays are inefficient and include repetitive reagent additions and washing steps, which greatly reduces their practicality. One way to address this problem is the utilization of microfluidic approaches. Zheng et al. developed a microfluidic device that enabled the in-channel micro-patterning of antibodies for HT immunoassays [153]. Antibody reactive sites were defined using embedded actuating button valves (Fig. 10a). Through an in-channel patterning process, rapid and highly sensitive detection of biomolecules were achieved with samples of only a few microliters (Fig. 10b). The developed microfluidic-based immunoassay chip showed comparable results to the conventional plate-based enzyme-linked immunosorbent assay (ELISA) with improved reproducibility. HT hydrogel microarrays can also be utilized to analyze cell-secreted proteins in 3D microenvironments. Fernandes et al. developed a reliable and reproducible HT immunoassay platform using microarrayed 3D alginate hydrogels [154]. Levels of hypoxia-inducible factor (HIF-1 α) produced by tumor cells resulting from chemical treatment with of 2-methoxyestradiol, which is a HIF-1 α inhibitor, were characterized. The measured HIF-1 α values with various 2-methoxyestradiol concentrations showed good agreement with standard Western blotting. Similarly, Yan et al. also recently developed a HT, versatile 3D immunoassay chip to detect cell-secretion proteins [155]. A dry immunoassay chip (3D immunoChip) was fabricated using antibody-containing lyophilized hydrogels. Hepatotoxicity responses to delivered drugs were evaluated by sandwiching drug-loaded 3D ImmunoChips with 3D Cell Culture Chip microarrays. The cell-secreted biomolecule detection performance of the device was comparable to the conventional ELISA kit (Fig. 10c).

Immunoassays mostly rely on fluorescently labeled biomarkers. However, the interference between molecular binding interactions and fluorescent labeling mechanisms often results in inaccurate measurements [156]. For the development of label-free sensors, several approaches have been employed. Among them, biosensors using surface plasmon resonance (SPR) have received attention due to their rapid and ultrasensitive detection properties [157]. In a study by Chang et al., a large-scale plasmonic nanohole array was introduced to quantitatively measure protein-protein interactions [158]. Signal-to-noise ratio and image acquisition time could be significantly enhanced by utilizing a dual filter imaging method. Recently, Kang et al. reported a label-free, HT screening method using surface-enhanced Raman scattering (SERS) measurements [159]. Polymer microbeads with protein-specific binders were decorated with 44 kinds of SERS active nanoparticles (Fig. 10d). The microbeads with SERS active nanoparticles can be used to identify protein binding (Fig. 10e). This system can be potentially used for the investigation of one-bead-one-compound libraries for various biomedical applications. Although the developed HT analytical systems have great potential for improving biomedical engineering and therapeutic applications, the validation of systems for multiplexed conditions is required. In addition, the integration of the HT analytical systems with other HT cell culture platforms is critical for advancing the

utility of these tools, as most current HT screening platforms analyze outcome measures using time- and material-consuming conventional non-HT approaches.

5. Conclusions

For the effective development of biomaterials for various biomedical applications and regenerative medicine, the comprehensive understanding of synergistic effects of biomaterials' properties on cellular behaviors is required. HT platforms are aiming to investigate the interactions between cells and biomaterials to find the optimal formulation of biomaterials for specific target applications. Here, we have reviewed recent HT strategies to screen and analyze cellular responses to biomaterials for advanced biomedical engineering and drug discovery applications. HT technologies have shown promise in analyzing 2D and 3D cellular responses with the analysis of thousands of samples in a single chip through systematic variation of microenvironmental parameters. Additionally, HT platforms provide rapid, precise and reproducible results with minimized use of reagents and biological samples. Conventionally, development of biomaterials has been focused on the engineering of biomaterials' chemical and physical properties to improve the function of engineered micro tissue construct. However, recent findings suggest that additional research directions on biomaterials could be made on the development of smart biomaterials that can temporally change their material properties to recapitulate the dynamic nature of native tissues. Therefore, it is anticipated that integrative HT platforms with real-time detect and analysis systems will significantly advance the field of biomaterials through the better understanding of dynamic interactions between cell and biomaterials. Moreover, the true success of HT platforms will lie on their translation into widely used tools, which requires close collaboration between medical professionals and bioengineers. Otherwise, the techniques will be used to just prove and explore their screening ability, which is not beneficial unless applied to investigate important questions regarding, for example, cell behavior, developmental processes or drug efficacy. In addition, development of advanced HT platforms for studying cell behavior would benefit from more *in vivo*-like microenvironments by incorporating, for example, external biophysical cues including dynamic mechanical strain and electrical stimulation. Implantable HT screening platforms containing biomaterial microarrays also need to be considered as another future avenue. The implantable HT screening platform can be potentially used to investigate immune responses and recruitments of inflammatory cells induced by biomaterials, which could provide insights to design fully functional implantable biomaterials and devices to treat chronic neurological and musculoskeletal disorders. These platforms would be valuable for a wide variety of research ranging from drug discovery, toxicology, pharmaceutical science and cellular therapies. HT technologies will substantially contribute to obtaining information about materials, technical options for tissue engineering and drug screening advances.

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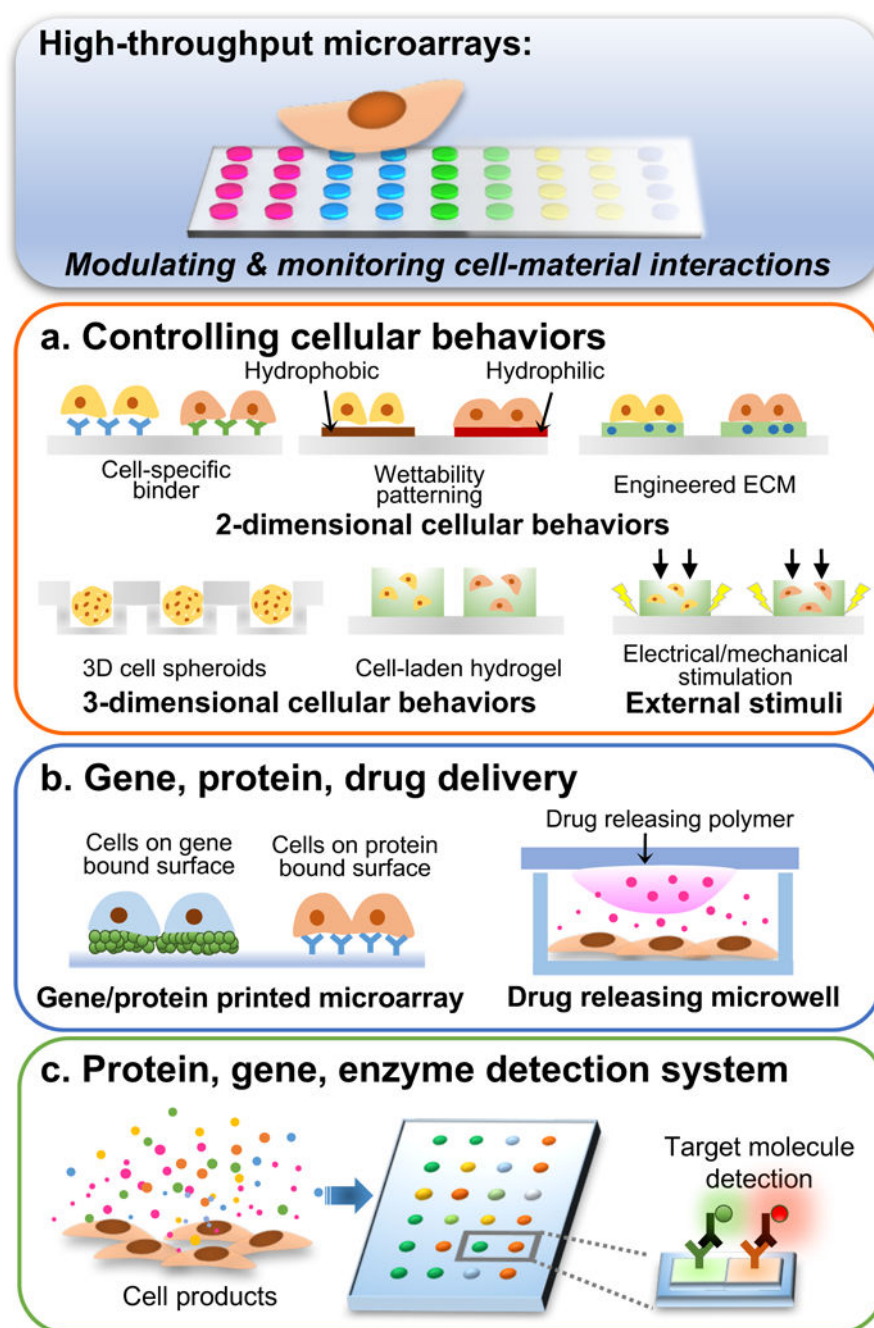


Figure 1.

Schematic illustration of HT approaches for modulating and monitoring cell-material interactions. (a) HT platforms for the screenings of 2D and 3D cellular behaviors with different microenvironments. 2D environments can be engineered by using cell-specific binding peptides, wettability patterning technique, and ECM coatings. Cell spheroids and 3D cell-laden hydrogels can be used to monitor the cell behaviors in 3D environments. In specific purpose, cells can be externally stimulated via electrical or mechanical stimulation. (b) HT can provide the region-specific cell inoculation and delivery of bioactive molecules including genes, proteins, and chemical drugs to the cells via microarray and microwell

methods. (c) The HT technique can also be utilized for the analysis of multiple cell-secreted factors and cellular products including proteins, genes, and enzymes.

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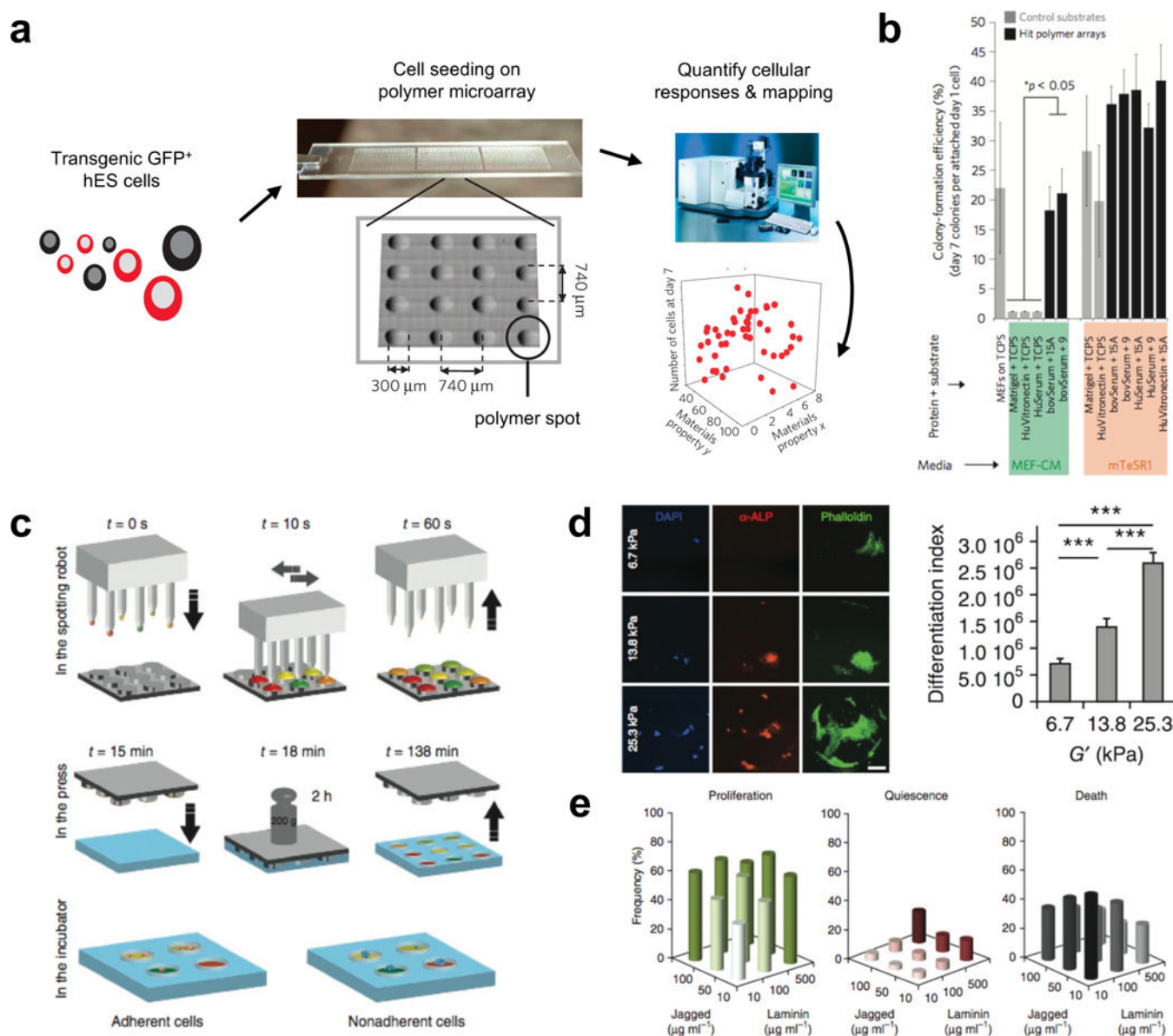


Figure 2.

HT approaches for the investigation of 2D stem cell behaviors. (a) HT screening platform to study the effects of cell-biomaterial interactions on human ESC behaviors, (b) which allowed for the comparison of human ESC colony formation efficiency by varying the composition of biomaterials and cell culture media. Combinatorial screening of three different cell culture substrate compositions (TCPS, hit-polymer 9, and hit-polymer 15A), four different protein coatings (matrigel, bovine serum (bovSerum), human serum (HuSerum), and human vitronectin (HuVitronectin)), and two different culture media (mouse embryonic fibroblast-conditioned media (MEF-CM) and chemically defined media (mTeSR1)) was conducted using the developed platform. (c) Fabrication of microarrays with engineered ECM allowed for investigating (d) stem cell behaviors as a function of hydrogel stiffness; and (e) extracellular matrix proteins. Figures adapted and reprinted with permission from (a, b) [34] and (c–e) [35].

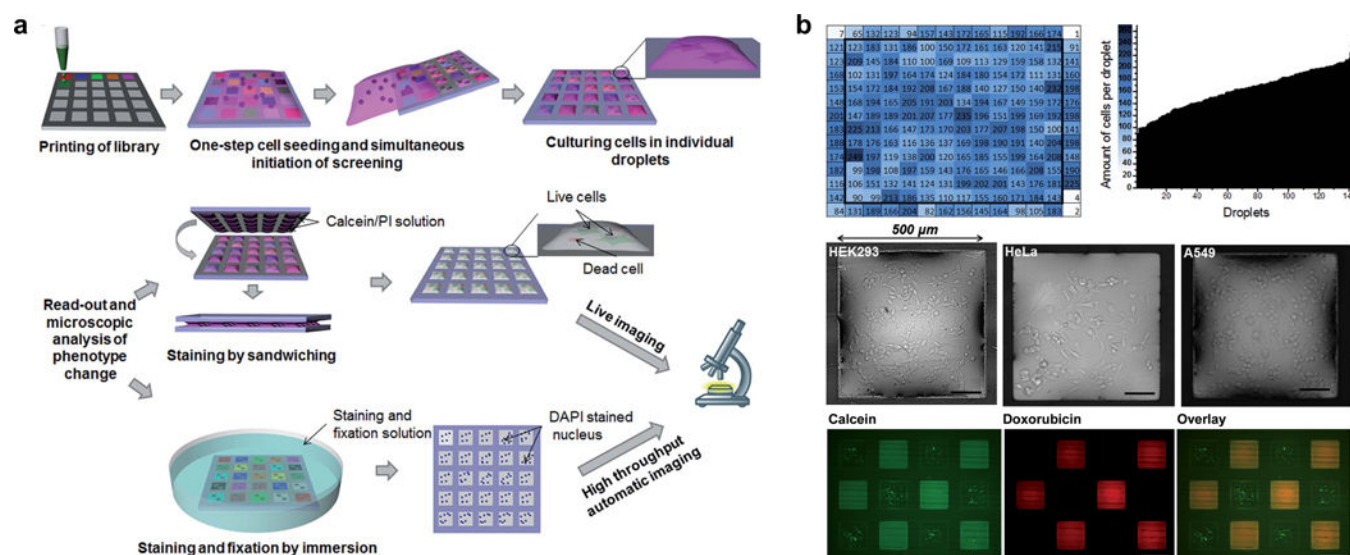


Figure 3. Superhydrophobic-superhydrophilic patterning for 2D HT screening platform. (a) Schematic illustration of the workflow of HT screening platform for the study of cellular behaviors with conventional image-based analytical methods. (b) Homogenous seeding of diverse cell types on the superhydrophobic-superhydrophilic patterned substrate for screening cell behaviors with delivered biomolecules. Figures adapted and reprinted with permission from [44].

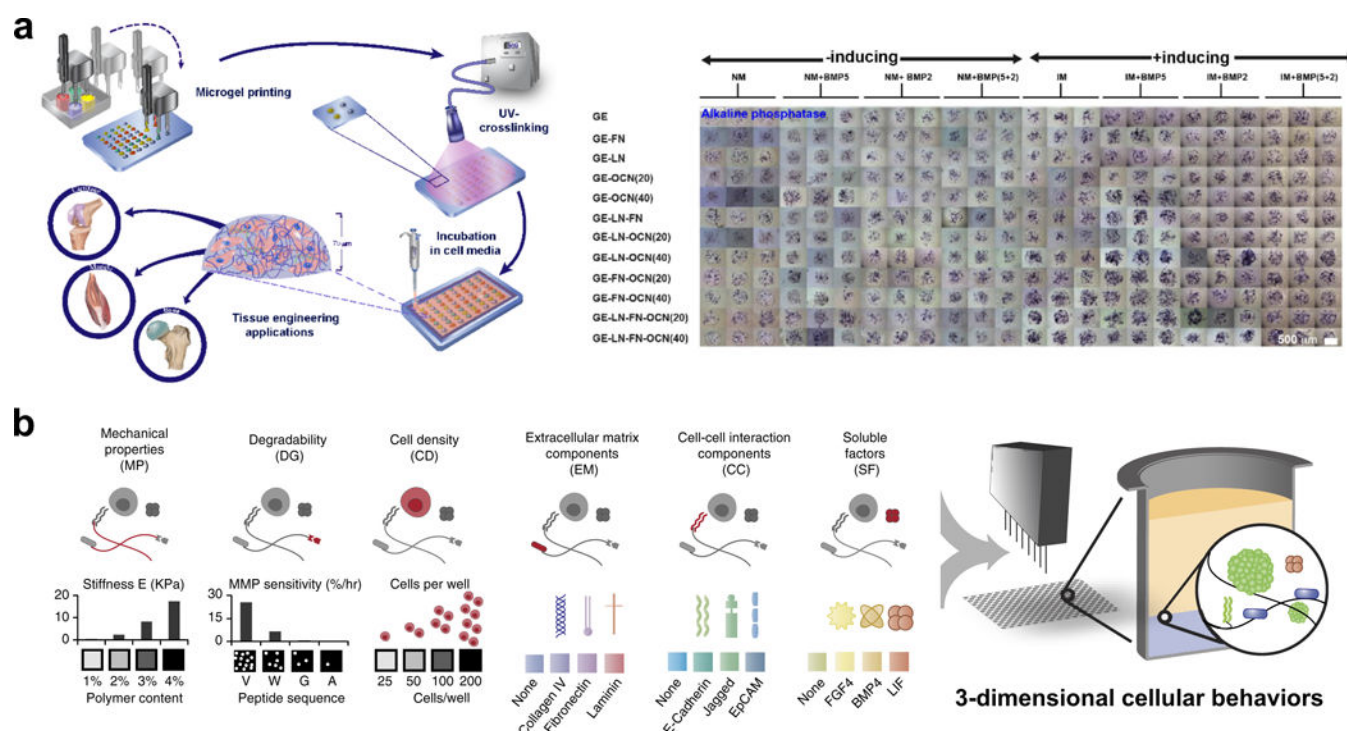


Figure 4.

HT approaches to investigate 3D cellular behaviors. (a) Cell-laden gelatin methacryloyl (GE) hydrogel microarrays were developed for the identification of the compositional effects of ECM proteins (laminin (LN, 40 μ g/mL), fibronectin (FN, 40 μ g/mL), osteocalcin (OCN, 20 and 40 μ g/mL)), culture media (normal media (NM) and osteogenic inducing media (IM)) supplement with different concentrations of bone morphogenic proteins (BMP-2 and BMP-5, 50 ng/mL). (b) High-throughput screening of mouse ESC behaviors within PEG 3D hydrogel microarrays in response to varying hydrogel stiffness and degradability, cell density, extracellular matrix components, cell-cell interactions, and soluble biomolecules. Figures adapted and reprinted with permission from (a) [39] and (b) [62].

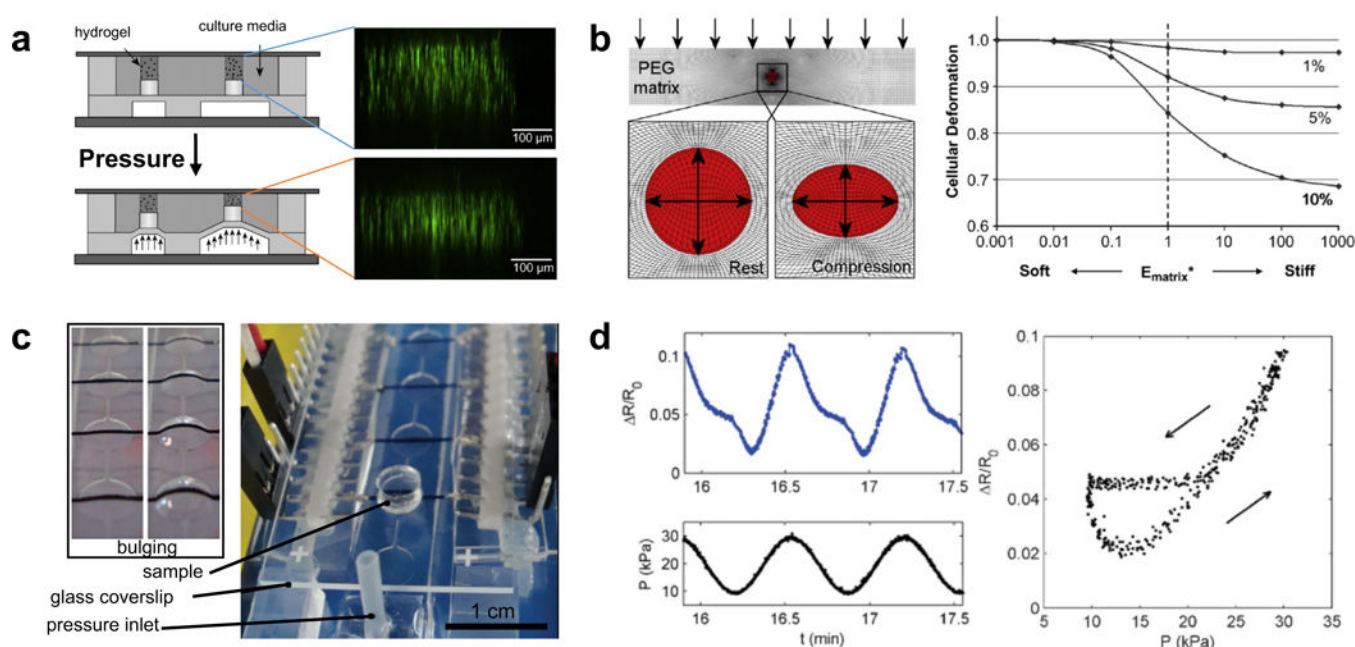


Figure 5. HT biomechanical stimulation systems. (a) Microarray platform for applying dynamic mechanical compressive strain across biomaterials such as PEG hydrogels. (b) Finite element simulations presenting the deformation of cell and hydrogel matrix under the compressive stress. According to the relative stiffness of cells and hydrogel matrix (E^*_{matrix}), the degree of cell deformations can be varied. (c) Photograph of bulging membranes with integrated strain sensor for the real-time monitoring of strain onto biomaterials. (d) Strain sensing of cyclic membrane bulging by using a carbon nanotube-based strain sensor. A time-dependent resistive strain of the sensors ($\Delta R/R_0$) demonstrated a good correlation with input pressure (P), although there is a hysteresis at loading and unloading strain in low input pressure regime. Figures adapted and reprinted with permission from (a, b) from [70] and (c, d) [73].

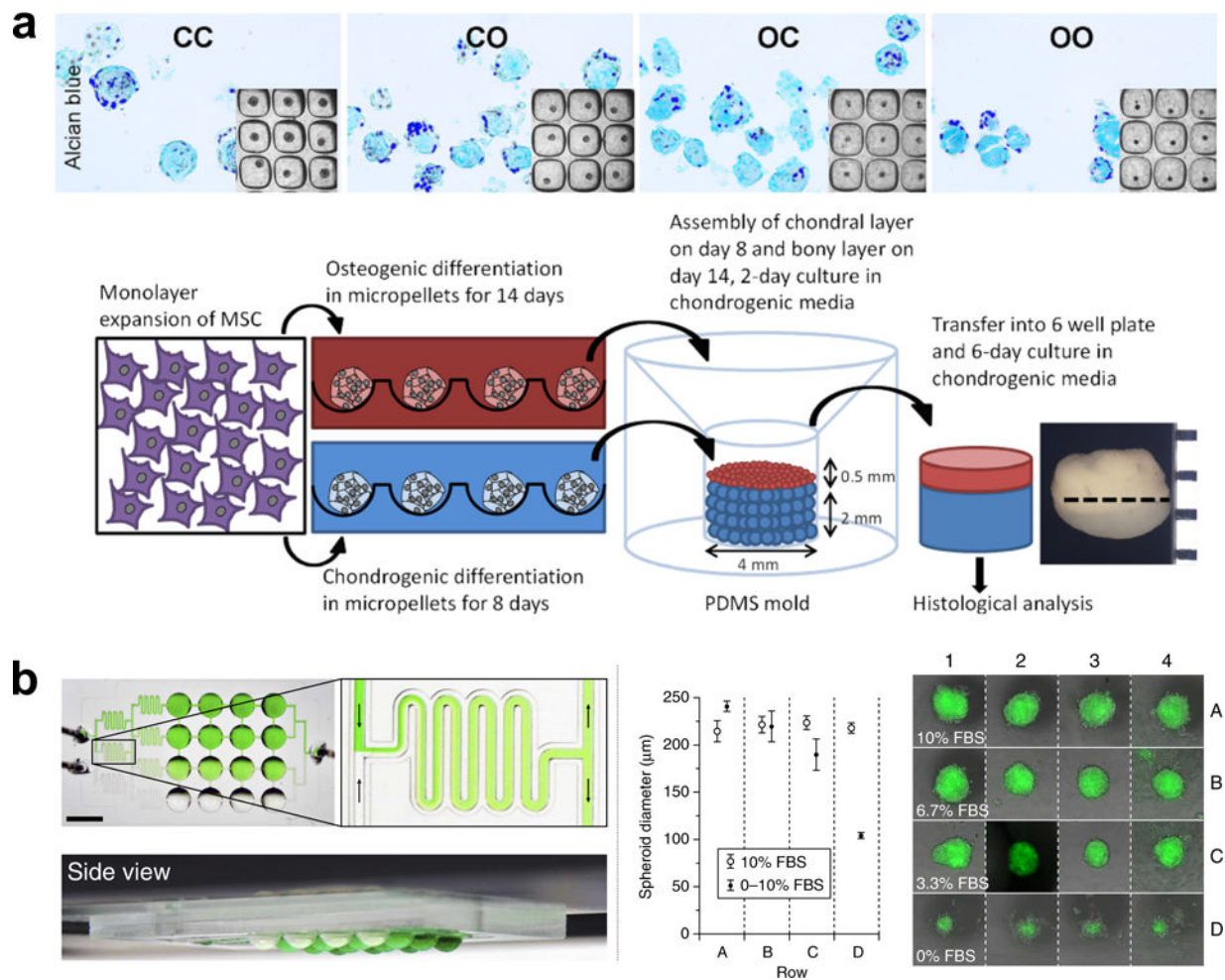


Figure 6.

HT platforms for 3D microtissue behaviors. (a) HT 3D spheroids were formed in microwells to function as microbuilding blocks to generate an artificial osteochondral tissue construct. Culture conditions are defined by the media type (chondrogenic media (C) or osteogenic media (O)) and culture duration for the first 8 days and following 6 days. For instance, CO indicates cell culture with chondrogenic media for 8 days followed by 6 days culture with osteogenic media. Osteochondral-like tissue structure was obtained by co-culture of pre-osteogenic and pre-chondrogenic differentiated hMSC spheroids. (b) Microfluidic approach for monitoring of 3D microtissue behaviors. A suspension of fluorescent human colorectal carcinoma cells was flowed through microchannels to form 3D spheroids in hanging-drop microwells. The effect of FBS concentration on spheroid formation was explored simultaneously. Figures adapted and reprinted with permission from (a) [88], (b) [95].

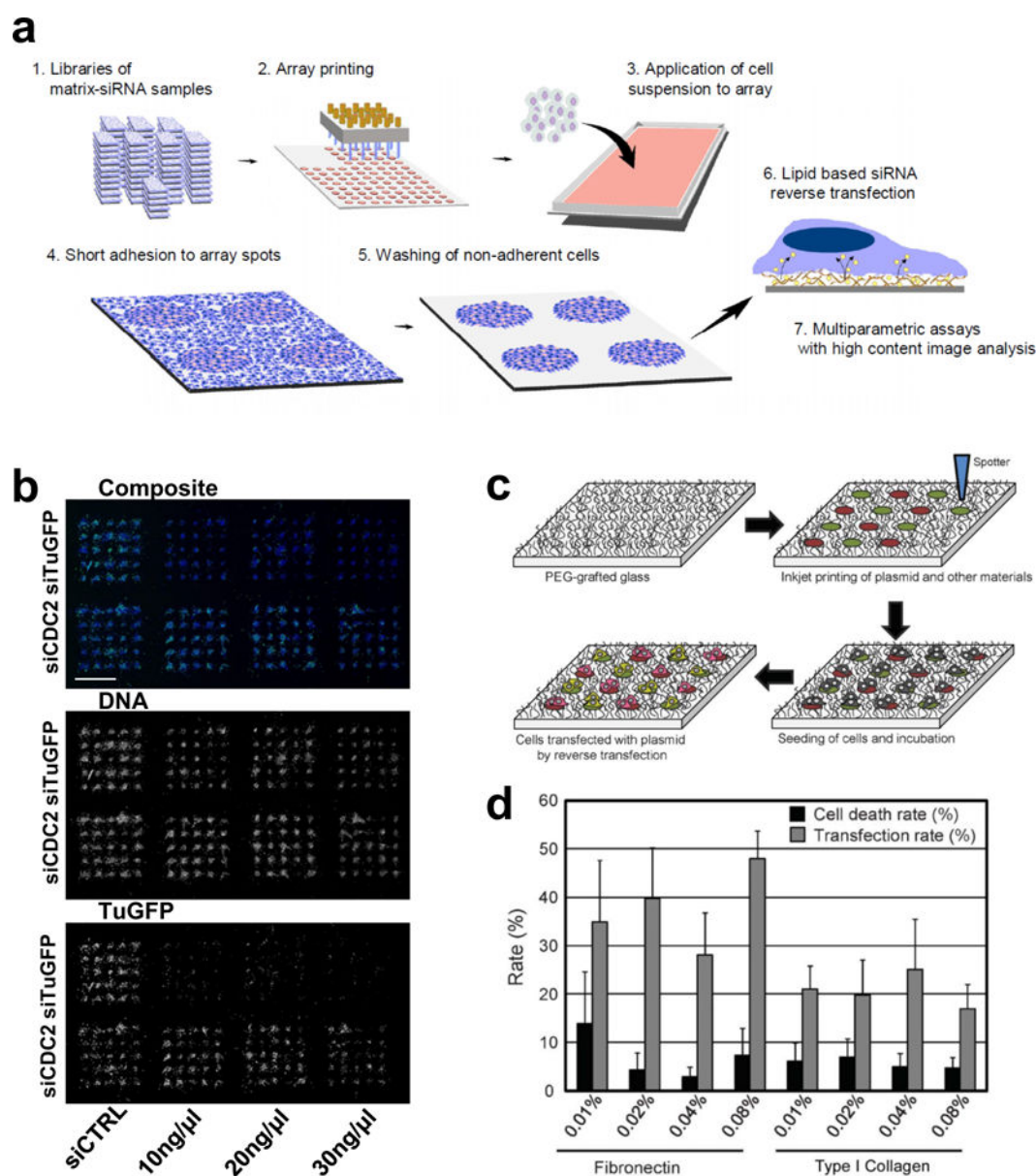


Figure 7.

Cell spot microarray methods for HT investigation of gene transfection. (a) Principle and workflow of the cell spot microarray method. (b) TurboGFP silencing of U-2OS sarcoma cells on cell spot microarray. Silencing efficacies of different siRNA concentrations were identified using a siRNA (siCTRL) as a negative group and a siRNA for CDC2 (siCDC2) as a control group. TuGFP = green, DNA = blue, scale bar = 2 mm. (c) Illustration of the patterning of a transfected cell microarray. (d) Average cell death rates (black columns) and the reverse transfection efficiency (gray columns) per one spot ($n = 8$). Figures adapted and reprinted with permission from (a,b) [105] and (c, d) [103].

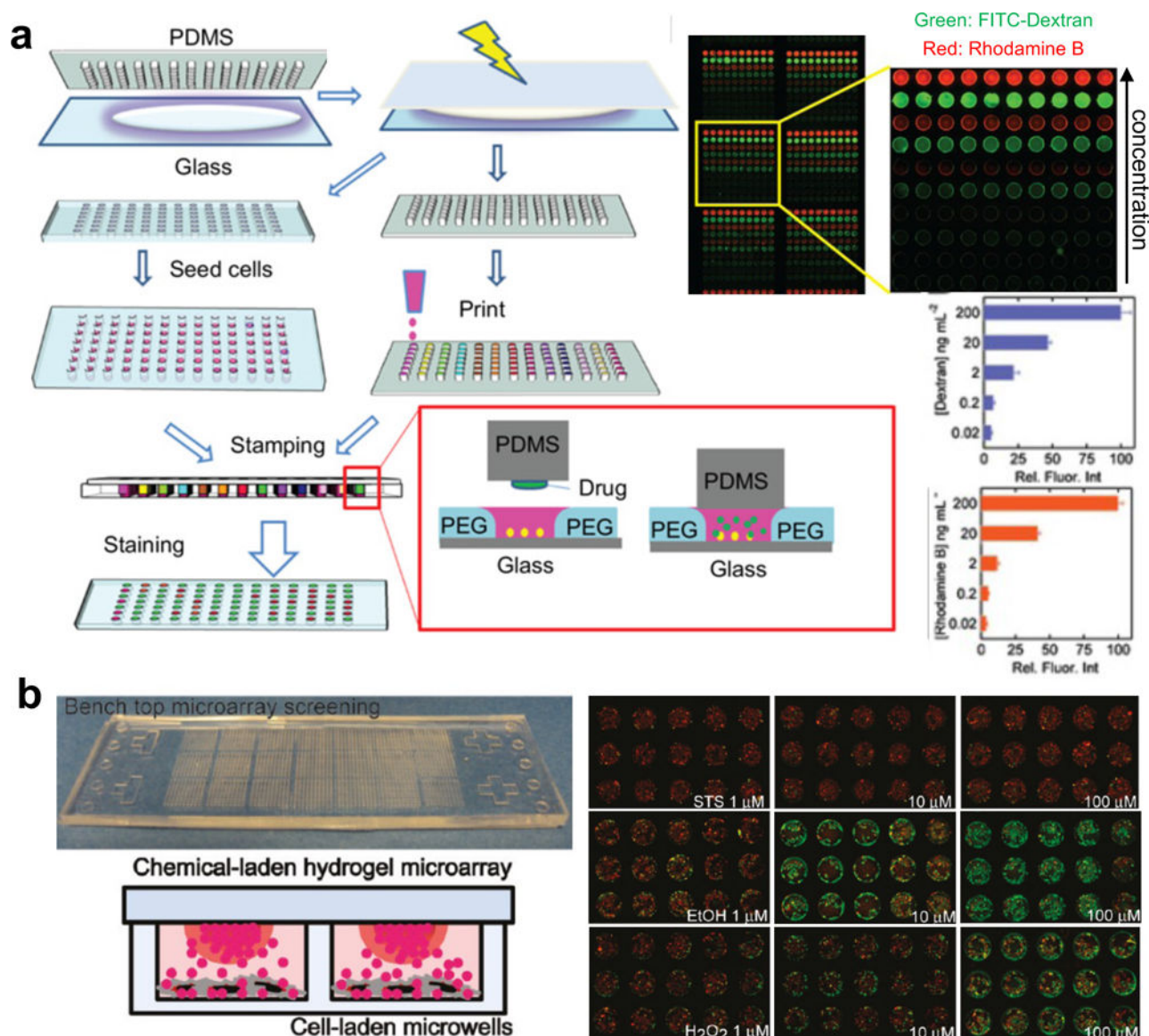


Figure 8.

HT systems for screening the effect of controlled delivery of drugs. (a) Schematic of the device fabrication and use: from fabricating the microwell array and chemical library printing to sandwiching the device for toxicological analysis. To validate the device, different concentrations of fluorescein isothiocyanate-dextran (FITC-Dextran) and Rhodamine B (0.02 to 200 ng/mL) were printed on PDMS posts and fluorescent intensities were evaluated after sandwiching. (b) A microarray system for released chemical screening (left) and resulting apoptosis and necrosis of MCF-7 cancer cells with various chemicals with different concentrations: staurosporine (STS), ethanol (EtOH), and hydrogen peroxide (H₂O₂). Apoptosis and necrosis were evaluated by annexin V-allophycocyanin and SYTOX orange, respectively. Figures adapted and reprinted with permission from (a) [113] and (b) [114].

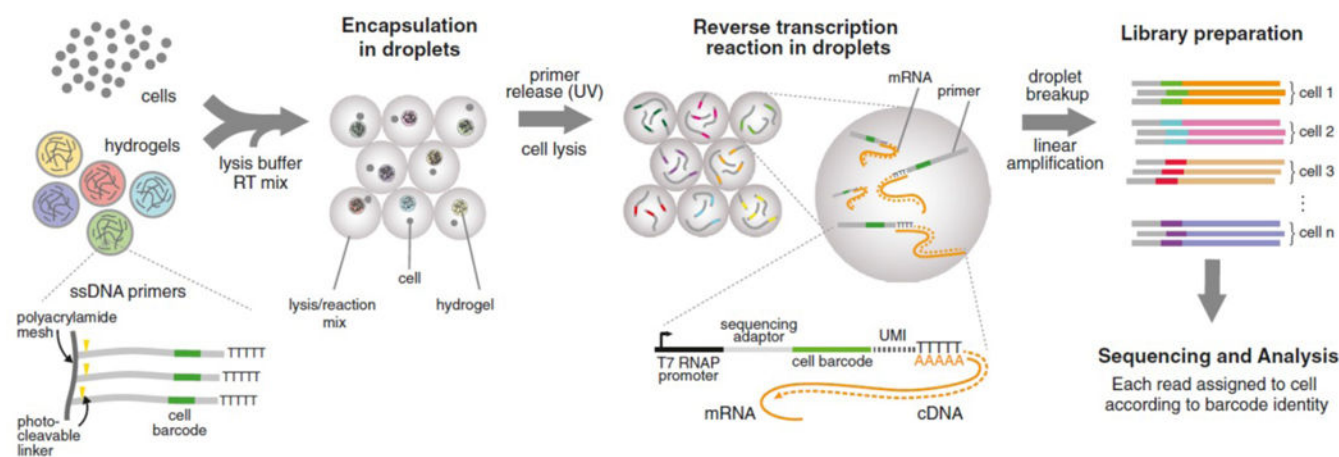


Figure 9. HT analysis of gene expression. A droplet-based DNA barcoding platform for RNA sequencing. UMI = unique molecular identifier. Figures adapted and reprinted with permission from [126].

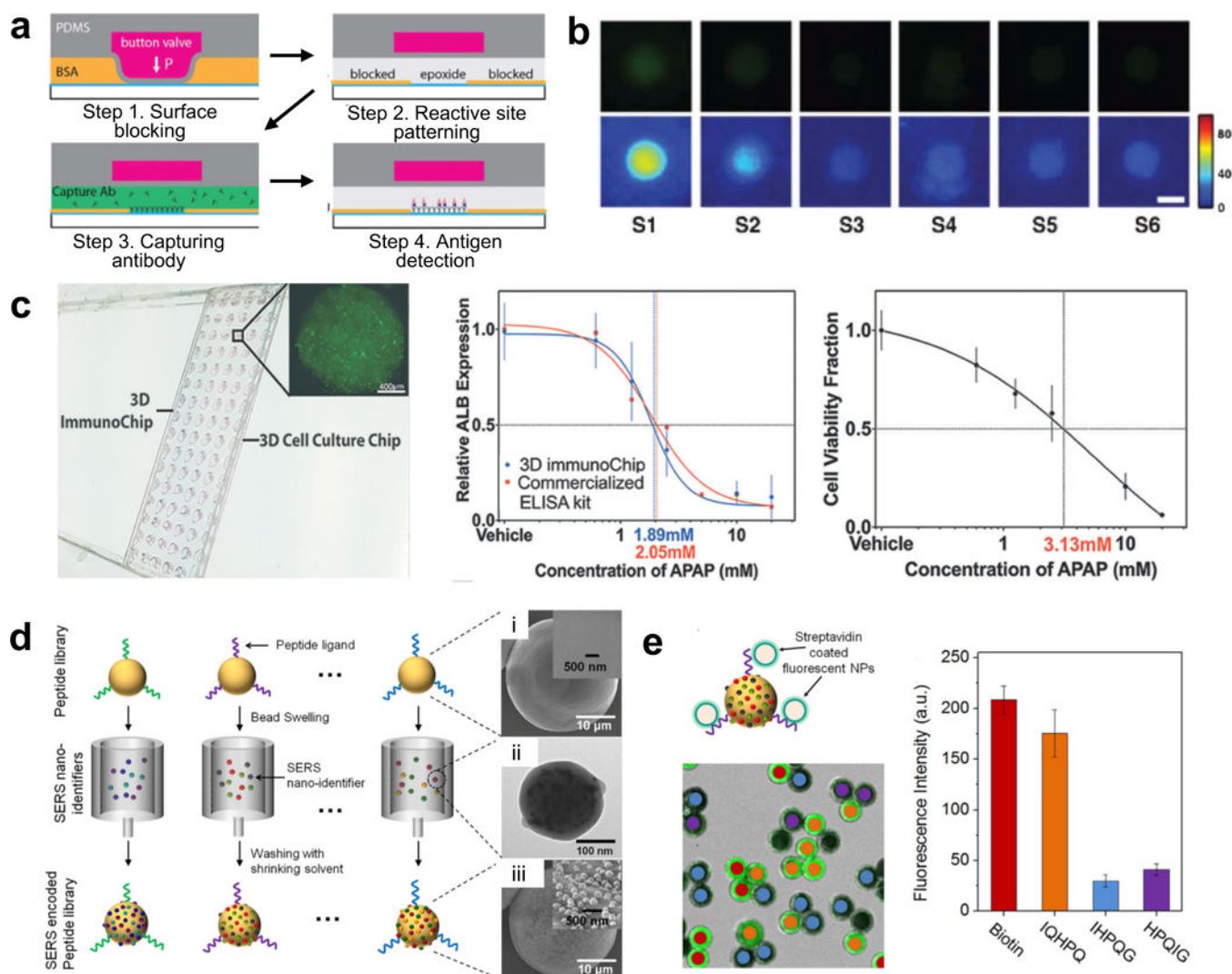


Figure 10.

HT immunoassays. (a) In-channel antibody patterning of the microfluidic device for the highly sensitive immunoassay platform. (b) Fluorescence-based detection of carcinoembryonic antigen from a human serum sample of 6 patients using the microfluidic device. (c) Drug hepatotoxicity evaluation using a coupled 3D immunoChip with a 3D cell culture chip. The detection capability of the 3D immunoChip platform is comparable to conventional ELISA analysis. (d) Schematic diagram of SERS active nanoparticles encoding onto polymer bead with protein specific binder and scanning electron microscopic images at each step (i-iii). (e) Label-free detection of four different bio-ligands (HPQIG, IHPQG, IQHPQ, and biotin) by combining the SERS barcodes and streptavidin binding assay. Figures adapted and reprinted with permission from (a, b) [153], (c) [155], and (d, e) [159].

Table 1

HT screening platforms for the investigation of cell-biomaterial interactions.

Screening device application	Screened factors on HT devices	Applied parameters/biomaterials in HT devices	Cell type	Refs.
Cell behavior monitoring in 2D	Cell adhesion and attachment	Synthetic polymers	None (Fibronectin adhesion)	[52]
		Peptides	Fibroblasts	[53]
	Biomaterial-stem cell interactions	ECM proteins, copolymer, hydrogels, growth factors	Neuroblastoma cells, fibroblasts	[54]
Cell behavior monitoring in 3D	Cell spreading, proliferation, and differentiation	Dynamic/static mechanical strains on cell-laden hydrogels ECM proteins, copolymer, hydrogels, growth factors	Human and mouse stem cells (MSCs, ESCs)	[32–35] [39, 62]
	Biomaterial-stem cell interactions		Mouse MSCs, fibroblasts, myoblasts Human and mouse stem cells (MSCs, ESCs)	[70–73] [32–35, 39, 62]
Gene delivery	Transfection efficiency of various types of RNAi	RNAi/transfection reagent	Human prostate cancer cells	[105]
	Cell adhesion and gene delivery	Plasmid DNA or siRNA/ECM proteins	HeLa cells	[103]
	Non-viral gene delivery carriers	DNA/polymer carrier concentrations	Monkey kidney fibroblast cell-line	[102]
Protein profiling	Profiling the proteins release into droplets from polymer matrices	Protein/hydrogel	None (protein release in PBS)	[106]
Drug delivery and screening (Spot printing without walls)	Fluorescent signal expression of cells	Rhodamine-tagged drugs/phospholipid multilayers	Mouse fibroblast cell line	[110]
	Synthetic lethality	12 compounds of small molecules/poly-(D,L)-lactide/glycolide copolymer (PLGA)	Mammalian cells (HeLa, A549 cells)	[111]
Drug delivery and screening (Sandwich microarray)	Cytotoxicity	Metabolic enzymes/prodrugs/sol-gel macromere Apoptotic drugs and chemicals/polyethylene glycol diacrylate hydrogels (PEGDA)	Human breast cancer cells Human breast cancer cells	[112] [113, 114]
Gene analysis	Gene expression level RNA sequencing	91 oligomers that can be detected in genetically modified plant materials barcoded primer/gels barcoded primer/microparticles	Genetically modified plant materials Mouse embryonic stem cells Human embryonic kidney cells, mouse fibroblasts	[125] [126] [127]
Carbohydrate analysis	Carbohydrate-mediated molecular recognition and anti-infection responses	Polysaccharides/nitrocellulose	None (Human serum specimens) Sheep erythrocytes, human serum specimens	[133] [144]
	Glucose/glycan detection	Magnetic nanoparticles Colorimetric/spectrophotometric substrates Indium tin electrodes/lectins detection oxide (ITO)	None (Human serum specimens) None (Glucose solution) Human chronic myelogenous leukemia	[145] [146] [147]
	Enzyme reactivity	Fluorogenic/chromogenic detection substrates	None (Enzyme solution)	[148, 149]
Protein immunoassays	Protein detection	Micro-patterning of antibodies in microfluidic channels Antibodies/3D hydrogel	None (Human serum specimens) Human pancreatic cancer cells	[153] [160] [155]

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Screening device application	Screened factors on HT devices	Applied parameters/biomaterials in HT devices	Cell type	Refs.
			Human hepatocytes	
	Protein-protein interactions	Plasmonic nanohole array Polymer microbeads for surface-enhanced Raman spectroscopic barcoding	None (Protein A/G, anti-Mouse IgG) None (peptides)	[158] [159]