Current Pharmaceutical Design, 2018, *24*, 5458-5470

REVIEW ARTICLE

Recent Advances in High-throughput Platforms with Engineered Biomaterial Microarrays for Screening of Cell and Tissue Behavior

Kijun Park[†], Yeontaek Lee[†] and Jungmok Seo*

School of Electrical and Electronic Engineering, Yonsei University, Seoul, 03722, Republic of Korea

ARTICLE HISTORY

Received: December 20, 2018
Accepted: February 2, 2019

DOI:
10.2174/1381612825666190207093438

Abstract: In the last decades, bioengineers have developed myriad biomaterials for regenerative medicine. Development of screening techniques is essential for understanding complex behavior of cells in the biological microenvironments. Conventional approaches to the screening of cellular behavior *in vitro* have limitations in terms of accuracy, reusability, labor-intensive screening, and versatility. Thus, drug screening and toxicology test through *in vitro* screening platforms have been underwhelming. Recent advances in the high-throughput screening platforms somewhat overcome the limitations of *in vitro* screening platforms via repopulating human tissues' biophysical and biomchemical microenvironments with the ability to continuous monitoring of miniaturized human tissue behavior. Herein, we review current trends in the screening platform in which a high-throughput system composed of engineered microarray devices is developed to investigate cell-biomaterial interaction. Furthermore, diverse methods to achieve continuous monitoring of cell behavior via developments of biosensor integrated high-throughput platforms, and future perspectives on high-throughput screening will be provided.

Keywords: High-throughput platforms, cell-biomaterial interactions, cellular microenvironments, biomaterial screening, organs-on-a-chip, myriad biomaterials.

1. INTRODUCTION

A myriad of microenvironmental factors has proven to influence the way cell behaves including adhesion, apoptosis, proliferation, and cell differentiation [1-7]. The cell microenvironments are often categorized into biochemical and biophysical factors [8]. Biochemical factors include chemical composition of extracellular matrix (ECM), soluble bioactive molecules, and cell-secreted proteins. Micro- and nano-topography, matrix elasticity, stiffness, and wetting properties are examples of biophysical factors. In addition to these passive microenvironmental cues, active mechanical and electrical inputs also affect the developmental process of various organ systems such as musculoskeletal and cardiovascular tissues [9, 10]. In an attempt to understand cellular behaviors by recapitulating the microenvironments of cells, researchers have investigated novel biomaterials with varying chemical and physical properties. The developed biomaterials have provided a facile way to temporally and spatially engineer microenvironments of cells resulting in different cell behaviors and functionalities for various therapeutic applications [11, 12]. Although traditional *in vitro* approaches to screening microenvironments are clumsy and inadequate for screening the vast number of microenvironmental factors, it has been found that the engineered microenvironmental cues allow an in-depth understanding of diverse cellular behavior. High-throughput screening technique is one of the most effective methods of screening the interaction between vast combinations of cellular microenvironments and cellular behaviors [13, 14]. In comparison to conventional approaches, high-throughput screening provides superior advantages including usage of minimal input materials and cells, expeditious screening and faster analysis, and detecting of multiple targeted samples on a single device. Diverse approaches have been taken to produce a comprehensive biomaterial library to consider the synergistic influences of biological microenvironments on cell responses. In an attempt to understand the cellular responses regarding their physical and chemical microenvironments, cellular behavior was first simplified into 2-dimensional (2D) platforms. However, the complex human cells interact with 3-dimensional (3D) microenvironments, and the 2D biomaterial microarrays are not adequate to represent the complexity of cell microenvironments in the human body. Therefore, there have been continuous efforts on developing high-throughput screening platforms with 3D biomaterial microarrays, which are capable of applying bioactive physical stimuli with temporally controllable biochemical cues by integrating microfluidics to understand accurate 3D cellular behaviors. For the continual monitoring of cell and tissue behavior according to the interaction between biomaterials and cells, research to integrate high-throughput screening platforms on developing biosensor has received significant attention. The developed high-throughput screening platforms with biosensors allow detailed observation and more predictable assessments of the interaction between microenvironments and dynamic responses of the cells. Moreover, biosensors which are capable of continuous and automated monitoring of cellular responses over a long period of time make it more suitable for testing chronic drug response [15].

In this review, we introduce the leading-edge developments of high-throughput approaches for monitoring interactions between vast combinations of microenvironments and cellular behaviors. Three approaches to high-throughput screening are introduced. Firstly, screening of biochemical microenvironments including 2D and 3D biomaterial microarrays with integrated microfluidic systems. Secondly, screening effects of biophysical environments covering the effects of micro/nano topography and stiffness, and active mechanical and electrical stimuli on cellular responses. Lastly, biosensors for the investigation of integrated cellular responses in vast combinations of microenvironments are reviewed. Furthermore, forthcoming challenges to developments of high-throughput platforms and future perspectives are discussed.

2. SCREENING OF BIOCHEMICAL MICROENVIRONMENTS

2.1. Biomaterial Microarrays

Tissue culture-polystyrene (TCPS) is currently regarded as a gold standard of cell culture platform for various cell types. How-

*Address correspondence to this author at the School of Electrical and Electronic Engineering, Yonsei University, Seoul, 03722, Republic of Korea;
E-mail: jungmok.seo@yonsei.ac.kr
[†]*These authors contributed equally to this work.*

1873-4286/18 $58.00+.00

ever, uncontrollable chemical and physical property of TCPS could regulate cell fates in unexpected directions. For example, Yang *et al*. lately verified the new finding that human mesenchymal stem cell (hMSC) fate could be regulated by their previous culture time on TCPS because cells memorize their past physical microenvironments via Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding domain (TAZ) signaling pathway. Consistent exposure to culture microenvironments activates YAP-TAZ signaling pathway which leads to constitutive activation promoting hMSC's multipotency towards osteogenesis [16]. In addition, cellular chemical microenvironments need to be controlled by coating TCPS with cell type-specific ECM proteins or adding soluble bioactive molecules into the culture medium for long-term preservation of pluripotent human embryonic stem cells (hESCs) [17]. In an attempt to solve the challenges of maintaining cellular microenvironments, and prolong its functionality, various biomimetic biomaterials have been investigated biomimetic of cell microenvironments to modulate its behavior. Particularly, hydrogels with synthetic or natural ECM proteins have received considerable attention due to their high cell viability, ease of chemical modification, and injectable property that can be used to various therapeutic applications [18, 19]. To analyze the cellular behaviors in biomaterials composed of different ECM proteins, various high-throughput approaches have been developed. High-throughput biomaterial screening platform, which is made of hydrogel microarray is capable to carry out simultaneous analysis of sophisticated interactions regarding vast microenvironmental factors and hydrogels. Fabrication of the high-throughput hydrogel microarrays for screening cell behavior involve various methods including a contact printing, soft-lithography, and superhydro-phobic-hydrophilic patterning [20-27]. The contact printing method employs capillary force exerted from hollow pin arrays to move biomaterials into the tips of the array, followed by a tip-substrate contact. Various high-throughput screening platforms have used the contact printing method to analyze interactions between cells and biomaterials, having different chemical compositions [28-30]. For instance, combinatorial screenings of cellular behaviors on the contact printed biomaterial arrays were conducted. Biomaterial arrays composed of different amounts of collagen I, II, IV, fibronectin, and laminin were spotted on to a substrate in order to investigate cell functions and differentiation of murine ESCs, and rat hepatocytes [28, 29]. Similarly, Mei *et al*. employed a high-throughput system for the examination of hESC behaviors on synthetic biomaterials [30]. Biomaterial microarrays with different chemical compositions were printed onto poly(2-hydroxyethyl methacrylate)-coated glass surface via contact printing method. Arrays consisted of 496 different biomaterial compositions was generated from the mixtures of 22 different acrylate-based-monomers with different concentrations and ratios. They investigated how self-renewal property of hESCs was regulated on 2D microenvironments including chemical properties, surface roughness, elastic modulus, and wetting properties. Polymers with high acrylate content with moderate wettability showed enhanced colony formation of hESCs, which represents self-renewal property of hESCs. Gobaa and colleagues developed high-throughput screening platforms using hydrogel microwells (Fig. **1a**) [23]. To fabricate hydrogel microwells, solutions containing different protein compositions were spotted onto silicon micropillars, followed by a contact printing onto partially crosslinked polyethylene glycol (PEG) hydrogel substrate. Through the approach, isolated 2D microenvironments could be generated onto the hydrogel. It was observed that mesenchymal stem cell (MSC) differentiation and proliferation properties could be affected by the composition of ECM proteins, stiffness, and cell densities.

The 2D microarrays of biomaterials could provide effective ways to monitor the interaction between cells and biomaterials. However, cells in the body interact with complex 3D microenvironments, which results in different cellular behaviors [31-34]. Thus, enormous efforts have been directed to advance high-throughput platforms for analyzing the influence of 3D microenvironments on cellular behaviors [22, 35-39]. Dolatshahi-Pirouze *et al*. investigated the correlation between osteogenic differentiation of hMSCs and bone tissue-associated ECM components including fibronectin, laminin, and osteocalcin, in cell-laden hydrogel arrays using the contact printing method (Fig. **1b**). The observation suggests that the incorporating ECM protein components significantly promote hMSCs' osteogenic differentiation. With the data collected from the observation, an optimal composition of the incorporated ECM proteins and growth factor have been figured out for osteogenic differentiation of hMSCs. Besides the contact printing technique, 3D biomaterial arrays also have been fabricated via wettability patterning. Formation of the 3D biomaterial arrays is achieved by applying wettability patterns on surfaces enabling the cell laden biomaterials to be isolated onto the hydrophilic area bordered by hydrophobic region. Superhydrophobic surfaces showing extreme water-repellent property have received attention for various biomedical applications due to their anti-biofouling property [40-43]. The patterning of hydrophilic arrays on superhydrophobic surface provides a facile way for the generation of 3D biomaterial arrays. An innovative protein delivery and profiling method has been proposed by Oliveira *et al* [44]. To overcome the limitations that a conventional analytical method possesses, the wettability patterning of superhydrophobic-hydrophilic regions was used to examine the protein releases from protein matrix. Bovine serum albumin (BSA) was encapsulated in alginate, and placed on the center of the superhydrophobic surface followed by crosslinking. After the mixture being cross-linked, a phosphate-buffered saline (PBS) was dispensed covering the alginate hydrogel on each patterned surfaces (Fig. **1c - i**). The quantity of the released protein was measured by florescence imaging technique obtaining comparable data to conventional on-chip protein releasing methods. Beside the quantification of protein released, the device is also capable of visualize the distribution of the proteins over time (Fig. **1c – ii**). The chip-based assay allowed precise control over protein release via contrasting wettability. Similarly, Salgado *et al*. fabricated microarray with alternating hydrophilic and superhydrophobic patterns to develop high-throughput screening platform [45]. Square-shaped hydrophilic array patterns were formed onto a superhydrophobic polystyrene (PS) substrate by using a plasma treatment. Two cell types including fibroblasts and pre-osteoblast cells were studied with 24 different hydrogel compositions, composed of a mixture of alginate, chitosan, collagen, and hyaluronic acid, to investigate cellular behavior. The two cells were encapsulated into the hydrogels and their cytocompatibility and metabolic activity were investigated. The study not only highlights the performance of the contrasting wettability patterns of superhydrophobic, and hydrophilic arrays, but also non-destructive analysis of the platform which is promising for complex investigation of 3D biomaterial microarrays-cells interaction. Similar wettability patterning methods have been developed to generate separated droplet microarrays, which can be potentially used to various high-throughput screening platforms [46-49]. Recently, Li *et al* developed surface-wettability guided assembly (SWGA) method which is used to create spatially engineered of 3D biological microenvironments (Fig. **1d**) [39]. In order to apply wettability patterns on hydrophilic glass surface, lithography was done to a microarray patterned thin polydimethylsiloxane (PDMS) layer assembling the layer with a glass substrate. Cell-laden biomaterial arrays were precisely patterned via wettability difference between the hydrophobic, and hydrophilic surfaces. The SWGA method enables fabrication of various shaped hydrogels containing different cell types. Furthermore, heterogenous microenvironments were achieved by assembling interconnecting hydrogel arrays from two substrates with varied shapes. The aforementioned methods for the manufacture of biomaterial microarrays provide the individually and spatially engineered cellular microenvironments, which are also useful techniques for the development of new biomaterials and drugs.

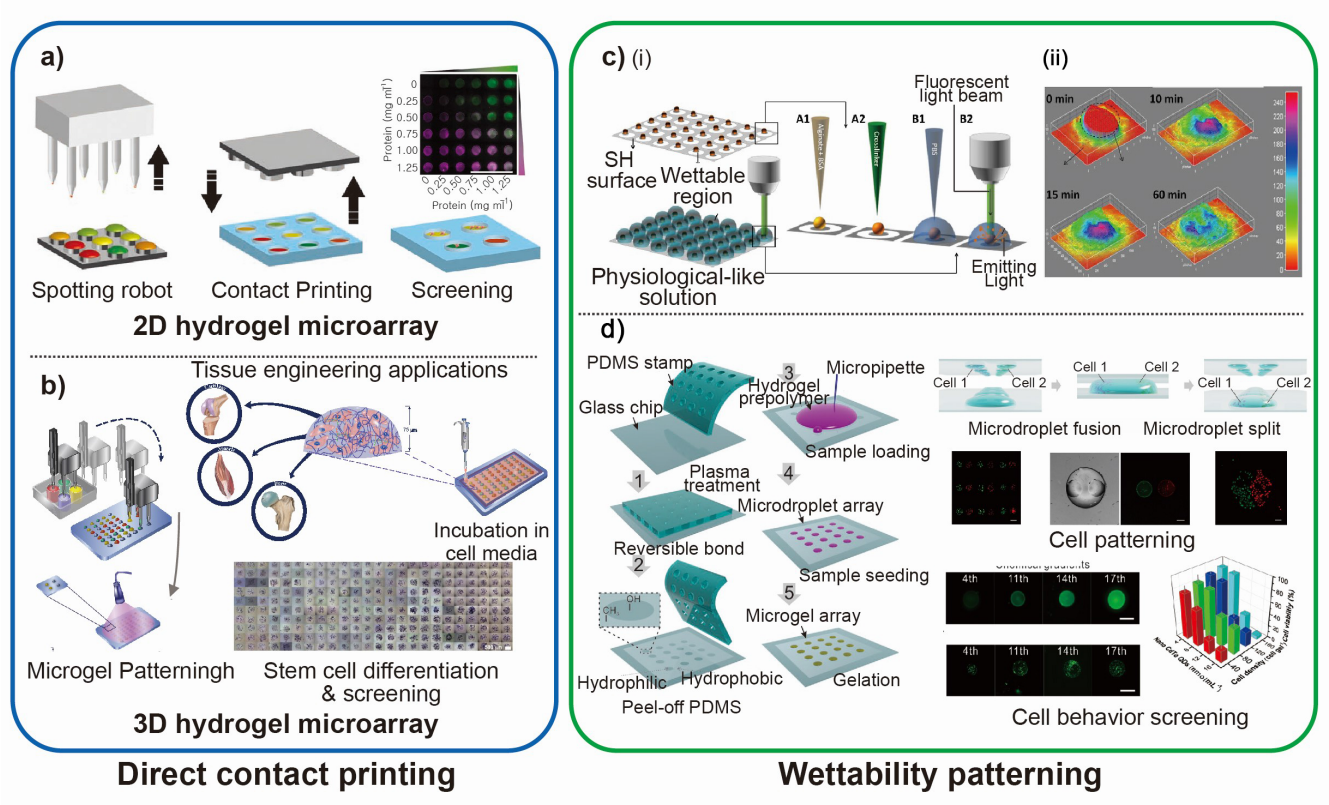

Fig. (1). Formation of biomaterial mircroarrays for high-throughput screening of cellular behaviors in different chemical microenvironments. **a**) Fabrication of 2D hydrogel microarrays using contact printing method to fabricate artificial isolated niches. **b**) Contact printed cell-laden 3D hydrogel arrays to investigate osteogenic differentiation of hMSCs. **c**) (i) 3D hydrogel array on superhydrophobic-hydrophilic patterned substrate for high-throughput screening of cellular behavior. (ii) distribution of proteins across 3D hydrogel over time. **d**) Surface-wettability-guided assembly (SWGA) of 3D hydrogel arrays for heterogeneous cell patterning and cell behavior screening. Reproduced with permission of: Nature Publishing Group, ACS Publications, and John Wiley & Sons.

2.2. Microfluidic Systems

For the last decade, basic biological studies and drug discovery have flourished due to newly developed microfluidic systems as emerging cell culture platforms, because the complexly designed microchannels mimic various biological environments that cannot be achievable by using conventional cell culture platforms [50-52]. Particularly, microfluidic systems can spatially and temporally control cellular biochemical microenvironments by flowing cell culture media with desired water-soluble bioactive molecules and chemical compounds. Recently, researchers have developed microfluidic high-throughput screening platforms to investigate cellular behavior in response to their spatially and temporally controlled chemical microenvironments. By utilizing advection, random Brownian motion, and laminar flow of liquids, well-controlled chemical gradients can be generated [53, 54]. The microfluidic high-throughput screening platform typically contains a microfluidic cell culture chamber, integrated with a chemical gradient generator to apply chemical gradients to cells [55, 56]. For example, Awwad *et al*. developed microfluidic high-throughput screening platform using a long perfused cell culture chamber with a chemical gradient generator [56]. The chemical gradient generator was created by using multiple ramified microchannels, connected to the cell culture chamber. The chemical gradient generator enabled the spatial control of chemical gradients inside the cell culture chamber by utilizing Laminar flow and diffusive mixing. The concentration of stimulant, Interleukin-1β (IL-1β), could be spatially controlled from 0.005 to 0.1 ng/mL within the cell culture chamber and the cellular response of Chinese Hamster Ovary (CHO) cells was investigated. The chemical gradient generator could also be integrated with hanging-drop culture platform for screening 3D cell spheroids behavior according to the spatially controlled chemical gradients. 3D cell-laden hydrogel construct into microfluidics was fabricated via diverse physical and chemical cross-linkage. Ostrovidov *et al*. reported a microfluidic device for fabricating hydrogels with a concentration gradient of target drugs [57]. In order to make drug concentration within the hydrogel prepolrymer solution, researchers injected PEG-diacrylate (PEGDA) hydrogel prepolymer solution with the drug into the chemical gradient generator. The microfluidic device contains a microfluidic gradient generator with its chamber, two inlets connected to a microchannels for controlled drug concentration via diffusion and an outlet **(Fig. 2a – i)**. After the injection of the PEGDA prepolymer solution, photocrosslinking process is carried out to make hydrogel constructs with desired drug concentration. They further characterized drug release profiles of drug-containing hydrogels and toxic effects of okadaic acid on cells **(Fig. 2a – ii)**. Frey *et al*. investigated various 3D cellular behavior including metabolic activity and cell-to-cell interactions according to the cell type and perfusion configuration via chemical gradient generator integrated hanging drop culture platform [58]. The hanging drop culture system is composed of a gradient generator connected to a four-by-four culture array for continuous flow **(Fig. 2b – i)**. Multicellular spheroids were formed at the bottom surface of 'hanging drop' culture platforms via injecting cells through cell loading ports. Spatially controlled chemical gradients could be perfused to the formed 3D cell spheroids through microfluidic channels **(Fig. 2b – ii)**. The time-dependent spheroidal growth, and its fluorescence imaging were also investigated over 60 hours **(Fig. 2b – iii)**. Similarly, Occhetta *et al*. developed microwell embedded microfluidic system to observe the effects of growth factors on 3D hMSC behavior in high-throughput manner [59]. They observed that the proliferation of hMSCs was significantly increased compared to the

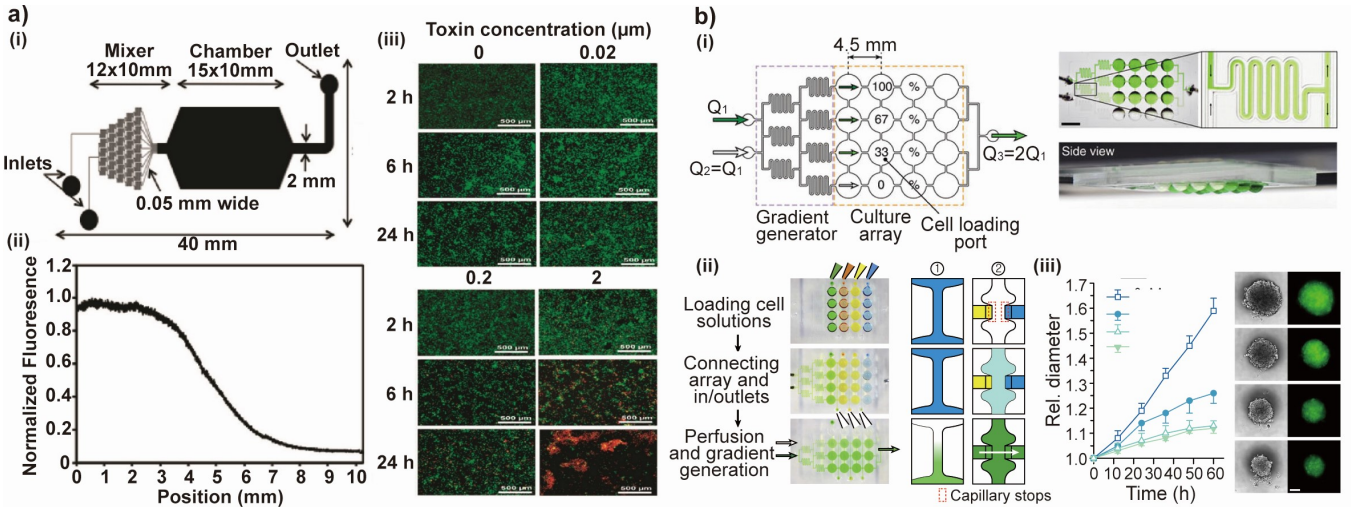

Fig. (2). Microfluidic high-throughput screening platforms a) Spatial control of biochemical microenvironments by using a microfluidic concentration gradient device for the investigation of single CHO/GFP-NFjBp65 cell nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) dynamics. **b**) (i) Microfluidic-based hanging-drop culture system for screening 3D cell spheroid behavior in a high-throughput fashion. (ii) Three-step working principle of the hanging-drop culture system. (iii) Metabolic activity of cell spheroids and inter-organ interaction were investigated by varying nutrient supply and chemical dosage. Reproduced with permission of: ACS Publications, and Nature Publishing Group.

static 2D culture and screened growth factors induced chondrogenic differentiation of hMSCs. Biomimetic 3D hydrogel arrays have introduced to microfluidics to induce spatially controlled chemical microenvironments [60, 61]. Similarly, human lung epithelial (A549) cells, and PEG hydrogel encapsulated hepanoma (HepG2) cells were generated in the microfluidic device via photolithography to investigate cellular responses to anticancer drugs by Gao *et al* [62]. Microfluidic devices allow a simple way to spatially control chemical microenvironments of cells via perfusion of a controlled drug and chemical compositions during cell culture. Further, microfluidic devices allow an in-depth understanding of various physiological processes in native tissues due to their microchannel mimicking biological environments. Thus, microfluidic system combined with biomaterial microarrays can be potentially used as a universal platform to investigate tissues' developmental process, drug efficacy, and stem cell differentiations regarding their temporally and spatially controlled chemical microenvironments in a high-throughput fashion.

3. SCREENING THE EFFECTS OF BIOPHYSICAL MICROENVIRONMENTS

Not only the biochemical microenvironments but also biophysical microenvironments regulate cellular behavior. Cells sense their physical microenvironments, such as surface topography, ECM stiffness, and elasticity through surface bounded focal adhesions that activate intercellular pathways. The activation process of intercellular pathways via the sensing of biophysical cues is termed mechanotransduction [9]. For example, the nano- and micro-topography of surface direct human neural stem cells (hNSCs) to functional neuronal cells through Rho-associated protein kinase pathway, and β1 integrin-mediated binding [63, 64]. Whereas, the stiffness of ECM can determine stem cell fate [65]. In addition to these static biophysical cues, active biomechanical and electrical stimuli could promote cell functions and stem cell differentiation efficiency [66, 67]. These biophysical cues synergistically affect to cells, however, conventional approaches are not suitable to investigate cellular behavior upon the influences of complex biophysical microenvironments. Here, we introduce recent advances in high-throughput screening platforms to aid investigation of the effects of biophysical microenvironments including topography, stiffness, and active mechanical and electrical stimuli on cellular behavior.

3.1. Micro- /Nano-topography and Stiffness

Micro- and nano-topography of substrate regulate differentiation of stem cells including human induced pluripotent cells (hiPSCs), hESCs, and hMSCs [68-70]. In addition, nanometer scale holes on cell-repellent surface direct cell migrations and organization [71]. To investigate the cellular behavior on the surfaces with micro- and nano-patterns, Unadkat *et al*. developed a 'Topochips' containing 2,176 different topography patterns from the combination of three primitive shapes (Fig. **3a**) [72]. Polyactic acid (PLA) films were used to fabricate micro-topography by using hot embossing methods. The combination of patterns which was able to keep its pluripotency or improved osteogenic differentiation of hMSCs was screened. Recently, Hu and his colleagues have developed 96-well plate platform to screen cellular behavior on various micro- and nano-topographic patterns (Fig. **3b**) [73]. The platform was fabricated by bonding micro- and nano-topography patterned PDMS layer with a commercial standard bottomless microplate via aminosilane-mediated treatment, and oxygen plasma treatment. The developed platform is compatible with conventional multiwall plated-based cell culture and analytical tools. The topography patterned surface was covered with antibodies including anti-CD3 and anti-CD28 to screen the activation and proliferation of T cells on different micro- and nano-topologies. The results demonstrated increased interleukin 2 (IL-2) cytokine production of T cells on trench-grid surfaces with 200 nm trench width and > 2.3 μm grating pitch. On the other hand, IL-2 production level diminished at the surface with 100nm width and <0.5 μm pitch. They found that IL-2 cytokine production could be further enhanced by adding blebbistatin. The platform could provide the insight to design biomaterials and scaffolding system that can enhance clinical efficacy of immunotherapy.

The stiffness of matrix and interfacial geometry of cells also direct cellular behavior. For example, Englar *et al*. investigated that stem cell lineage specification process depends on substrate stiffness [74]. They investigated the stem cell differentiation of hMSCs on polyacrylamide gels having three different stiffness (1, 10, and 100 kPa) for mimicking physiologically relevant stiffness of brain, muscle, and bone, respectively. Cell morphologies and gene expression showed that hMSCs differentiate to specific cell linages on hydrogels with relevant stiffness. As a high-throughput approach, Mih *et al*. fabricated polyacrylamide hydrogel microarrays with

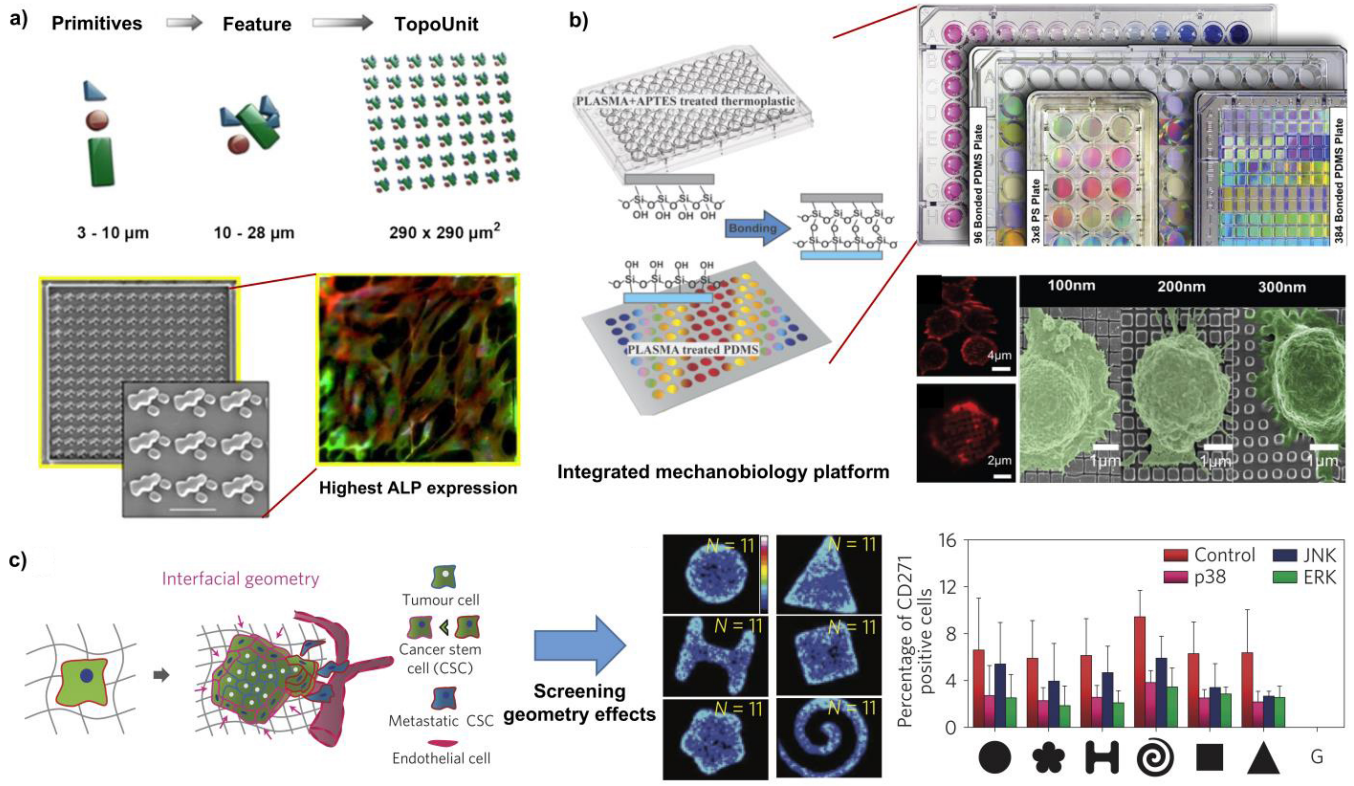

Fig. (3). High-throughput approaches to investigate cellular behavior in response to their topology. a) Fabrication of micro-topography patterns by using three types of primitive shapes to find the best combination for osteogenic differentiation of hMSCs by evaluation ALP expression, an early osteogenic differentiation marker. Scale bar = 20 μm. **b**) Mechanobiology screening platform by the integration of micro- and nanotopography patterns with commercial well plates. **c**) Screening cancer tissue behavior according to their geometric features. The interfacial geometry modulates cell shape, adhesion, and pluripotency of cancer cells. Reproduced with permission of: United States National Academy of Sciences, and Nature Publishing group.

different substrate stiffness in commercial 96 and 384 well plates [75]. By adjusting crosslinking ratio, a substrate stiffness ranging from 0.3 to 55 kPa could be achieved. Using the platform, cellular behavior of 7 different cell types was investigated.

Cancer stem cells (CSCs) has known that it plays crucial role during the metastases process of cancer, although only small percentage of CSCs are existed within a tumor [76]. This small population of CSCs in tumor is believed to be causing recurrence of cancer after surgical treatment and chemotherapy [77, 78]. Thus, understanding cell behavior of CSCs in response to their microenvironments are crucial to predict disease progression and develop effective anticancer drugs. Recent findings suggest that the biophysical cues, such as stiffness and interfacial geometry can influence CSCs behavior in a diverse way [79, 80]. Lee *et al*. developed micropatterned hydrogel array platform to investigate synergistically effects of perimeter geometric cues and stiffness on cellular behavior of CSCs [81]. Micropatterned hydrogels with 11 different shapes and stiffness was used to investigate how the synergistic biophysical cues influences CSC behavior and tumorigenicity (Fig. **3c**). They observed that the population of CSCs is highly dependent on geometric features of tumor tissue, which increased tumorrigenicity both *in vivo*, and *in vitro*. Regardless of the microhydrogel shapes, level of CSC markers was increased. This is a consequence of the localized concave, and convex structure at the periphery. Spiral shaped hydrogel showed the most increased expression of CSC marker, compared to other shapes. Stiffness of the matrix was not significantly affect to the CSC marker expression. The results demonstrate that how the geometrical features of tumor tissue drive normal cancer cells toward CSC-like phenotype, which may help to investigate cancer metastasis and develop personalized anticancer drugs.

3.2. Active Mechanical and Electrical Stimuli

In addition to the aforementioned passive microenvironmental cues, temporarily applied external stimuli, such as biomechanical strains and electrical signaling cues affect cell behavior [82-84]. In the body, many organs dynamically move during the daily life and organ functions are governed by electrical signaling from the brain. Thus, screening effects of external active stimuli on cell behavior is crucial to design new biomaterials for therapeutic applications. It has been investigated that the stem cell differentiation efficiency of MSCs into functional of load bearing musculoskeletal tissues could be significantly enhanced by mechanical stimulation [85-87]. However, the relationship between the applied strain (magnitude, time, and frequency) and corresponding cellular behavior is still unclear. To investigate the correlation between the two, high-throughput biomechanical stimulation device has been developed by Moraes *et al*. Application of compressive strains to 3D cell-laden hydrogel microarrays was achieved by the developed high-throughput system [88]. The device contains individually controllable actuating micropillars, which enables applying precisely controlled compressive strains to cell-laden hydrogels. Deformation of cell nuclear under compression was characterized by applying dynamic compressive strain ranging from 0% to 26% to PEG hydrogel. Furthermore, continuous tensile stress was applied onto 3D cell encapsulated hydrogels which act as human tissues including muscles, blood vessels, hearts. To investigate changes in 3D cell encapsulated hydrogel arrays upon applied tensile stress, Liu *et al*. developed a deformable membrane device [89]. To adhere hydrogel to the actuating PDMS membrane, a tiol-ene reaction was utilized between 3D PEG norbornene (PEG-NB) hydrogel and off-stoichiometry tiol-ene based PDMS. Due to the stable and strong bonding between the

hydrogel and membrane, cell-laden hydrogel arrays were stable under dynamic strains for several days. The developed arrays allowed in-depth examination of the 3D cellular behavior of hMSCs. Recently, Li *et al*. fabricated a hydrogel array platform which can control applied strain via magnetic force varying from 0 to 60%. The developed platform can cover almost all physiological ranges of tissues **(Fig. 4a – i)** [90]. cell behavior in the 3D microenvironments were then compared with the cell in static condition, and it showed significant difference in behavior of fibroblsts and myofibers including proliferation, spreading, and differentiation **(Fig. 4a – ii)**. In addition, researchers studied time-dependent behavior of 3D fibroblasts in dynamic strains (~60%) over 7 days **(Fig. 4a – iii)**. The results provide an insight on how cells behave upon active biomechanical stimuli in 3D.

Significant efforts have been made to perform combinatorial screening of complex cell behavior in response to active mechanical stimuli. However, the aforementioned high-throughput screening platforms with mechanical loading ability only address few combinations of active mechanical input and are not able to screen synergistic effects of biomaterial composition and dynamic mechanical stimulation. To address it, Seo *et al*. developed interconnectable bioreactors containing multiple actuating microposts operated by gas pressure. The actuating microposts allow accurate screening of 3D hydrogel with different material compositions by precisely controlled compressive strains. The combinatorial high-throughput screening of 3D cellular responses can provide accurate prediction on how cell differentiate in dynamic mechanical stimulation [91]. Each stack of a device is fabricated in three different components including a 3-(trimethoxysilyl) propyl methacrylate (TMSPMA) treated glass substrate, a media chamber, and a nitrogen gas (N_2) pressure chamber, which make hydrogel adhesively stable during dynamic compression **(Fig. 4b – i, ii)**. For hydrogel array, the patterning process is done by injecting prepolymer mixture containing Gelatin methacryloyl (GelMA) and hMSCs into the media chamber then UV light and a photomask are applied to selectively photocrosslink the mixture onto the posts. 3D hMSC responses such as cell spreading and osteogenic differentiation were screened in GelMA hydrogels with varying concentration via the interconnectable bioreactors. The result based on cellular imaging, biochemical analysis, gene expression analysis and histological analysis demonstrates the optimal conditions of cells to initiate cell spreading and osteogenic differentiation, and such behavior is significantly influenced by the changes in GelMA hydrogel concentrations and dynamic compression **(Fig. 4b – iii)**. The developed high-throughput bioreactor in which combinatorial cellular response can be screened can potentially allow diverse applications including *in vitro* organ-on-a-chip device, which specifically mimic mechanically active soft tissues including blood vessels, muscles, cartilage, and tendons or drug screening and toxicological applications due to its compatibility with a various hydrogels composition.

Beside active mechanical stimulation, the cell behavior including cell proliferation and differentiation can be influenced by electrical stimulation [92, 93]. In addition, several studies have shown that naturally occurring endogenous electrical cues can involve tissue morphogenesis during developmental process. Consequently, the electrical stimulation is one of the most significant microenvironmental factors that can affect direction of cell fate and function. Recently, Jin and colleagues investigated on reprogramming process of fibroblasts to functional neuronal cells and role of electrical stimulation in the process [94]. The Triboelectric nanogenerator utilizes kinetic energy from frictional movement to generate biphasic electrical current pulse (1 Hz, ~270 nA). The research has shown that the *in vivo* and *in vitro* cell reprogramming efficiency can be improved via electrical stimuli and a nonviral delivery of specific transcription factors for neuronal lineage.

As mentioned above, the electrical stimuli is a significant factor in understanding cellular responses. However, investigation of optimum electrical stimuli improving cellular behavior in high-throughput manner has not conducted, yet remained unexplored. In order to develop a high-throughput platform that can monitor electrical signals, while applying electrical stimulation, Dai *et al* fabricated 3D flexible nanoelectronics interfacing 3D human cardiac tissue **(Fig. 4c - i)** [95]. The nanoelectonic devices are consisted of electrode arrays with sensors which can individually address electrical stimuli to apply electric signals across 3D cardiac tissue contruct **(Fig. 4c – ii)**. Due to the array configuration, action potential propagation of human heart tissue was spatially manipulated via electrical stimuli (1 V, 1.25 Hz) which can possibly address current limitations of sensing platforms including optical voltage sensing. The miniaturization of the nanoelectronic platform can provide various advantages in drug discovery and developing new regenerative medicine as in-depth investigation on 3D engineered tissue models such as blood brain barrier, blood vessel regeneration, neuronal network became possible.

Current High-throughput approach focus on screening the influence of active biophysical stimuli, stiffness, and ECM compositions towards 2D and 3D cellular behavior. The promising results allow in-depth research on the cells and surrounding 3D biological microenvironments with minimal resources and efforts required. However, screening and analysis of cellular behavior at the same time still remains as challenges. For developments of true high-throughput platform, standardized platform which is compatible with prevalent cell culture techniques, and analysis methods, and capable of *in situ* monitoring is highly demanding. In addition, closer collaboration among medical professions, biologist, and engineers is needed from the designing step to widespread high-throughput screening platform. The advanced high-throughput platform can provide new insights for a variety of research area from basic biology study to translational clinics.

4. INTEGRATION OF BIOSENSOR WITH ORGANS-ON-A-CHIP PLATFORMS

As the development of high-throughput screening platforms provides an efficient tool for combinatorial screening of cellular behavior, there have been growing interest and needs to develop microfluidic biosensor and organs-on-a-chip platforms with integrated sensor enabling continual monitoring of cellular behavior for *in vitro* sequential analysis of cell behavior upon the changes of dynamic microenvironments [96]. Changes in physiochemical properties are known to cause unwanted variations in the physiology of the organoids, which are crucial in predicting cellular responses from organs-on-a-chip models. For example, changes in extracellular acidity (pHe) may cause malfunction of biological processes including contraction of cardiac muscles, and immune function [97]. In addition, oxygen delivery plays an significant role in maintaining cell function [98]. For these reasons, maintaining, and monitoring the levels of such physiochemical properties are essential to ensure reliable prediction of the complex cellular responses to the 3D microenvironments successfully. Seyed *et al*. developed optical sensing module for analysis of multiple analytes with a microfluidic bioreactor [96]. The developed module is a single chip composed pH and oxygen sensor, which is packed by using PMMA plates and double-sided adhesive. Real-time pH monitoring is achieved by the pH sensor detecting the level of light absorbed by culture medium which contains phenol red, and the oxygen sensor made of gas-permeable PDMS simultaneously monitoring the changes in oxygen level by measuring the level of quenching in a fluorophore, [Ru(dpp)3]2+Cl2–tris(4,7-diphenyl-1,10-phenanthroline)-ruthenium(II) chloride **(Fig. 5a – i)**. In addition, pH sensitivity was tested conducting step experiment and it showed its great sensitivity, as well as selectivity in changing pH **(Fig. 5a - ii)**. The developed optical sensor allows real time and continual monitoring of physiochemical properties of cell production with minimal labor involved in a non-invasive manner.

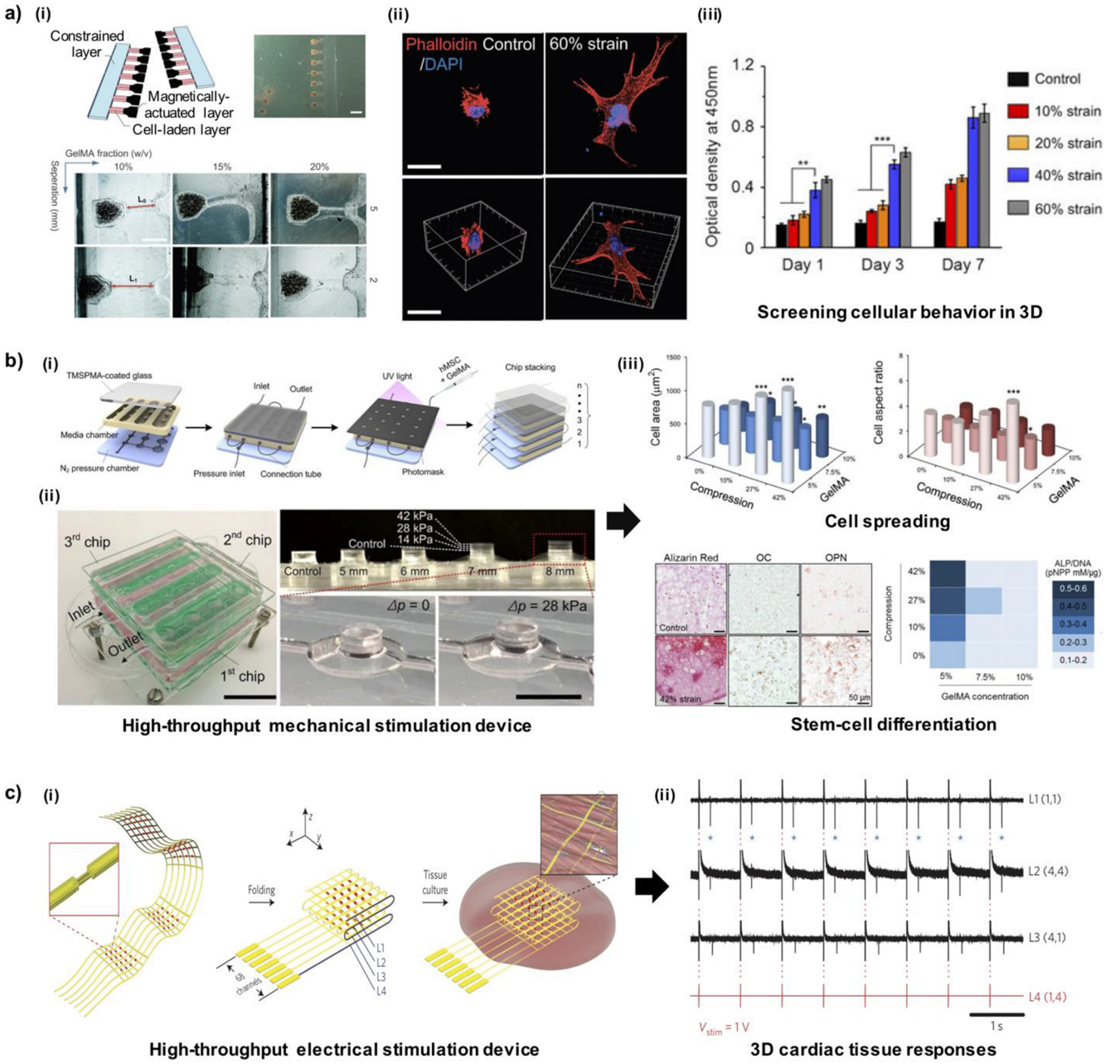

Fig. (4). High-throughput screening platforms to investigate cellular responses to applied biomechanical and electrical stimuli.
a) (i) Magnetic stretching and degrees of separation from applied magnet of μMACs in different GelMA concentration (ii) 3D image of encapsulated fibroblasts under different strain condition (iii) Changes in cell proliferation according to time and strain level (~40%) **b**) (i) Fabrication of interconnectable bioreactor to investigate mechanical strain-induced cellular responses in 3D (ii) schematic of magnetically actuated microarrays for precise control of dynamic compression (iii) cellular behavior in dynamic compression **c**) (i) Flexible nanoelectronics for mapping and regulation of 3D tissue construct. (ii) Time-dependent responses from nanoelectronics under biphasic stimulation. Reproduced with permission of: Nature Publishing Group, and ACS Publications.

Despite of recent improvement in microfluidics and screening platforms, continual and automated monitoring of the sample especially in long-term 89culture or drug screening processes remains as a development in progress. Information about cell-secreted biomarkers and status of these organoids is important in understanding and maintaining microenvironments for accurate prediction of human cell and micro-tissue behavior [99]. Hence, prosperous probing method to detect biomarker secretion during long-term culture is required. Riahi *et al*. investigated capability of continual and long-term screening platform by developing a programmable bead-based microfluidic electrochemical (EC) immunosensor [100]. The EC immunosensor utilizes magnetic microbeads (MB) in which the behavior of the MB can be controlled with magnets. Use of disposable MB allows continual monitoring by simply disposing the microbeads after each analysis and larger surface areas to immobilize the targeted biomolecules in large quantities. the MB gets functionalized with primary antibody which then is loaded and immobilized in the reaction chamber. The immobilized MB is led into the chip forming covalent bond do an enzyme called Streptavidin HRP (SA-HRP) resulting in oxidation of 3,3',5,5'-tetramethylbenzidine (TMB). Introduction of the oxidized TMB into the detection chamber results in detecting the amounts of biomarkers present in the

sample via sensing electrodes. The result of the study demonstrated a limit of detection of 0.03 ng/mL, that is significantly lower compared to enzyme-linked immunosorbent assay (ELISA) (0.2ng/mL). Traditional methods including ELISA and mass spectroscopy (MS) are served as a viable tool for label-free biosensing of biomarker detection, however, these existing methods have limited usage due to its insufficient sensitivity and selectivity while conducting experiments on complex biological environments. Therefore, high sensitivity and selectivity is demanded for biosensors monitoring cellular responses and corresponding microenvironments. Shin *et al*. adapted an aptamer instead of antibody on biosensor to detect myocardial injury biomarker known as creatine kinase (CK-MB) from microfluidic heart-on-a-chip organoid **(Fig. 5b – i)** [101]. Aptamers, which are nucleic acids widely used to detect and quantify various analytes including proteins, have few advantages over conventional antibodies when measuring low levels of soluble biomarkers [102-109]. In comparison to conventional antibodies, aptamers have more uniform deposition over the surface of the sensor due its aggregation [110, 111]. Furthermore, functional groups can be easily attached to aptamers giving desirable properties. Also, aptamer-based sensor demonstrates relatively stable sensitivity and selectivity regarding the changes in environments such as pH, temperature, and interferents [112, 113]. The biosensor is composed of a microfluidic cardiac reactor containing cardiac spheroids cultured from ESC derived cardiomyocytes (ESC-CMs) in a hydrogel. The cardiac spheroids are then photo-crosslinked at the bioreactor surface generating cardiac-spheroids-based heart-on-chip platform. In order to monitor the changes in level of CK-MB which is expected to be secreted upon the exposure of cardiomyocytes to cancer drug, doxorubicin (DOX) is one of the common cancer drug introduced to the platform. DOX-dosed organoid demonstrated decreased level of beating rates and cell viability, and the amounts of CK-MB secreted **(Fig. 5b – ii, iii)** Heart-on-a-chip device was then integrated with the biosensor for *in situ* monitoring allowing easy usage and screening in automated manner. The developed aptamer-based microfluidic biosensor, which is the first integrated sensor for detection of cell-secreted biomarker without any off-chip processing, demonstrates significantly high sensitivity and rapid measurements compared to conventional antibody-based biosensors. In addition, the aptamer-based approach can be applied to other organ-on-a-chip device beside heart-on-a-chip in which continual in situ monitoring of biomarker with high sensitivity is required.

Recent approaches to biosensor integrated high-throughput platforms not only emphasize the sensitivity and selectivity of the biosensor but also its regenerative features. Conventional biosensing platforms such as ELISA and MS, as well as electrochemical, and fluorescence-based sensor involve excessive proteins and interfering compounds causing inherent saturation of the surface with target molecule bindings [114]. Therefore, the microfluidic biosensor with incorporated surface regeneration capability is required for fully-automated and continual monitoring of biomolecules or human cells under complex biological microenvironments. Shin *et al*. developed label free electrochemical microfluidic biosensors which can terminate the saturated reaction products of antibody-antigen reaction after cycles of screening in an automated manner [115]. EC biosensor utilizes microelectrodes to continually monitor biomarkers secreted from liver organoids including albumin, and glutathione-S-ransferase-alpha (GST-α). The EC sensor involves strong binding interactions including self-assembly monolayer (SAM), SPV -biotin, and antibody-antigen. In order to monitor target biomolecules in both continual and automated manner, the saturated electrode surface requires cleaning process. Therefore, excessive remaining molecules on the electrode gets removed with thin Au layers by EC desorption method, which applies etching solutions onto the electrode surface and electrical potential at >±1V. They further investigated the way to optimize the cleaning process, and they treated the surface with H_2SO_4, and $K_3FE(CN)_6$ exposure under electrical sweep. With appropriate applied potential and use of sulfuric- and cyanide-based cleaning solutions, majority of the thiol-Au bonding was broken resulting in removing most of the antibody/antigens. In addition, the electrical current of the regenerated electrode a was found to be close to 100% of the electrical potential of the as-fabricated electrode sets. Although the microelectrodes that have been regenerated only last for finite cycles of the screenings due to diminish in thickness, it contributes to automation of the EC biosensor allowing continual monitoring of the biomarkers in complex microenvironments. The finding demonstrates unprecedented and efficient approach to solve inherent surface saturation due to biomolecules-surface interaction by regenerating steps involving etching solutions and electrolysis.

Traditional approaches to microfluidic-integrated biosensors enable comprehensive evaluation of the cellular behavior observed in single organs-on-a-chip device, however, such system is not adequate to represent complex interactions between human organs, and the complex human cell behavior from the interaction. Thus, the prediction based on the biosensor may turns out to be inaccurate. To address the issue, multisensor-integrated organs-on-a-chip device was developed [116]. The modular organs-on-a-chip platform controls microfluidic flows in a breadboard via built-in microfluidic channels and pneumatic valves. In addition, it contains microbioreactors for culture of organoids, a set of different sensing modules including sensing modules for microenvironment parameters such as pH and temperature, and multiple EC sensing devices detecting cell-secreted biomarkers from organoids in housing microbioreactors, and a bubble trap **(Fig. 5c - i)**. The fully automated screening was achieved by integrated microchips specifically designed to allow high sensitivity, and long-term observation on organoids in the platform. In addition, it adopts the two-step regeneration processes involving etching the electrode surfaces breaking bonds between gold electrode and antibodies and removing the Au atom layer on the surface, and then applying electrical current to regenerate the electrode surfaces. In the research, two organ platforms were constructed to monitor chronic drug responses and acute toxicity of biomarkers involving Albumin, GST-α, and CK-MB upon exposure to acetaminophen (APAP), a non-steroidal anti-inflammatory drug, which causes hepatotoxicity via necroapoptotic mechanisms **(Fig. 5c-ii)** [117]. For the observation, Cell viability of the cell-secreted biomarkers upon different amounts of toxic doses were screened for up to 5 days. Interestingly, biomarkers secreted from liver organoids including albumin, and GST- α, demonstrated dose-dependent behavior resulting in decreases and increases in secretion level. Whereas, level of secreted CK-MB from cardiac organoid showed minor elevation. Furthermore, the liver-and-heart-on-a-chip device was investigated on DOX dose which is a popular chemotherapeutic drug for diverse cancer. The non-invasive EC biomarker analysis allow continual monitoring of changes in dual-organ platforms in an automated manner, which also can be applied to other organ platforms promoting better drug screening performances. Meanwhile, the several limitations such as usage of PDMS which is not suitable for the organ-on-a-chip device as potential adsorption and absorption of molecules such as drugs can occur. In addition, the highly complex device with relatively large size should be acknowledged and require further research to improve.

Current biosensor including EC biosensor based on microelectrodes measures signal from single or multiple organ models at a time, and it allows label-free, sensitive, and continuous monitoring of cellular responses in unaffected complex biological conditions. The investigation on single or multiple cell behavior has promoted further integration of microelectrode, which is a sensing component, into microarrays for accumulations of a comprehensive library of cell behavior from engineered biomaterial microarrays. Zhang *et al*. introduced high-density microarray with individually addressable electrodes [118]. The electrochemical droplet microarray (eDMA) is composed of Au bands which plays a role as wire and electrode for up to 20 electrochemical cells, and other two sets of

electrodes. The surface of the electrode bands sets is coated by a chemically functionalized permeable porous polymethacrylate layer which forms contrasting superhydrophobic-superhydrophilic patterns on the surface. Due to the wettability differences between the spots and the background, discontinuous dewetting occurs which leads to a quick and easy formation of numerous droplets in every superhydrophilic surface by simply dragging source droplets over the microarray while stopping ion transport and analyte diffusion. The one-step separation of the microdroplets allows individual addressability of the eDMA and prevents cross-contamination intrinsically. The changes in EC behavior of the coated electrode sets was investigated in aqueous electrolytes, and it demonstrated the full solvation of the polymers by the aqueous electrolyte, thus the EC behavior remains unchanged. The EC microarrays with integrated electrodes demonstrate various advantages of the platform over conventional microarrays including reversibility of the EC property, simple droplet manipulations via porous coating layer, and individual addressability of the sensor allowing various applications in drug screening, biochemical, and living cell monitoring.

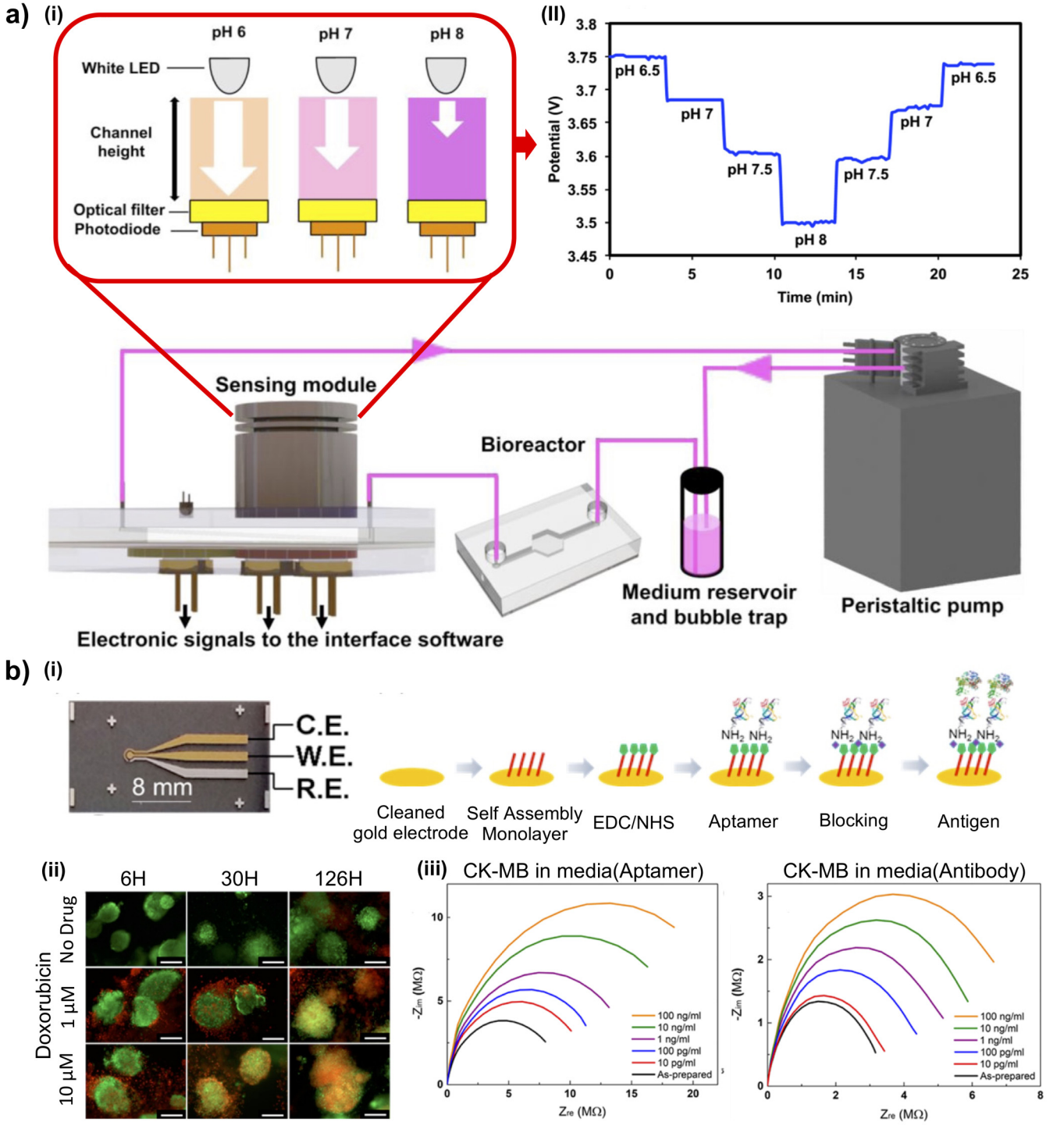

Fig. (5) Contd….

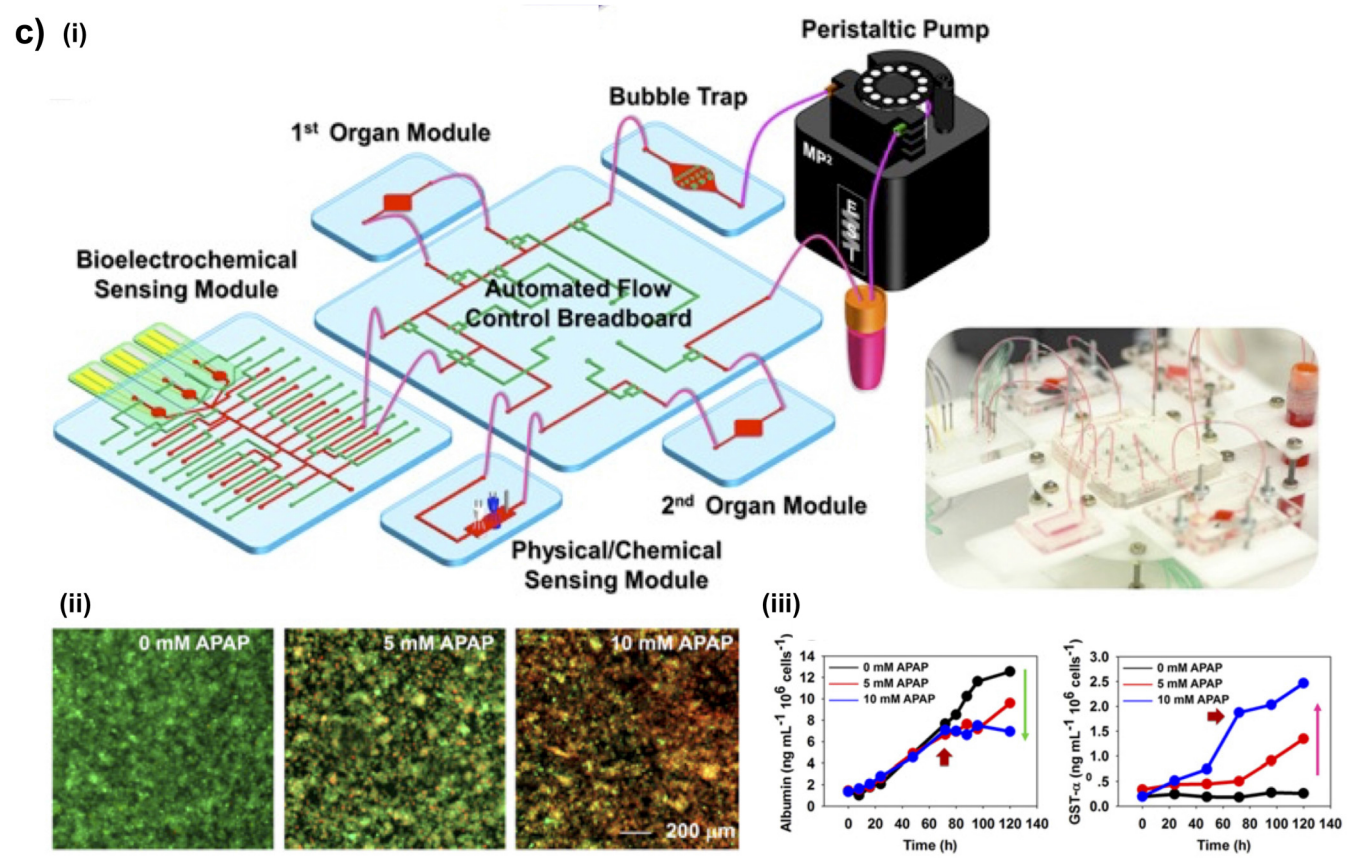

Fig. (5). a) (i) Schematic of the microfluidic optical sensing platform detecting absorbed light detection in culture medium via solid-state optics. (ii) step experiment result showing sensitivity of the pH sensor **b**) (i) Fabricated microelectrode set, and schematic of aptamers being immobilized onto microelectrode surface. (ii) Live/dead staining of cardiomyoctes after its exposure to DOX up to 126h. (iii) Comparison between aptamer, and antibody-based sensor under different concentration of CK-MB **c**) (i) microfluidic integrated biosensor composed of multiple organ modules, and sensing modules. (ii) Live/dead cell staining of liver-on-chips model upon treatment with 0, 5, 10 mM of APAP at 120h. (iii) Automated EC measurements of changes in hepatic organoids secreted albumin, and GST-α. Reproduced with permission of: American Institute of Physics, John Wiley & Sons, and United States National Academy of Sciences.

Droplet manipulation achieved by superhydrophobic-hydrophilic patterned surface provides great flexibility and versatility to biosensor application including the absence of cross-contamination, reproducibility, and reusability. However, manipulation of droplets including droplet transporting, mixing, and dispensing has been incomplete as the droplet-based microsystem usually operates by applying high voltage which makes it less applicable, and versatile for biological applications [119, 120]. Seo *et al*. developed a single-droplet multiplex colorimetric bioassay platform with an unprecedented droplet manipulation method utilizing reversible local deformation of superamphipohbic (SPO)-superamphiphilic (SPI) patterend PDMS [121]. As the PDMS substrate which is used as colorimetric bioassay library is loaded onto the droplet manipulation device, the single droplet sample composed of multiple analytes can be controlled through the dimple structure generated by applied vacuum pressure beneath the substrate. For colorimetric analysis, the residual liquid from single-droplet moving to adjacent SPI spot via operation of a vacuum tip. The SPI patterned surface is coated with the colorimetric reagent including enzymes and a color indicator. Thus, it could indicate the level of detected analytes by different concentration of color. By developing facile single-droplet manipulation integrated colorimetric bioassay platform, the target analytes can be easily detected using only single droplet. In addition, the multiplex bioassay promotes the growth in point-of-care devices which can operate full-range manipulation of a single droplet of various liquids such as water, ethylene glycol (EG), blood, dimethyl sulfoxide (DMSO), and hydrogels.

The advancements in microfluidics integrated biosensor have been gaining attention due to increasing demands for greater accuracy and flexibility of detecting methods. The biosensors enable continual monitoring of complex cellular responses in 3D complex microenvironments via detection of changes in level of physiochemical parameters or biomarkers upon stimulation. In addition, recent developments of biosensor which is capable of regenerating its surface by breaking thiol-Au bonds on the electrode surfaces allows automated screening processes with minimal labors involved. Despite of the current advances, development of microfluidics integrated high-throughput biosensor has not yet been achieved. Further research is needed to integrate conventional approaches to the biosensors to develop high-throughput biosensor which is capable of continual monitoring of multiple biological materials with high accuracy and reusability for more in-depth investigation of complex human cellular behavior in biomaterials.

CONCLUSION AND FUTURE PERSPECTIVE

Current drug screening and toxicology applications are usually arranged *in vivo* with animals, however, investigations on human-specific diseases have been underwhelming due to the absence of appropriate screening platforms. Thus, *in vitro* screening platform, which allows in-depth investigations of cellular behavior in complex microenvironments, is in high demand. To address the issues

in the current technique, label-free and regenerative EC microfluidic biosensor was developed which could establish a firm ground for continual monitoring which is essential for a biosensor. In addition, integration of the regenerative processes into multisensor organs-on-chip platform via microfluidic breadboard significantly enhances reusability, and accuracy of the sensor while making automated screening possible. Furthermore, single-droplet based multiplex bioassay platform demonstrates diverse applicability of the microfluidic-integrated biosensor with improved flexibility. Recent advances in screening techniques enabled label-free and continual monitoring of cellular behaviors in vast biological microenvironments. And yet, screening of cellular behavior under dynamic microenvironments via high-throughput biosensor remains unexplored. Current research on high-throughput system is on the way to integrate the system into organs-on-a-chip developing a platform capable of continuous monitoring on biomaterials-cell interaction independent of the microarrays. We believe further efforts on integrated high-throughput system allow reliable screening platform providing accurate predictions of complex human behavior, and testing drug efficacy and toxicity.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT) (2019R1C1C1006720).

REFERENCES

[1] Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol 2008; 214(2): 199-210.

[2] Ingber DE. Mechanical control of tissue morphogenesis during embryological development. Int J Dev Biol 2006; 50(2-3): 255-66.

[3] Place ES, Evans ND, Stevens MM. Complexity in biomaterials for tissue engineering. Nat Mater 2009; 8(6): 457-70.

[4] Hubbell JA. Biomaterials in tissue engineering. Biotechnology (N Y) 1995; 13(6): 565-76.

[5] Leijten J, Khademhosseini A. From Nano to Macro: Multiscale Materials for Improved Stem Cell Culturing and Analysis. Cell Stem Cell 2016; 18(1): 20-4.

[6] Leijten J, Rouwkema J, Zhang YS, Nasajpour A, Dokmeci MR, Khademhosseini A. Advancing Tissue Engineering: A Tale of Nano-, Micro-, and Macroscale Integration. Small 2016; 12(16): 2130-45.

[7] Jabbari E, Leijten J, Xu Q, Khademhosseini A. The matrix reloaded: the evolution of regenerative hydrogels. Mater Today 2015; 19(4): 190-6.

[8] Alsberg E, von Recum HA, Mahoney MJ. Environmental cues to guide stem cell fate decision for tissue engineering applications. Expert Opin Biol Ther 2006; 6(9): 847-66.

[9] Ricca BL, Venugopalan G, Fletcher DA. To pull or be pulled: parsing the multiple modes of mechanotransduction. Curr Opin Cell Biol 2013; 25(5): 558-64.

[10] Bursac N, Papadaki M, Cohen RJ, *et al.* Cardiac muscle tissue engineering: toward an *in vitro* model for electrophysiological studies. Am J Physiol 1999; 277(2): H433-44.

[11] Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol 2005; 23(1): 47-55.

[12] Leijten J, Seo J, Yue K, *et al.* Spatially and temporally controlled hydrogels for tissue engineering. Mater Sci Eng Rep 2017; 119: 1-35.

[13] Fernandes TG, Diogo MM, Clark DS, Dordick JS, Cabral JMS. High-throughput cellular microarray platforms: applications in drug discovery, toxicology and stem cell research. Trends Biotechnol 2009; 27(6): 342-9.

[14] Chin VI, Taupin P, Sanga S, Scheel J, Gage FH, Bhatia SN. Microfabricated platform for studying stem cell fates. Biotechnol Bioeng 2004; 88(3): 399-415.

[15] Seo J, Shin J-Y, Leijten J, *et al.* High-throughput approaches for screening and analysis of cell behaviors. Biomaterials 2018; 153: 85-101.

[16] Yang C, Tibbitt MW, Basta L, Anseth KS. Mechanical memory and dosing influence stem cell fate. Nat Mater 2014; 13(6): 645-52.

[17] Mahlstedt MM, Anderson D, Sharp JS, *et al.* Maintenance of pluripotency in human embryonic stem cells cultured on a synthetic substrate in conditioned medium. Biotechnol Bioeng 2010; 105(1): 130-40.

[18] Lund AW, Yener B, Stegemann JP, Plopper GE. The natural and engineered 3D microenvironment as a regulatory cue during stem cell fate determination. Tissue Eng Part B Rev 2009; 15(3): 371-80.

[19] Jarrahy R, Huang W, Rudkin GH, *et al.* Osteogenic differentiation is inhibited and angiogenic expression is enhanced in MC3T3-E1 cells cultured on three-dimensional scaffolds. Am J Physiol Cell Physiol 2005; 289(2): C408-14.

[20] Seo J, Lee S, Lee J, Lee T. Guided transport of water droplets on superhydrophobic-hydrophilic patterned Si nanowires. ACS Appl Mater Interfaces 2011; 3(12): 4722-9.

[21] Neto AI, Demir K, Popova AA, Oliveira MB, Mano JF, Levkin PA. Fabrication of Hydrogel Particles of Defined Shapes Using Superhydrophobic-Hydrophilic Micropatterns. Adv Mater 2016; 28(35): 7613-9.

[22] Dolatshahi-Pirouz A, Nikkhah M, Gaharwar AK, *et al.* A combinatorial cell-laden gel microarray for inducing osteogenic differentiation of human mesenchymal stem cells. Sci Rep 2014; 4: 3896.

[23] Gobaa S, Hoehnel S, Roccio M, Negro A, Kobel S, Lutolf MP. Artificial niche microarrays for probing single stem cell fate in high throughput. Nat Methods 2011; 8(11): 949-55.

[24] Lee M-Y, Kumar RA, Sukumaran SM, Hogg MG, Clark DS, Dordick JS. Three-dimensional cellular microarray for high-throughput toxicology assays. Proc Natl Acad Sci USA 2008; 105(1): 59-63.

[25] Hull R, Chraska T, Liu Y, Longo D. Microcontact printing: new mastering and transfer techniques for high throughput, resolution and depth of focus. Mater Sci Eng C 2002; 19(1): 383-92.

[26] Seong TW, Seo J, Lee KH. Full length histone H3 conjugated electrochemical biosensor for extracellular proteolytic Cathepsin L activity detection. Sens Actuators B Chem 2018; 267: 237-44.

[27] Oliveira MB, Salgado CL, Song W, Mano JF. Combinatorial on-chip study of miniaturized 3D porous scaffolds using a patterned superhydrophobic platform. Small 2013; 9(5): 768-78.

[28] Flaim CJ, Teng D, Chien S, Bhatia SN. Combinatorial signaling microenvironments for studying stem cell fate. Stem Cells Dev 2008; 17(1): 29-39.

[29] Flaim CJ, Chien S, Bhatia SN. An extracellular matrix microarray for probing cellular differentiation. Nat Methods 2005; 2(2): 119-25.

[30] Mei Y, Saha K, Bogatyrev SR, *et al.* Combinatorial development of biomaterials for clonal growth of human pluripotent stem cells. Nat Mater 2010; 9(9): 768-78.

[31] Caiazzo M, Okawa Y, Ranga A, Piersigilli A, Tabata Y, Lutolf MP. Defined three-dimensional microenvironments boost induction of pluripotency. Nat Mater 2016; 15(3): 344-52.

[32] Camci-Unal G, Cuttica D, Annabi N, Demarchi D, Khademhosseini A. Synthesis and characterization of hybrid hyaluronic acid-gelatin hydrogels. Biomacromolecules 2013; 14(4): 1085-92.

[33] Jabbarzadeh E, Blanchette J, Shazly T, Khademhosseini A, Camci-Unal G. T Laurencin C. Vascularization of Biomaterials for Bone Tissue Engineering: Current Approaches and Major Challenges. Curr Angiogenes 2012; 1(3): 180-91.

[34] Seo J, Lee JS, Lee K, *et al.* Switchable water-adhesive, superhydrophobic palladium-layered silicon nanowires potentiate the angiogenic efficacy of human stem cell spheroids. Adv Mater 2014; 26(41): 7043-50.

[35] Ranga A, Gobaa S, Okawa Y, Mosiewicz K, Negro A, Lutolf MP. 3D niche microarrays for systems-level analyses of cell fate. Nat Commun 2014; 5: 4324.

[36] Fernandes TG, Kwon SJ, Bale SS, *et al.* Three-dimensional cell culture microarray for high-throughput studies of stem cell fate. Biotechnol Bioeng 2010; 106(1): 106-18.

[37] Meli L, Barbosa HS, Hickey AM, *et al.* Three dimensional cellular microarray platform for human neural stem cell differentiation and toxicology. Stem Cell Res (Amst) 2014; 13(1): 36-47.

[38] Popova AA, Demir K, Hartanto TG, Schmitt E, Levkin PA. Droplet-microarray on superhydrophobic–superhydrophilic patterns for high-throughput live cell screenings. RSC Advances 2016; 6(44): 38263-76.

[39] Li Y, Chen P, Wang Y, *et al.* Rapid Assembly of Heterogeneous 3D Cell Microenvironments in a Microgel Array. Adv Mater 2016; 28(18): 3543-8.

[40] Shin S, Seo J, Han H, Kang S, Kim H, Lee T. Bio-Inspired Extreme Wetting Surfaces for Biomedical Applications. Materials (Basel) 2016; 9(2): 116.

[41] Sahoo B, Yoon K, Seo J, Lee T. Chemical and Physical Pathways for Fabricating Flexible Superamphiphobic Surfaces with High Transparency. Coatings 2018; 8(2)

[42] Seo J, Lee S-K, Lee J, *et al.* Path-programmable water droplet manipulations on an adhesion controlled superhydrophobic surface. Sci Rep 2015; 5: 12326.

[43] Leslie DC, Waterhouse A, Berthet JB, *et al.* A bioinspired omniphobic surface coating on medical devices prevents thrombosis and biofouling. Nat Biotechnol 2014; 32(11): 1134-40.

[44] Oliveira MB, Mano JF. On-chip assessment of the protein-release profile from 3D hydrogel arrays. Anal Chem 2013; 85(4): 2391-6.

[45] Salgado CL, Oliveira MB, Mano JF. Combinatorial cell-3D biomaterials cytocompatibility screening for tissue engineering using bioinspired superhydrophobic substrates. Integr Biol 2012; 4(3): 318-27.

[46] Feng W, Li L, Du X, Welle A, Levkin PA. Single-Step Fabrication of High-Density Microdroplet Arrays of Low-Surface-Tension Liquids. Adv Mater 2016; 28(16): 3202-8.

[47] Hirtz M, Feng W, Fuchs H, Levkin PA. Click-Chemistry Immobilized 3D-Infused Microarrays in Nanoporous Polymer Substrates. Adv Mater Interfaces 2016; 3(6): 1500469.

[48] Molla MR, Levkin PA. Combinatorial Approach to Nanoarchitectonics for Nonviral Delivery of Nucleic Acids. Adv Mater 2016; 28(6): 1159-75.

[49] Popova AA, Schillo SM, Demir K, Ueda E, Nesterov-Mueller A, Levkin PA. Droplet-Array (DA) Sandwich Chip: A Versatile Platform for High-Throughput Cell Screening Based on Superhydrophobic-Superhydrophilic Micropatterning. Adv Mater 2015; 27(35): 5217-22.

[50] Barata D, van Blitterswijk C, Habibovic P. High-throughput screening approaches and combinatorial development of biomaterials using microfluidics. Acta Biomater 2016; 34: 1-20.

[51] Domachuk P, Tsioris K, Omenetto FG, Kaplan DL. Biomicrofluidics: biomaterials and biomimetic designs. Adv Mater 2010; 22(2): 249-60.

[52] Whitesides GM. The origins and the future of microfluidics. Nature 2006; 442(7101): 368-73.

[53] Kim S, Kim HJ, Jeon NL. Biological applications of microfluidic gradient devices. Integr Biol 2010; 2(11-12): 584-603.

[54] Berthier E, Beebe DJ. Gradient generation platforms: new directions for an established microfluidic technology. Lab Chip 2014; 14(17): 3241-7.

[55] Byrd TF IV, Hoang LT, Kim EG, *et al.* The microfluidic multitrap nanophysiometer for hematologic cancer cell characterization reveals temporal sensitivity of the calcein-AM efflux assay. Sci Rep 2014; 4: 5117.

[56] Awwad Y, Geng T, Baldwin AS, Lu C. Observing single cell NF-κB dynamics under stimulant concentration gradient. Anal Chem 2012; 84(3): 1224-8.

[57] Ostrovidov S, Annabi N, Seidi A, *et al.* Controlled release of drugs from gradient hydrogels for high-throughput analysis of cell-drug interactions. Anal Chem 2012; 84(3): 1302-9.

[58] Frey O, Misun PM, Fluri DA, Hengstler JG, Hierlemann A. Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis. Nat Commun 2014; 5: 4250.

[59] Occhetta P, Centola M, Tonnarelli B, Redaelli A, Martin I, Rasponi M. High-Throughput Microfluidic Platform for 3D Cultures of Mesenchymal Stem Cells, Towards Engineering Developmental Processes. Sci Rep 2015; 5: 10288.

[60] Baker BM, Trappmann B, Stapleton SC, Toro E, Chen CS. Microfluidics embedded within extracellular matrix to define vascular architectures and pattern diffusive gradients. Lab Chip 2013; 13(16): 3246-52.

[61] Malda J, Visser J, Melchels FP, *et al.* 25th anniversary article: Engineering hydrogels for biofabrication. Adv Mater 2013; 25(36): 5011-28.

[62] Gao D, Liu J, Wei H-B, Li H-F, Guo G-S, Lin J-M. A microfluidic approach for anticancer drug analysis based on hydrogel encapsulated tumor cells. Anal Chim Acta 2010; 665(1): 7-14.

[63] Yang K, Jung H, Lee H-R, *et al.* Multiscale, hierarchically patterned topography for directing human neural stem cells into functional neurons. ACS Nano 2014; 8(8): 7809-22.

[64] Yang K, Lee J, Lee JS, *et al.* Graphene Oxide Hierarchical Patterns for the Derivation of Electrophysiologically Functional Neuron-like Cells from Human Neural Stem Cells. ACS Appl Mater Interfaces 2016; 8(28): 17763-74.

[65] Allen JL, Cooke ME, Alliston T. ECM stiffness primes the TGFβ pathway to promote chondrocyte differentiation. Mol Biol Cell 2012; 23(18): 3731-42.

[66] Davisson T, Kunig S, Chen A, Sah R, Ratcliffe A. Static and dynamic compression modulate matrix metabolism in tissue engineered cartilage. J Orthop Res 2002; 20(4): 842-8.

[67] Nunes SS, Miklas JW, Liu J, *et al.* Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. Nat Methods 2013; 10(8): 781-7.

[68] Dalby MJ, Gadegaard N, Tare R, *et al.* The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. Nat Mater 2007; 6(12): 997-1003.

[69] Ankam S, Suryana M, Chan LY, *et al.* Substrate topography and size determine the fate of human embryonic stem cells to neuronal or glial lineage. Acta Biomater 2013; 9(1): 4535-45.

[70] Kingham E, White K, Gadegaard N, Dalby MJ, Oreffo RO. Nanotopographical cues augment mesenchymal differentiation of human embryonic stem cells. Small 2013; 9(12): 2140-51.

[71] Jeon H, Koo S, Reese WM, Loskill P, Grigoropoulos CP, Healy KE. Directing cell migration and organization via nanocrater-patterned cell-repellent interfaces. Nat Mater 2015; 14(9): 918-23.

[72] Unadkat HV, Hulsman M, Cornelissen K, *et al.* An algorithm-based topographical biomaterials library to instruct cell fate. Proc Natl Acad Sci USA 2011; 108(40): 16565-70.

[73] Hu J, Gondarenko AA, Dang AP, *et al.* High-Throughput Mechanobiology Screening Platform Using Micro- and Nanotopography. Nano Lett 2016; 16(4): 2198-204.

[74] Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell 2006; 126(4): 677-89.

[75] Mih JD, Sharif AS, Liu F, Marinkovic A, Symer MM, Tschumperlin DJ. A multiwell platform for studying stiffness-dependent cell biology. PLoS One 2011; 6(5): e19929.

[76] Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 2008; 8(10): 755-68.

[77] Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 2005; 5(4): 275-84.

[78] Mehlen P, Puisieux A. Metastasis: a question of life or death. Nat Rev Cancer 2006; 6(6): 449-58.

[79] Liu J, Tan Y, Zhang H, *et al.* Soft fibrin gels promote selection and growth of tumorigenic cells. Nat Mater 2012; 11(8): 734-41.

[80] Tan Y, Tajik A, Chen J, *et al.* Matrix softness regulates plasticity of tumour-repopulating cells via H3K9 demethylation and Sox2 expression. Nat Commun 2014; 5: 4619.

[81] Lee J, Abdeen AA, Wycislo KL, Fan TM, Kilian KA. Interfacial geometry dictates cancer cell tumorigenicity. Nat Mater 2016; 15(8): 856-62.

[82] Ehrlich PJ, Lanyon LE. Mechanical strain and bone cell function: a review. Osteoporos Int 2002; 13(9): 688-700.

[83] Tandon N, Marsano A, Maidhof R, *et al.* Surface-patterned electrode bioreactor for electrical stimulation. Lab Chip 2010; 10(6): 692-700.

[84] Sathaye A, Bursac N, Sheehy S, Tung L. Electrical pacing counteracts intrinsic shortening of action potential duration of neonatal rat ventricular cells in culture. J Mol Cell Cardiol 2006; 41(4): 633-41.

[85] Wang D-L, Jiang S-D, Dai L-Y. Biologic response of the intervertebral disc to static and dynamic compression *in vitro*. Spine 2007; 32(23): 2521-8.

[86] Pelaez D, Huang CY, Cheung HS. Cyclic compression maintains viability and induces chondrogenesis of human mesenchymal stem cells in fibrin gel scaffolds. Stem Cells Dev 2009; 18(1): 93-102.

[87] Rath B, Nam J, Knobloch TJ, Lannutti JJ, Agarwal S. Compressive forces induce osteogenic gene expression in calvarial osteoblasts. J Biomech 2008; 41(5): 1095-103.

[88] Moraes C, Wang G, Sun Y, Simmons CA. A microfabricated platform for high-throughput unconfined compression of micropatterned biomaterial arrays. Biomaterials 2010; 31(3): 577-84.

[89] Liu H, Usprech J, Sun Y, Simmons CA. A microfabricated platform with hydrogel arrays for 3D mechanical stimulation of cells. Acta Biomater 2015.

[90] Li Y, Huang G, Gao B, Li M, Genin GM, Lu TJ, *et al.* Magnetically actuated cell-laden microscale hydrogels for probing strain-induced cell responses in three dimensions. NPG Asia Mater 2016; 8(1): e238.

[91] Seo J, Shin J-Y, Leijten J, *et al.* Interconnectable Dynamic Compression Bioreactors for Combinatorial Screening of Cell Mechanobiology in Three Dimensions. ACS Appl Mater Interfaces 2018; 10(16): 13293-303.

[92] Heidi Au HT, Cui B, Chu ZE, Veres T, Radisic M. Cell culture chips for simultaneous application of topographical and electrical cues enhance phenotype of cardiomyocytes. Lab Chip 2009; 9(4): 564-75.

[93] Chen MQ, Xie X, Wilson KD, *et al.* Current-controlled electrical point-source stimulation of embryonic stem cells. Cell Mol Bioeng 2009; 2(4): 625-35.

[94] Jin Y, Seo J, Lee JS, *et al.* Triboelectric Nanogenerator Accelerates Highly Efficient Nonviral Direct Conversion and *In Vivo* Reprogramming of Fibroblasts to Functional Neuronal Cells. Adv Mater 2016; 28(34): 7365-74.

[95] Dai X, Zhou W, Gao T, Liu J, Lieber CM. Three-dimensional mapping and regulation of action potential propagation in nanoelectronics-innervated tissues. Nat Nanotechnol 2016; 11(9): 776-82.

[96] Mousavi Shaegh SA, De Ferrari F, Zhang YS, *et al.* A microfluidic optical platform for real-time monitoring of pH and oxygen in microfluidic bioreactors and organ-on-chip devices. Biomicrofluidics 2016; 10(4): 044111.

[97] Thies R. Physiology (Oklahomar notes). 4th ed: Springer New York; 1995 02/23/1995.

[98] Brennan MD, Rexius-Hall ML, Elgass LJ, Eddington DT. Oxygen control with microfluidics. Lab Chip 2014; 14(22): 4305-18.

[99] Massa S, Sakr MA, Seo J, *et al.* Bioprinted 3D vascularized tissue model for drug toxicity analysis. Biomicrofluidics 2017; 11(4): 044109.

[100] Riahi R, Shaegh SAM, Ghaderi M, *et al.* Automated microfluidic platform of bead-based electrochemical immunosensor integrated with bioreactor for continual monitoring of cell secreted biomarkers. Sci Rep 2016; 6: 24598.

[101] Shin SR, Zhang YS, Kim D-J, *et al.* Aptamer-Based Microfluidic Electrochemical Biosensor for Monitoring Cell-Secreted Trace Cardiac Biomarkers. Anal Chem 2016; 88(20): 10019-27.

[102] Du L, Wu C, Peng H, Zou L, Zhao L, Huang L, *et al.* Piezoelectric olfactory receptor biosensor prepared by aptamer-assisted immobilization. Sens Actuators B Chem 2013; 187: 481-7.

[103] Kara P, de la Escosura-Muñiz A, Maltez-da Costa M, Guix M, Ozsoz M, Merkoçi A. Aptamers based electrochemical biosensor for protein detection using carbon nanotubes platforms. Biosens Bioelectron 2010; 26(4): 1715-8.

[104] Liu Y, Tuleouva N, Ramanculov E, Revzin A. Aptamer-based electrochemical biosensor for interferon gamma detection. Anal Chem 2010; 82(19): 8131-6.

[105] Tombelli S, Minunni M, Mascini M. Aptamers-based assays for diagnostics, environmental and food analysis. Biomol Eng 2007; 24(2): 191-200.

[106] Lai RY, Plaxco KW, Heeger AJ. Aptamer-based electrochemical detection of picomolar platelet-derived growth factor directly in blood serum. Anal Chem 2007; 79(1): 229-33.

[107] Xiao Y, Lubin AA, Heeger AJ, Plaxco KW. Label-free electronic detection of thrombin in blood serum by using an aptamer-based sensor. Angew Chem Int Ed Engl 2005; 44(34): 5456-9.

[108] McCauley TG, Hamaguchi N, Stanton M. Aptamer-based biosensor arrays for detection and quantification of biological macromolecules. Anal Biochem 2003; 319(2): 244-50.

[109] Liss M, Petersen B, Wolf H, Prohaska E. An aptamer-based quartz crystal protein biosensor. Anal Chem 2002; 74(17): 4488-95.

[110] Ruigrok VJB, Levisson M, Eppink MHM, Smidt H, van der Oost J. Alternative affinity tools: more attractive than antibodies? Biochem J 2011; 436(1): 1-13.

[111] Stoltenburg R, Reinemann C, Strehlitz B. SELEX--a (r)evolutionary method to generate high-affinity nucleic acid ligands. Biomol Eng 2007; 24(4): 381-403.

[112] Sun H, Zhu X, Lu PY, Rosato RR, Tan W, Zu Y. Oligonucleotide aptamers: new tools for targeted cancer therapy. Mol Ther Nucleic Acids 2014; 3(8): e182.

[113] Jayasena SD. Aptamers: an emerging class of molecules that rival antibodies in diagnostics. Clin Chem 1999; 45(9): 1628-50.

[114] Tia SQ, He M, Kim D, Herr AE. Multianalyte on-chip native Western blotting. Anal Chem 2011; 83(9): 3581-8.

[115] Shin SR, Kilic T, Zhang YS, *et al.* Label-Free and Regenerative Electrochemical Microfluidic Biosensors for Continual Monitoring of Cell Secretomes. Adv Sci (Weinh) 2017; 4(5): 1600522.

[116] Zhang YS, Aleman J, Shin SR, *et al.* Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. Proc Natl Acad Sci USA 2017; 114(12): E2293-302.

[117] Zhang Y-F, He W, Zhang C, *et al.* Role of receptor interacting protein (RIP)1 on apoptosis-inducing factor-mediated necroptosis during acetaminophen-evoked acute liver failure in mice. Toxicol Lett 2014; 225(3): 445-53.

[118] Zhang H, Oellers T, Feng W, *et al.* High-Density Droplet Microarray of Individually Addressable Electrochemical Cells. Anal Chem 2017; 89(11): 5832-9.

[119] Zhang Y, Wang T-H. Full-range magnetic manipulation of droplets via surface energy traps enables complex bioassays. Adv Mater 2013; 25(21): 2903-8.

[120] Pollack MG, Fair RB, Shenderov AD. Electrowetting-based actuation of liquid droplets for microfluidic applications. Appl Phys Lett 2000; 77(11): 1725-6.

[121] Han H, Lee JS, Kim H, *et al.* Single-Droplet Multiplex Bioassay on a Robust and Stretchable Extreme Wetting Substrate through Vacuum-Based Droplet Manipulation. ACS Nano 2018; 12(2): 932-41.