Review

Advances in cell-based biosensors using three-dimensional cell-encapsulating hydrogels

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Cell-based biosensors (CBBs) have emerged as promising biotechnical tools whereby various cell types can be used as basic sensing units to detect external stimuli. Specifically, CBBs have been applied in environmental monitoring, drug screening, clinical diagnosis and biosecurity. For these applications, CBBs offer several advantages over conventional molecular-based biosensors or living animal-based approaches, such as the capability to better mimic physiological situations, to enhance detection specificity and sensitivity, and to detect unknown compounds and toxins. On the other hand, existing CBBs suffer from several limitations, such as weak cell-substrate attachment, two-dimensional (2D) cell microenvironment, and limited shelf life. An emerging method for scaffold-free three-dimensional (3D) cell culture uses hydrogels to encapsulate cells. Advances in novel biomaterials and nano/microscale technologies have enabled encapsulation of cells in hydrogels to fabricate 3D CBBs, which hold great potential for addressing the limitation in existing 2D CBBs. Here, we present an overview of the emerging hydrogel-based CBBs, their applications in pathogen/toxin detection, drug screening and screening of cell-biomaterials interaction, and the associated challenges and potential solutions.

1 Introduction

Over the past few decades, tremendous progress has been made in the development of biosensors for applications in medicine, environment monitoring and food safety. Biosensors in general consist of three components: a sensing unit for identifying targets, a transducer for converting biological reactions to chemical/ electrical/ optical signals, and an output system for signal amplification and readout [1]. According to the nature of the sensing units, biosensors can be categorized as molecule-based biosensors (MBBs), cell-based biosensor (CBBs), and animal-based biosensors (ABBs). MBBs rely on specific interactions between antibodies, nucleic acids, enzymes or ion channels and their corresponding cognitive moieties [2], such as DNA biosensor [3]. ABBs are based on the use of live animals (e.g., cat-

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Abbreviations: ABB, animal-based biosensor; **CBB**, cell-based biosensor; **ECM**, extracellular matrix; **MBB**, molecule-based biosensor

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tle, fish sheep, etc.) as sensing units to probe environmental hazards. For example, canaries have been used to monitor carbon monoxide to improve the safety of coal mines [4]. In contrast, living cells such as bacterial or eukaryotic cells are included in CBBs to sense agents of interest at a cellular level.

Among these three types of biosensor, CBBs offer several advantages for monitoring and screening pathogens/toxic substances over MBBs and ABBs [5–9]. The advantages include the capability to closely mimic physiological situations and to detect unknown compounds and toxins [10, 11]. Although MBBs have high sensitivity and specificity, they are usually designed using established mechanisms and thus have limited capability to identify unknown stimuli. ABBs cannot be easily applied on site for large-scale applications due to limited portability [4]. On the other hand, CBBs can reduce cost and complexity associated with animal testing for monitoring the environment. It has been calculated that 3200 animals are needed to assess the toxicity of a single chemical compound, which highlights the need for cost-effective CBBs for toxicological assessment [12]. CBBs can be readily coupled with high-throughput proteomics and genomics analytical methods to identify the responses to unknown toxins and to screen for drug candidates [13]. In addition, cellular responses induced by test analytes can be singled-out using specific cell types that do not interfere with other cell types or the whole sensing system [14].

Despite the advantages of CBBs, several limitations are associated with existing CBB systems, such as weak cell–substrate attachment, 2D cell microenvironment, and limited shelf life. Also, some mammalian cell lines, especially neurons, are susceptible to detachment, and thus easily get washed away during cell culture [15]. Most cells used in existing CBBs are cultured on hard 2D glass or plastic substrates that do not mimic their in vivo counterparts. In addition, the sensing cells need to be immobilized and patterned on the sensors with mi-

croscale spatial resolution to monitor cellular response to external stimuli [16]. Recently, significant advances in novel biomaterials and nano/micro engineering methods have been made it possible to immobilize cells using scaffold-free 3D methods [17]. For instance, biocompatible hydrogels have emerged that are water-swellable with structure similar to native extracellular matrix (ECM), allowing oxygen, nutrients and metabolic products to diffuse, thus offering cells a controllable 3D microenvironment [18, 19]. These advances have enabled the encapsulation of cells in 3D hydrogels [20–22], which has been widely used in tissue engineering. Cell encapsulation in hydrogels can also be used as platforms for CBBs and hold great potential to address the challenges for the existing CBBs. In this review, we present an overview of CBBs and then focus on the applications of hydrogel-based CBBs in medicine, food safety, environmental monitoring and biosecurity. We also discuss the challenges for hydrogel-based CBBs and potential solutions.

2 CBBs based on cell-encapsulating hydrogels

2.1 Three elements in CBBs

A typical CBB is consisted of an input system, an output system, and host cells [23], as illustrated in Fig. 1. The input system includes soluble factors (standardized culture medium, metabolic substrates, vitamins, antibiotics, etc.) and insoluble factors (ECM or neighbor cells), as well as experimental stimuli (cytokines, growth factors, hormones, and putative therapeutic candidates). Soluble factors, which are often added to the culture medium, are key factors for maintaining cell growth and controlling cell behavior. ECM binds soluble factors and transfers cues to cells to regulate cell proliferation, survival, migration and differentiation [24,
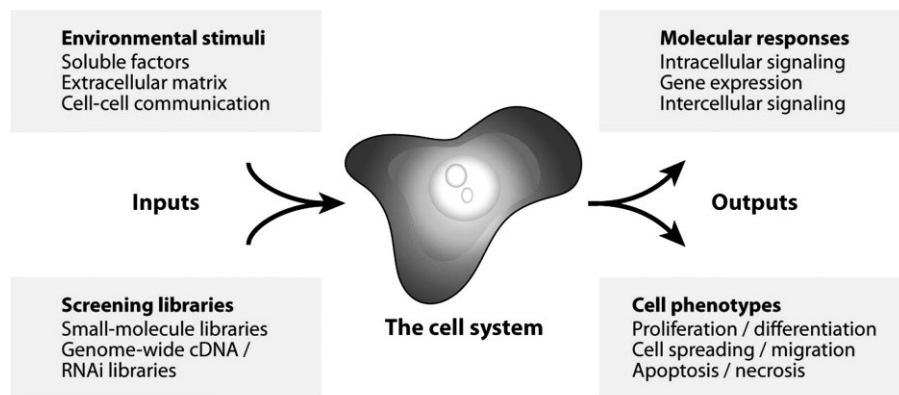

Environmental stimuli
Soluble factors
Extracellular matrix
Cell-cell communication

Molecular responses
Intracellular signaling
Gene expression
Intercellular signaling

Inputs

Outputs

The cell system

Screening libraries
Small-molecule libraries
Genome-wide cDNA /
RNAi libraries

Cell phenotypes
Proliferation / differentiation
Cell spreading / migration
Apoptosis / necrosis

Figure 1. Schematic of three basic components of a CBB, an input system, an output system, and host cells [23]. The input system consists of soluble factors and insoluble factors, as well as experimental stimuli. The output system includes molecular responses and cell phenotypes. Reprinted from [23] with permission from Annual Reviews, Inc.

25]. Cell behaviors and activities are also regulated by signals that arise from cell microenvironment via cell-cell contact, cell-ECM interactions, and cell–soluble factor interactions [26, 27].

Cell type and cell density play important roles in CBBs for a variety of applications. For instance, prokaryotic cell (e.g., *E. coli*) [28] and eukaryotic cell [29] have been utilized in CBBs. *E. coli* have a fast reproduction rate (double every 20 min) and do not require a demanding culture environment. In addition, *E. coli* can be genetically engineered for bioluminescent property, enabling integration with electronic circuits in a single chip for convenient detection [30–32]. However, *E. coli* responds to external stimuli significantly differently from mammalian cells, which limits their application in practical applications to mimic human cell response for clinical diagnosis. Mammalian cells, especially human cells, can report human health information, such as cellular metabolism and physiologic responses [4]. However, mammalian cells require more delicate manipulations compared to bacteria. In addition to cell type, cell density also plays an important role since signals can be detected from both single cells and a population of cells. The average signal from a large cell population represents cellular response at a system level via cumulative change (e.g., fluorescence). In contrast, CBBs based on single cells can offer spatial and temporal information (e.g., cell morphology, motility change) [33].

The output system of CBBs is used to measure cell responses, generally via optical or electrical signals, depending on the nature of the biological reactions. Traditionally, optical methods are mainly used to measure absorbance, luminescence, or fluorescence signals that are related to cell metabolites and cell morphology. Alternatively, electrical methods can also report cellular responses. Based on the optical or electrical detection, a number of methods have been utilized to study cell responses to external stimuli, such as live-cell imaging [34], cell viability [35], cell metabolism [36], impedance change [37, 38], cell motility and adhesion [39].

2.2 Cell-encapsulating hydrogels as the host cell system of CBBs

Cell-encapsulating hydrogels offer a number of attractive features for developing CBBs, including enhanced 3D cell stabilization, ease of handling, and the ability to mimic in vivo cellular environment. For instance, cells encapsulated in hydrogels can avoid the issue of cell detachment (e.g., neurons and stem cells) seen with 2D sensor surfaces due to weak cell–substratum attachment (e.g., induced by frequent media change in prolonged cul-

ture periods). In addition, cell-encapsulating hydrogels can provide efficient mass transfer essential for prolonged cell viability, which determines the shelf life of CBBs. Moreover, the mechanical properties of hydrogels can protect the cells from environmental perturbations to some extent [15].

Hydrogel-based CBBs can potentially solve the challenges for existing 2D CBB culture systems. For instance, 2D cell culture systems can not accurately recapitulate the structure, function, or physiology of a native 3D cell microenvironment [40], such as the distribution of oxygen, nutrients, metabolites and signaling molecules [40, 41]. In contrast, biomaterials such as collagen, hyaluronic acid, and other natural hydrogels can mimic native 3D physiological conditions. Cells can be encapsulated in 3D hydrogels as platforms to develop CBBs [42, 43], which enables better reflection of cell-stimuli responses (e.g., gene expression, cell differentiation and biological activities) in vivo compared to 2D culture systems [5]. For instance, a significant difference in intracellular calcium concentration change in response to high K^+ (50 mM) depolarization was observed between cells cultured in 2D flat dishes (monolayer) and 3D hydrogels [33, 44, 45].

Owing to the advantages offered by 3D hydrogel cell culture, there is accumulating evidence demonstrating the advantage of hydrogel-based CBBs, as summarized in Table 1. For example, collagen hydrogel was able to support cell growth and maintain cell viability for several weeks without media exchange [46]. O'Connor and Andreadis [46] investigated the capability of 3D gels to culture neural cells, where embryonic rat cortical neurons and astrocytes were entrapped in the sugar poly(acrylate) hydrogel and collagen gels. Mao and Kisaalita [15] investigated the proliferation, morphology, intracellular calcium changes in a human neuroblastoma cell line encapsulated in collagen with different mechanical properties. CBB- based cell-encapsulating hydrogels_have been used to screen for drug candidates and to identify unknown toxins [47, 48]. A 3D cellular microarray comprising human cells entrapped in collagen and alginate was used to analyze the toxicity of drug candidates and their cytochrome P450- generated metabolites [47]. The microarray obtained similar responses compared to conventional 96-well plates, and the dose of drug candidates was reduced by 2000-fold. The detection time was shortened to 7 h, whereas conventional drug toxicology studies requires 24–168 h to report the results. Bhunia et al. [48] developed a CBB platform with Ped-2E9 cells encapsulated in collagen to detect the pathogenic *Listeria* and *Bacillus* species, and the toxins from these organisms. Their results showed that Ped-2E9 cells

Table 1. List of the cell encapsulating hydrogels in CBBs

Hydrogels	Cell types	Input system	Output system	Potential applications	Ref.
Collagen	IMR-32 neuroblastoma cell	Standardized cell culture medium	Ca^{2+} concentration Cell morphology Membrane potential	High-throughput drug screening	[15]
	B-lymphocyte origin Ped-2E9 hybridoma cell line	Bacterium	Alkaline phosphatase Cell apoptosis and necrosis	Rapid detection of pathogens and toxins	[48]
	Embryonic rat cortical neurons	Standardized cell culture medium	Calcium imaging Cell growth Cell electrophysiology	Assessment of cell biomaterials interactions	[46]
	Astrocytes				
	Neural progenitor cells				
	MCF7 cells	Drug candidates Drug metabolites	IC_{50}[a]	High-throughput toxicology assays Drug screening	[47]
	SH-SY5Y human neuroblastoma cells	Standardized cell culture medium	Membrane potential Calcium imaging		[45]
Alginate	Myeloblasts	Anti-cancer drugs	Cell viability Cell morphology	Drug screening	[78]
	MCF7 cells	Drug candidates Drug metabolites	IC_{50}	High-throughput toxicology assays Drug screening	[47]
Matrigel	Tumor cell HCT-116	Anti-cancer drugs	Cell viability	Drug screening	[78]
	Hepatoma cells HepG2/C3A		Cell morphology	Drug toxicity	
PEG	*E. coli*	Hydrogel shape	DNA detection Assay	Screening of biological materials	[81]
	Murine fibroblasts	Standardized cell culture medium	Cell viability	Manipulate cell–cell interactions at the micrometer scale	[16]

a) Half maximal inhibitory concentration.

could be immobilized in a collagen gel matrix without compromising the sensing capability of Ped-2E9 to detect pathogenic *Listeria* and *Bacillus* toxins. Ped-2E9 cells encapsulated in collagen can reduce the centrifugation step in 2D assays, which is important for integrating cells in a biosensor platform. Table 1 lists the examples of CBBs based on cell encapsulating hydrogels. All these studies indicate that cell-encapsulating hydrogels can be used as a cell-based sensing system to sense pathogens and toxins, and to screen for drug candidates.

3 Potential applications of CBBs based on cell-encapsulating hydrogels

CBBs based on cell-encapsulating hydrogels are increasingly being used in pathogen testing, toxicology assays, and high-throughput drug screening. In this section, we present their applications in pathogen and toxin testing, drug screening and cell-biomaterials interaction screening.

3.1 Pathogen and toxin testing

CBBs that can detect pathogens and toxins become increasingly important in biosecurity, food safety and disease control. For example, rapid detection of food-borne pathogens such as *E. coli*, *Salmonella* or *Vibrio cholerae* can facilitate the provision of appropriate medical care and prevent further disease spreading. The outbreak of cholera in Haiti in 2010 threatened the lives of 3 million people and caused over 500 deaths. Every year, malaria kills nearly 1 million people and affects another 250 million people's health. In developing countries, the condition is especially serious. It is estimated that more than 95% of deaths are caused by infectious diseases [49, 50]. Food safety is related to global health, and food-borne infectious diseases have a significant impact on public health and economic stability [51]. Several comprehensive literature reviews on detection of pathogens and toxins have been published [12, 14, 52]. Conventional methods such as culture, immunoassays and polymerase chain reaction (PCR) are sensitive and specific.
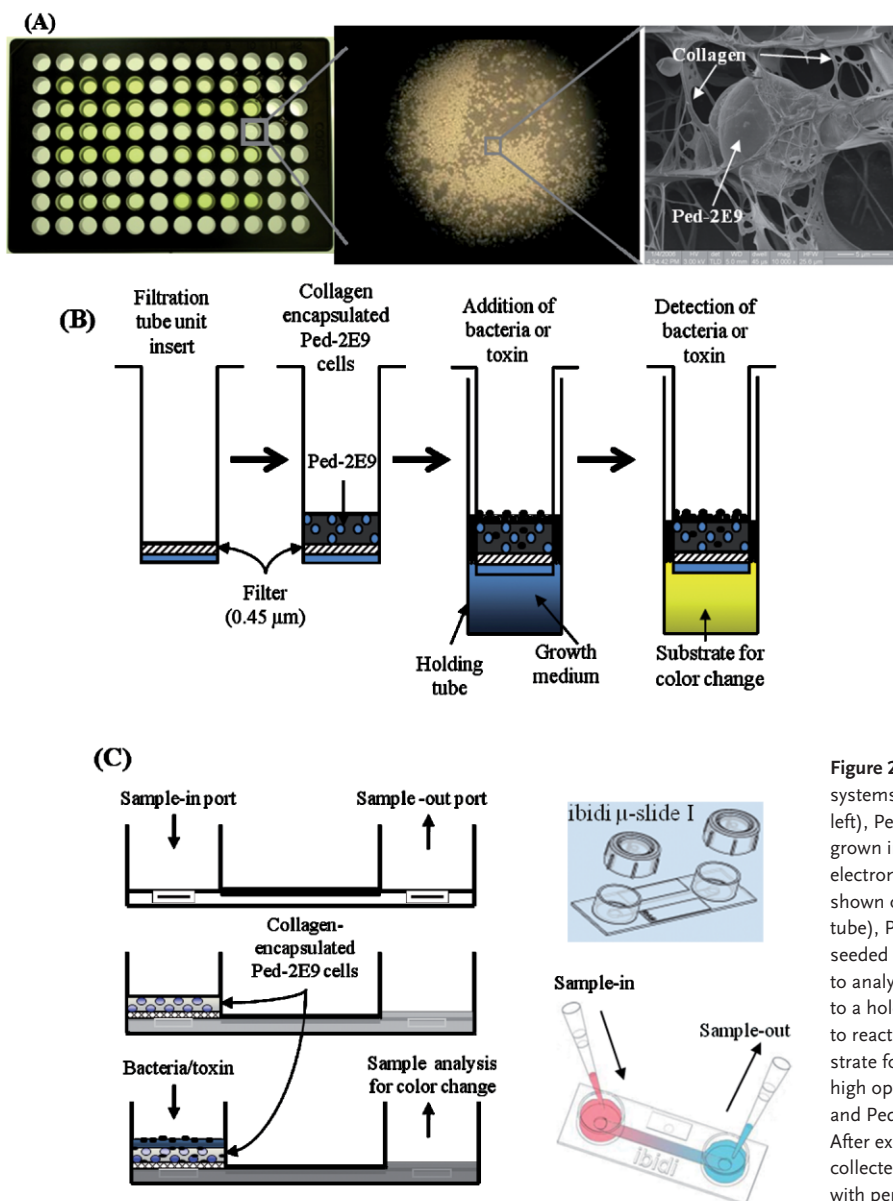
Figure 2. Different types CBB devices in 3D cell culture systems [54]. (**A**) In Device I (i.e., 96-well plate on the left), Ped-2E9 cells encapsulated in collagen were grown in a microwell (middle). The image of scanning electron micrograph of cell embedded in collagen was shown on the right panel. (**B**) In Device II (Filtration tube), Ped-2E9 cells encapsulated in collagen were seeded in a filtration tube. After the cells were exposed to analyte, the device was centrifuged to collect liquid to a holding tube. The collected liquid was transferred to react with alkaline phosphatase (ALP) liquid substrate for color reaction. (**C**) Device III was built on a high optical quality slide. A filter was placed in a well and Ped-2E9-collagen was dispensed into well port. After exposure to the analyte, the diffused liquid was collected and reacted with ALP. Reprinted from [54] with permission from Elsevier.

However, these methods have several limitations, including inability to provide the biological activity of the analyte, lack of robustness and portability, and long turnaround time from several hours to a few days [53].

Banerjee and coworkers have developed three types of collagen-based CBB devices [48, 54]. These CBB utilize B-cell hybridoma, Ped-2E9, B lymphocyte and Ped-2E9 cells as sensing units to screen for tens of pathogens and toxins such as *Listeria monocytogenes*, enterotoxigenic bacillus, vibrio and micrococcus (Fig. 2) [54]. Their results demonstrated the ability of the cell-encapsulating hydrogel-based CBBs to rapidly detect multiple pathogens and toxins.

3.2 Drug screening

High-throughput screening technologies have been highly successful in identifying hit and lead compounds for drug discovery. However, the increase in new chemical entities and potential targets identified by proteomics and genomics has resulted in a gap between the potential screening space and the cost [55, 56]. To address these limitations, miniaturized CBB platforms have been developed to reduce the cost of drug discovery. Bailey et al. [57] established a high-throughput CBB platform to screen for lethal small molecules to mammalian cells. In this platform, chemical candidates were first mixed with poly(lactic-co-glycolic acid) (PLGA) and ar-
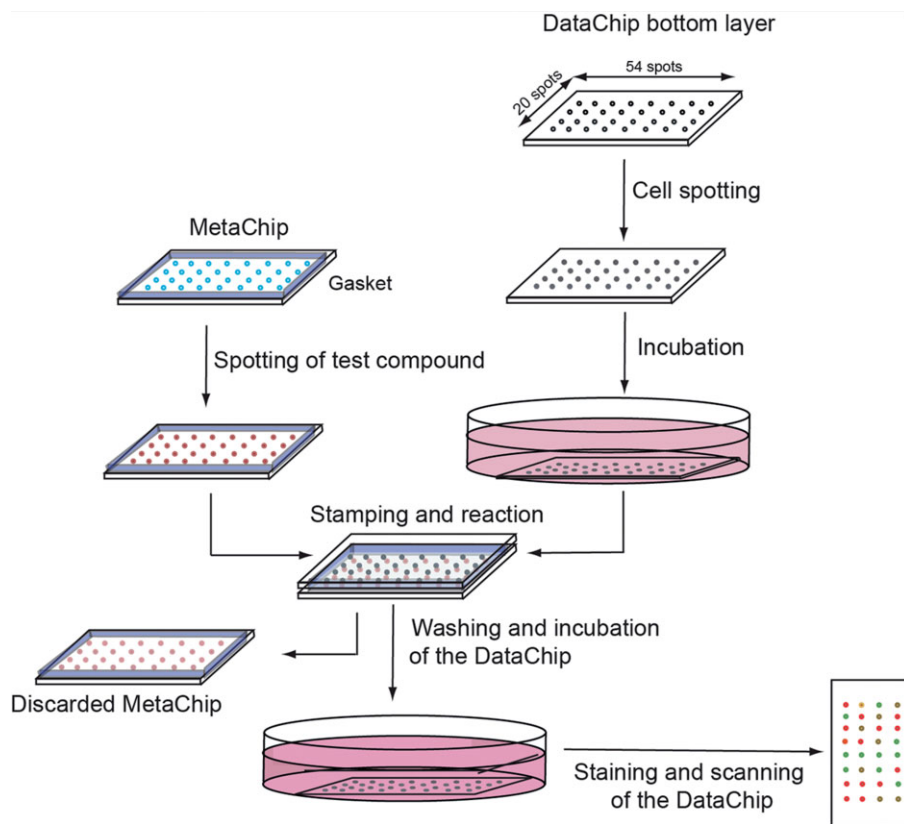
Figure 3. Schematic of the cell array platform to screen for drug candidates [47]. The platform is a microarray consisting of collagen or alginate spots with encapsulated MCF7 cells. Reprinted from [47] with permission from the National Academy of Sciences, USA.

rayed on a glass slide. HeLa cells were then seeded on the top of the arrayed PLGA matrix and cultured in cell media. Due to the degradability of the polymer, small molecules diffused out, affecting the proximal cells. Via this CBB platform, one chemical was identified (macbecin II) and showed lethal effect in cells with decreased expression of tuberous sclerosis 2 [57]. Lee et al. [47, 58] reported a method for high-throughput screening for metabolite toxicity of chemical compounds using hepatocytes. The mixture of drugs, metabolic enzyme and sol-gel were first arrayed on a microscope glass slide and stamped on a cell array (Fig. 3). Drugs in the sol-gel were metabolized by enzyme and then diffused out to affect cell growth. These CBB platforms can significantly increase the throughput, while reducing the overall cost of screening by reducing the amount of expensive reagents and materials used in each assay [59].

The increased number of chemical entities does not necessarily increase new drug approvals by the FDA, partly because of drug-drug interactions [60]. Drug-drug interactions are known to cause many adverse drug reactions and treatment failure. Drug-drug interactions may occur during absorption, distribution, metabolism and excretion. Therefore, there is an unmet need to develop high-

throughput cell-based assays to better understand drug-drug interactions in target cells prior to clinical evaluation. Wu et al. [61] developed a cell-based microarray sandwich system to study drug-drug interactions. This system included drug combination arrays and cell arrays. Drug combinations were printed on a PDMS post array and sandwiched to the cell-seeded microwells by stamping. In this way, drug-drug interactions were evaluated in a sealed chamber via this assembled array, and three chemicals were found to interact with verapamil.

3.3 Screening cell-biomaterials interactions

Natural and synthetic biomaterials have been extensively used in many biological applications, such as tissue engineering and regenerative medicine. Biomaterials provide microenvironment with signals arising from the cell-to-cell, ECM and soluble factor to support cell adhesion, growth and differentiation. It has been accepted that cell microenvironment (e.g., temperature, pH, components and humidity) is closely related to biomaterial properties. There are numerous parameters of biomaterials that regulate cell behavior by influencing cell proliferation, survival, shape, migration, differentiation, and gene expression (Fig. 4) [62].
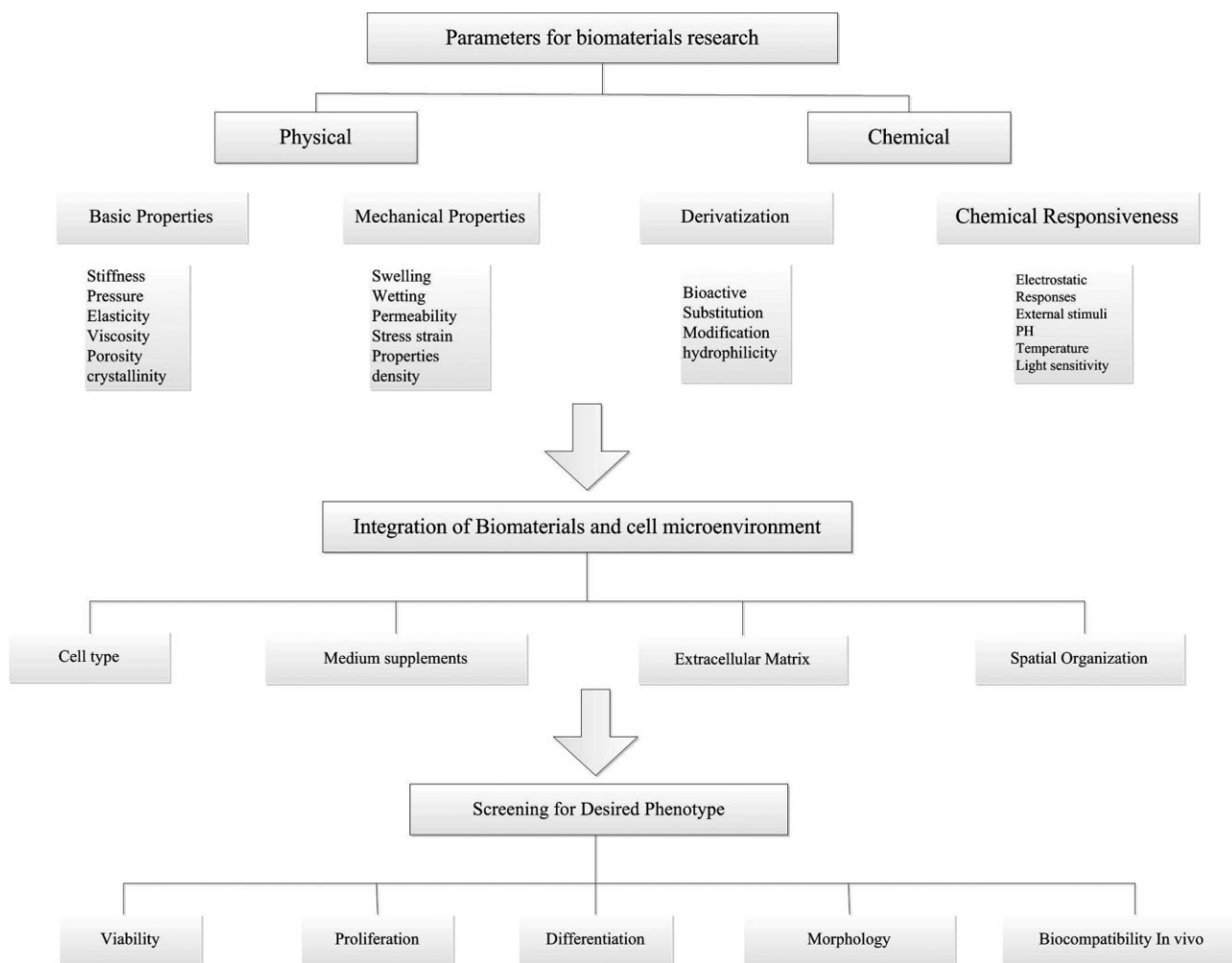
Figure 4. Parameters of biomaterials affect cellular behavior and microenvironment [62]. Reprinted from [62] with permission from Elsevier.

Therefore, it would be helpful to screen cell-biomaterial interactions.

To date, most of the studies on cell-biomaterials interactions have been based on 2D surfaces methods. For instance, Nakajima et al. [63] screened the interactions (cell adhesion, proliferation, promotion of neuronal and glial specification) of neural stem cells with growth factors, polymers, and combinatorial proteins using 2D microassays. The interactions of mesenchymal and embryonic stem cells with polymers that supported cell attachment and proliferation have been investigated [64, 65]. Despite these advances, a number of studies have demonstrated that 2D examination of cell behavior was definitely inadequate [20, 66, 67]. Many factors need be controlled simultaneously when investigating 3D cell behavior [68]. However, researchers have not found an efficient way to control these factors at the same time. Therefore, it is essential to develop 3D high-throughput screening models to

study cell-biomaterial interactions. CBBs based on cell encapsulating hydrogels in a microarray format may provide such an opportunity to rapidly test material-cell interactions.

4 Current challenges and potential solutions

Currently, hydrogel-based CBBs have been used to test pathogen and toxin, to screen for cell-drug and cell-biomaterial interactions. However, CBBs are still in their infancy and some challenges need to be addressed. First, improvement needs to be made in terms of long-term stability and probability. The US army center for environmental health research reported that no CBBs can be used to monitor the safety of drinking water [38]. Preserving long-term cell functions is essential to collect cellular responses over the time, especially for in vitro toxicity studies. Secondly, the properties of hydrogels for

cell encapsulation need to be optimized for their success in developing CBBs, including biocompatibility, mechanical strength and chemical stability. The composition and properties of some existing natural hydrogels are not stable [69], and some synthetic hydrogels (e.g., polyethylene glycol, PEG) are not ideal biomaterials for culturing cells because of poor cell affinity. For example, Arg-Gly-Asp peptides needed to be added as a cell adhesion component when PEG hydrogels were used to culture cells [70]. We have reported that synthetic negatively charged hydrogels, such as poly(2-acrylamido-2-methyl-propane sulfonic acid sodium salt) (PNaAMPS), and poly(sodium *p*-styrene sulfonate) (PNaSS), can support endothelial cells to form confluence [71, 72]. These hydrogels are potential materials for hydrogel-based CBBs. Thirdly, diffusion in hydrogels is restricted, limiting oxygen and nutrients availibility to within 200 µm from the diffusion surface [73]. To overcome the limited diffusion capability, hydrogels with larger pore sizes and microchannel dimensions need to be developed for CBBs [18].

To address these shortcomings of hydrogel-based CBBs, intense collaborative efforts are being made, including the use of modified growth conditions [74], protease inhibitors [75], and cell-cycle inhibitors in growth media [76]. A promising method to preserve long-term cell functions has been shown to be combining 3D cell culture within microfluidic devices [23]. Introducing microfluidic channels into CBBs may help overcome the diffusion limitation by expanding the fluidic networks [77]. Using a microfluidic device with 3D hydrogel cell culture has enabled the assessment of the cytotoxic effect of a drug over the time, which could not be achieved by conventional methods using a 96-microwell plate [78]. Similarly, a portable CBB was also developed to test drinking water using microfluidics-based CBBs [79]. More importantly, micro/nanofluidics can offer a huge potential for biosensors as well as diagnostics of infectious diseases in both developing and developed countries [80]. Furthermore, microfluidic biochip technologies can preserve cellular function in vitro over a long period of time up to 4 months. Thus, integration CBB with microfluidic techniques holds great potential for addressing the challenges of CBBs based on cell-encapsulating hydrogels.

5 Conclusions and perspectives

In summary, here we have presented the state-of-the-art advances in CBB development using cell-encapsulating hydrogels. Because of their 3D features, these CBBs hold the promise of long-term cell viability and close simulation of cell responses in vivo. CBBs have been utilized to investigate in vitro cell-cell interactions, high-throughput drug screening, and detection of pathogens and/or toxins. Although the development of CBBs in these applications is still at the proof-of-principle stage, CBBs hold great potential to become a broadly applicable biotechnical tool. It should be also noted that current CBBs are designed to evaluate/measure single parameters, which can only reveal one single aspect of the overall cell responses to external stimuli. If multiple parameters could be measured simultaneously in a single CBB device, it would reveal a comprehensive landscape of cell responses to pathogens, cell-cell interactions in coculture, and pathways used or affected by drug candidates. However, solutions to the fundamental issues and further CBB development rely on basic advances and integration of biology, material science and engineering. Creating collaboration opportunities and training programs across multiple disciplines would facilitate individuals engaged in these fast-growing areas, and thus create new applications of CBBs in medicine.

The authors declare no conflict of interest.

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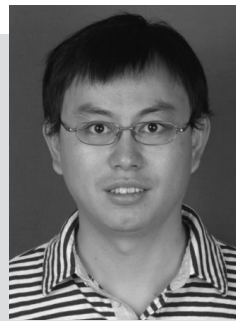
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