Chapter 12

Cell-Encapsulating Hydrogels for Biosensing

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Cell-based biosensors (CBBs) are emerging as a sensing platform in which live cells are utilized to sense external stimuli including physical, chemical, and biological changes. Till now, CBBs have demonstrated a broad range of applications including diagnostics, drug screening, environment monitoring, and biosafety monitoring. Although promising, current CBBs are normally based on cells cultured in two-dimensional (2D) surfaces, which brings challenges such as ease of contamination and limited capability for long-time preservation. In addition, cells grown in 2D culture environments cannot fully represent microenvironment in three-dimensional (3D) native tissues and may result in analytical variations such as in drug screening. One potential strategy to overcome these challenges is to incorporate cells in 3D hydrogels,

which provide cytocompatible microenvironments for prolonged cell preservation and improved phenotypic similarity with cells in native tissues. In this chapter, we present an overview of cell-encapsulating hydrogel based biosensors (CHBBs) and highlight the unique features of CHBBs as opposed to traditional CBBs.

1. Introduction

Cell-based biosensors (CBBs), also named as whole-cell biosensors, are analytical devices incorporating whole cells (e.g., native cells, genetically modified cells, synthetic cells, etc.) with a signal transducer (e.g., electronics, optics).[1–7] In CBBs, cells function as sensing units that interact with analytes and respond in a cytophysiological manner. The transducer then converts cellular responses into readable signals. By correlating these signal readouts with the original stimulus, characterization of biological samples such as sample type and target concentration can be quantitatively or qualitatively obtained (Fig. 1).

In most of CBBs, cells are patterned/cultured on two-dimensional (2D) surfaces. Although surface-based cell culture brings advantages such as ease of implementation, and compatibility for optical/electric detection, they are associated with issues such as ease of contamination for field applications, and incompatibility for long-time preservation of mammalian cells or for immobilization of bacteria. Moreover, when cells are cultured in a monolayer, they exhibit significant differences in phenotypes compared to cells in native tissues. Hence, cells on 2D surfaces do not effectively represent cells in complex three-dimensional (3D) tissue environments.[8–10]

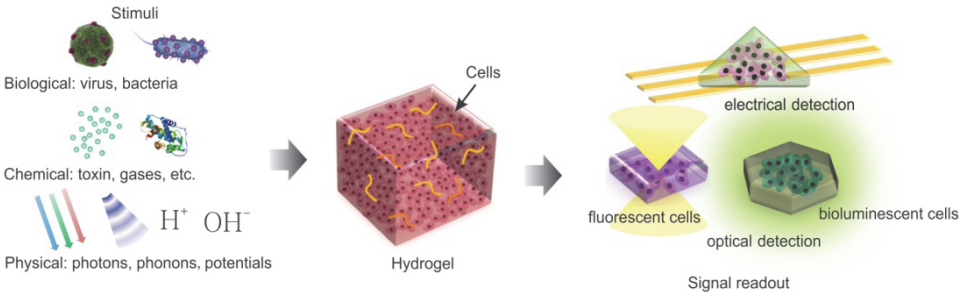

Fig. 1. Schematic of a cell-encapsulating hydrogel-based biosensor. CHBBs are composed of three basic components, namely, cell, hydrogel and signal transducer. Cells function as a sensing unit in response to physical, chemical and biological stimuli in the surrounding microenvironment. Hydrogels provide a 3D ECM. Cellular responses are converted to readable signals by optical/electronic transducers.

Hydrogels (such as collagen, fibrin, alginate, and polyethylene glycol (PEG)) are extensively used as biomaterials to mimic extracellular matrix (ECM) due to their cytocompatibility, moldability, tunability in mechanical properties, high-water content, and porosity.[10–12] The strategy of encapsulating cells within 3D hydrogel constructs has been successfully used in multiple applications such as creating 3D tissue structures for bottom-up tissue engineering, minimizing contamination, and reducing immune rejection for cell delivery *in vivo*.[10,13–16] By bringing together hydrogels, cells, and signal transducers, cell-encapsulating hydrogel-based biosensors (CHBBs) present improved functionality similar to cells in native tissues and are promising to become a new paradigm for CBBs. In this chapter, we first discuss three basic components of CHBBs, namely, hydrogels, cells and signal readout methods, and we then present various applications of CHBBs. Emphasis is placed on the unique features of CHBBs compared to traditional CBBs. Current challenges and their potential solutions of CHBBs are also discussed.

2. Components of Cell-Encapsulating Hydrogels

2.1. *Hydrogels*

2.1.1. *Hydrogel materials*

Hydrogels have attracted tremendous research interest over years in both scientific and industrial applications because of high-water content and biocompatibility. Cell-encapsulating hydrogels provide mimicries for 3D ECM, which improves cell viability, proliferation and maintains metabolic activities such as secretion of cell specific cytokines. Hydrogels can be categorized into natural and synthetic hydrogels.[16,17] Natural hydrogels are extracted from animal tissues such as collagen, gelatin, fibrin, hyaluronic acid, chitosan, as well as plants such as seaweeds. Compared to natural hydrogels, synthetic hydrogels provide a better control over reproducibility with minimal batch-to-batch variations, which is critical in biosensing applications. PEG, poly(vinyl alcohol) and poly(hydroxyethyl methacrylate) are the most widely used synthetic polymers for cell encapsulation.[18] Here, we discuss hydrogel materials that have been demonstrated for 3D cell encapsulation for biosensing applications.

Collagen — Collagen is the most abundant protein in ECM, comprising approximately 25% to 35% of the total protein mass in animal body.[19] Collagen is among the most popular natural hydrogels for cell encapsulation due to its biocompatibility and availability. Cell homing and bio-mimicry of the collagen hydrogels

provide highly sensitive cellular responses in biosensor studies. Collagen type-I is the most widely used among all collagen types. Polymeric structure of the collagen has peptide domains with amino acid sequences such as Asp–Gly–Glu–Ala (DGEA) and Gly–Phe–Gln–Glu–Arg (GFQGER) that regulate cell adhesion and phenotypic activities. However, collagen hydrogels have poor mechanical properties. They are soft with elastic moduli, ~5 kPa. Mixing collagen with other hydrogels is a common practice to enhance the mechanical rigidity as well as specializing hydrogels for culturing sensitive cell types such as neurons.[20] Due to its biocompatibility in biosensing applications, collagen has been used to encapsulate a wide variety of cells (e.g., stem cells and cancer cells). In addition, collagen is used to coat the surface of biosensor rods to increase biocompatibility and to prevent immune rejection for *in vivo* transplantation.[22]

PEG — PEG is a synthetic hydrophilic polymer with good biocompatibility, non-immunogenicity, and high resistance to protein adsorption. These properties make PEG one of the most popular synthetic polymers in medicine and biomedical applications. PEG can be synthesized in different chain lengths for specific purposes. PEG can be chemically modified with methacrylate groups, which becomes photo-crosslinkable, flexible to form the desired shape and spatial organization. Further, PEG hydrogels can be modified with various biological agents such as short peptides (i.e., Arg–Gly–Asp, RGD), enzymes (i.e., matrix metallopeptidase 13, MMP-13), growth factors (i.e., vascular endothelial growth factor, VEGF) or polymers to induce degradation (i.e., disulfide group, polyester, and fumarate). The mechanical properties of PEG hydrogels vary by molecular weight and crosslinking efficiency. The synthesis and modifications of PEG hydrogels can be standardized to enhance reproducibility. In this regard, biologically modified PEG hydrogels are promising materials for encapsulating cells for biosensing applications.[23] Cell-encapsulating PEG hydrogels have been developed as oxygen sensors utilizing fluorescent particles.[24] Moreover, PEG hydrogels can be utilized to respond to changes in pH[24] or cytokines[26] as well as to study the binding kinetics of antibodies.[27,28]

Alginate — Alginate, also known as alginic acid, is derived from brown algae. It is an anionic polysaccharide with high-water absorption capacity. Alginate is biocompatible and is widely used in food industry, drug delivery, and wound dressing applications. In long-term cell encapsulation studies, biological properties of alginate might be tuned with addition of peptides and/or growth factors to generate more favorable microenvironments for cells. RGD peptides are widely used to promote cell adhesion and to functionalize encapsulated cells. Due to its easy handling and crosslinking properties, alginate also finds applications in cell-encapsulating hydrogels for biosensing. Due to its anionic nature, alginate is sensitive to electric changes, which makes it a good candidate for electric sensing. In industrial applications, fungal cells have been encapsulated in alginate to detect changes in pH,[29]

and fibroblast encapsulating hydrogels have been used for detection of specific chemical agents.[30]

Agarose — Agarose is a polysaccharide derived from seaweed and is one of the main components of agar. The stiffness and porosity of agarose gels can be tuned by varying densities and concentrations. It is mostly used in gel electrophoresis to separate proteins and nucleic acids. Cell-encapsulating agarose can be used for migration assays and growing bacterial cells. Agarose hydrogels create favorable microenvironments for bacterial cells to detect chemical contaminations in drinking water.[31]

2.1.2. *Design and fabrication of hydrogels for biosensing applications*

The design of a cell-encapsulating hydrogel should take into account that the hydrogel should be biocompatible, porous and provide an essential for the cell survival and functionality. Besides, the hydrogel should not interfere with the read out signal, introducing background signal noise or luminescence shielding of the cell response. In addition, hydrogels should be highly standardized, and the batch-to-batch variability should be kept at a minimum range. Commercialization efforts for such systems require the accessibility, simplicity in use, cost efficiency and high throughput in mass production as well as long shelf life. Merging more than one biosensing features to biosensor design can significantly promote the wide applicability of the biosensing devices.

Assembly approaches — Fabrication techniques for cell-encapsulating hydrogels use a similar approach to have cells suspended in hydrogel prepolymer solution followed by crosslinking. The general cell-encapsulating approach is to mix the cells with the liquid form of polymer, and crosslink by temperature (e.g., collagen, agarose), chemicals (e.g., crosslinking alginate in calcium chloride solution) or with ultraviolet (UV) light (e.g., methacrylated PEG). There is a growing interest in engineering 3D living constructs from microscale cell-encapsulating hydrogel units[16] using different assembly principles such as magnetic,[32–35] acoustic,[36] surface tension,[37] liquid-based template,[38] ratchet-based,[39] microfluidic,[40–42] and robotic principles.[43] Advantages and disadvantages of these microscale assembly approaches were discussed in previous chapters.[44–48]

Bioprinting — Bioprinting emerged as a powerful method to pattern cell-encapsulating droplets on a receiving surface for biological applications, including tissue-like structures[49] and biosensors,[50–52] or cell-free polymer blocks for numerous applications such as 3D printed micro-battery[53] or bionic ear.[54] Here, we focus on the use of bioprinting technologies in developing cell-based biosensors, and illustrate underlying mechanisms of bioprinting to deposit cell-encapsulating droplets. Bioprinting offers high throughput, computerized xyz controlled deposition of

multiple bioactive factors and cells to a receiving substrate. Bioprinting has been demonstrated in several applications, including tissue engineering, microphysiological system engineering, as well as biosensor fabrication.[55] With these capabilities, bioprinting can reduce the cost of fabricating living biosensors. A biosensor platform was presented with a lensless charge-coupled device (CCD) and bioprinted smooth muscle cells on a microfluidic chip.[50,51] The CCD was utilized to evaluate cellular changes in morphology and viability to an external stimulus. Cell alignment and orientation were quantified in seconds without labeling. Recently, bioprinting has been also used to pattern mouse myotubes onto micronized cantilevers and ultimately to fabricate muscle-powered biosensors[52] (Fig. 2A). Results showed that mature myotubes were formed only after 4 days in the bioprinted samples, whereas the same process took >14 days in the control group of randomly seeded cells. Synchronous responses of cells to electrical and chemical stimuli were also investigated. These results are promising to demonstrate fabrication of biosensors at high throughput via bioprinting.

Computational simulations can help to understand how parameter, prior to actual experimentation, affect cell encapsulation or post-printing cell proliferation and viability.[56–58] Encapsulation of a cell or multiple cells into pico/nano-liter droplets is a probabilistic phenomenon[59,60] (Fig. 2B). Statistical models were presented to provide an understanding of cell-encapsulation process.[59] Results were presented as probability distributions, P(Xt) as a function of different target cell densities and types of cell loading (Fig. 2C). These statistical functions evaluated the probability of a single target cell suspended in a heterogeneous cell mixture to be encapsulated in an ejected droplet. While the ratio of target cells and homogeneity decreased in cell suspension, each probability function followed a Poisson distribution (Fig. 2C).

During the landing of ejected cell-laden droplets, mechanical aspects such as hydrodynamic pressure, capillary forces, and shear stress, might cause deformation of droplet and cell surface[61,62] (Figs. 2D and 2E). Eventually, this process can lead to programmed cell death (apoptosis). However, these experimental dynamics can be adjusted by decreasing/increasing ejection velocity or by replacing encapsulating fluids with better combination of material properties. Cell fate may depend on the hydrophobicity/hydrophilicity of the receiving surface, which is highly correlated with contact angle between droplet medium and surface. Prediction of cell fate using simulations can provide more parametric control over biosensor designs as well as complex viable tissue surrogate designs.[32–36,44,63] A finite-difference/front-tracking simulation model was reported for deposition of viscous compound droplets onto a receiving surface as a model for cell printing process.[64–66] By simulation, physical parameters were optimized for minimum cell deformation. Analyses were carried out for a set of dimensionless parameters, i.e., Weber

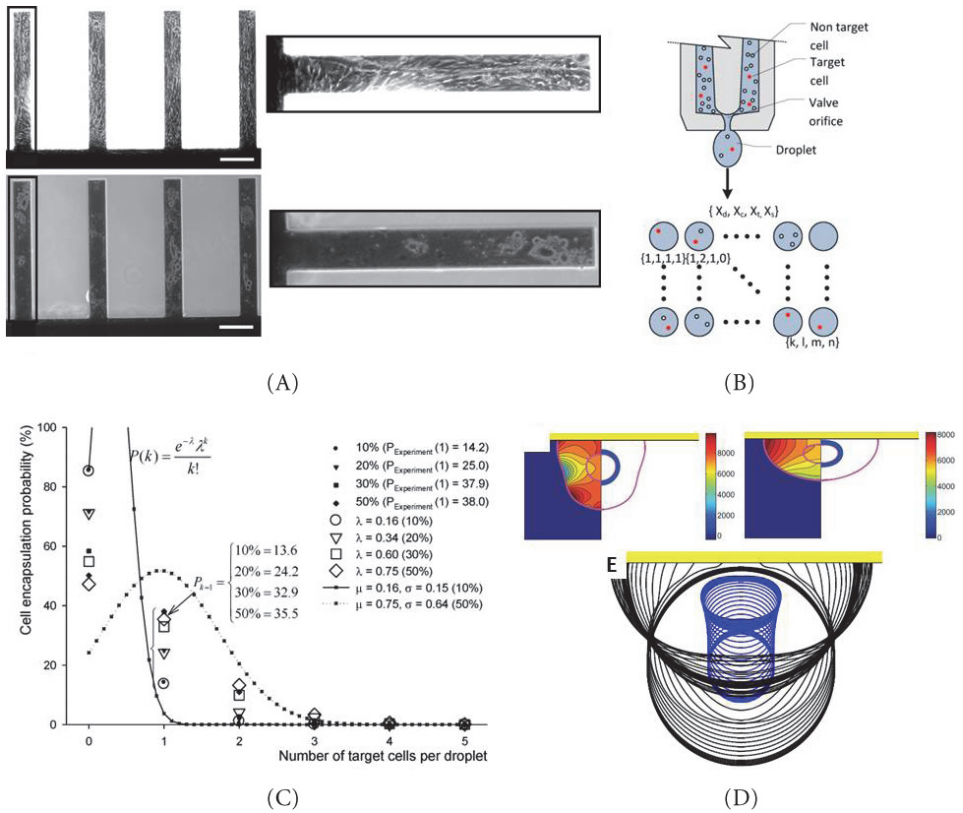

Fig. 2. (A) Biosensor fabrication via bioprinting cells onto cantilevers. Mouse muscle myoblast cells bioprinted on cantilevers. (top) Bioprinted cells formed myofibers on all cantilevers after 4 days in culture. (bottom) Non-printed cells randomly distributed on cantilevers and no myofibers were observed after 7 days. Scale bars 200 μm. (B–E) Statistical and computational modeling of cell encapsulation and printing process. (B) A droplet ejector was filled with heterogeneous mixture, including target and non-target cells for random cell encapsulation process. (X_d) the number of droplets that contain cells, (X_c) number of cells per droplet, (X_t) number of target cells, and (X_s) droplets encapsulating a single target cell, were mapped onto a matrix of cell-encapsulating droplets. (C) Cell encapsulation probability, $P(X_t)$, as a function of number of target cells per droplet for cell concentration = 1.5×10^5 cells ml^{-1}. (D) Simulation of cell printing process. Pressure contours and pressure distribution on the cell were plotted at the left half and the right half, respectively. Governing non-dimensional numbers are: We = 0.5, Re = 30, $d_o/d_i = 2.85$, $\sigma_o/\sigma_i = 2541$, $\mu_c/\mu_d = 10$. (E) Sequential impact images of cell-encapsulating droplet. (*A*) *is reproduced with permission,*[52] (*B*) *with permission,*[59] (*C*) *with permission,*[66] *and* (*D, E*) *with permission.*

number (We), diameter ratio (d_o/d_i), viscosity ratio (μ_c/μ_d), Reynolds number (Re), surface tension ratio (σ_o/σ_i), and equilibrium contact angle (θ_e). We and Re are widely-used non-dimensional numbers in fluid dynamics[67] to evaluate the influence of inertial forces compared to surface tension and viscous forces,

respectively.[68–70] The computational results demonstrated that the geometrical deformation of cell monotonically increased: (i) as d_o/d_i decreased; (ii) as q_e decreased (iii) as μ_o/μ_I increased; (iv) as Re increased; or (v) as μ_c/μ_d decreased. On the other hand, a local minimum, at least, of maximum geometrical deformation was obtained at We = 2. Results demonstrated that θ_e and μ_c/μ_d were strongly correlated with cell fate.

Such computational models can accelerate the development of more precise and reliable biosensors via bioprinting. Next-generation models should incorporate non-Newtonian features of fluid flows,[71,72] smaller contact angles, microstructured models of cells, and multiple patterning of droplets.

2.2. *Cell-based sensing units*

2.2.1. *Introduction of cell-based sensing units*

Cell is the basic unit of structure and function in most organisms, spontaneously perceives physical, chemical, and biological changes in its surrounding microenvironment, and responds in a cytophysiologically relevant manner.[2] Cell membrane is a selectively permeable interface that consists of a phospholipid bilayer with embedded proteins (e.g., receptors, channel protein), permitting intracellular–extracellular mass exchange. Of these proteins, receptors serve as microsensors and monitor environmental changes. Activation of the receptors by an external stimulus gives rise to a series of intracellular signaling cascade, which may result in complex cell physiological changes at a wide spatiotemporal scale (from the molecular level to the cellular level ranging from nanoseconds to hours). For example, folding of β-hairpins occurs in microseconds[73]; activation of G protein-coupled receptors (GPCRs),[74] inositol triphosphate receptor (IPR) mediated calcium release,[75] cytoplasmic protein and membrane protein translocation[76] ion transmembrane flux through ligand-gated ion channel[77] can be completed in milliseconds; chemotaxis induced neutrophil migration to an inflammation site takes seconds[78]; activation of protein- and lipid-mediated kinase cascades can be completed in minutes; and phenotypic changes generally take hours and even days. By correlating cell physiological changes with external stimuli, cells can be explored as a sensing unit to detect the existence of pathogens and toxins in food, clinical and environmental samples. In addition, these cytophysiological changes can also be used to deduce functional information (e.g., toxicity and efficacy of pharmacological agent) of a known stimulus, which can be useful for drug screening.

Biological macromolecules such as antibodies, enzymes, and nucleic acids have been widely used as sensing units in broad applications owing to their specificity and sensitivity. Although CBBs explore the same molecular recognition

mechanism with molecule-based biosensors (MBBs) via interactions between cellular membrane proteins and external stimuli, CBBs have their own unique features. First, some cell types are more robust than biomolecules under harsh conditions (e.g., thermophiles at high temperatures and acidophilus under high acidic conditions), whereas biomolecules, including proteins and deoxyribonucleic acid (DNAs) may lose their functions as a result of denaturation or degradation when exposed to the similar conditions. Second, CBBs enable spatiotemporal amplification of stimulation signals via intracellular signaling networks, which make it possible to detect subtle interactions between cells and stimuli. On the other hand, MBBs may require specific labeling using materials such as gold nanoparticles and quantum dots for signal amplification. Third, CBBs, especially bacteria based biosensors, are more cost-effective than MBBs. Bacteria can rapidly proliferate, which reduces the assay time and cost. Fourth, CBBs are capable of responding to a variety of biohazard substances (e.g., toxins, pathogens) in a cytophysiologically relevant manner akin to human beings and animals, thus offering an alternative platform for drug screening.[2] Fifth, CBBs allow more sophisticated applications than MBBs by bioengineering cells with functional exogenous genes or synthesizing artificial cells.[79–81] Comparison of MBBs and CBBs is given in Table 1.

Table 1. Comparison of cell-based and molecule-based biosensors. Mammalian-cell based biosensors and bacteria-based biosensors are listed separately due to their significant difference.

	Cell-based biosensors		
	Eukaryotic cells (e.g., Mammalian cells)	**Prokaryotic cells (e.g., bacteria)**	**Molecule-based biosensors**
Pros	1. Highly specific receptors 2. Spontaneous signal amplification 3. Provide functional information	1. Long-term stability and shelf life 2. Inexpensive to expand bacterial population 3. Spontaneous signal amplification	1. High specificity 2. Easy to immobilize 3. Linear response and suitable for quantitative analysis.
Cons	1. Expensive to expand mammalian cell population 2. Vulnerable to environmental changes 3. Difficult to deduce original stimulus in case of downstream cytophysiological events	1. Complex signaling transduction pathway 2. Difficult to deduce original stimulus	1. Expensive to manufacture 2. Easy to be degraded and denatured at the field

2.2.2. *Strategies for cell-based biosensing*

Bacteria and mammalian cells have been widely used as a sensing unit in CBBs. Bacteria are robust to harsh microenvironments, which make them suitable for applications such as deployment on the field. In addition, it is not expensive to maintain and expand a bacterial population. Compared to CBBs that immobilize bacteria on a 2D surface, bacterium-encapsulating hydrogels have advantages, including improved immobilization/packaging, minimization of contamination from other microorganisms and ease of deployment. However, wild-type bacteria usually develop comprehensive panels of membrane receptors with complex signaling cascade networks to survive in nature. Therefore, one downstream cytophysiological change in bacteria can be initiated by multiple upstream cytophysiological events, which brings difficulty in analyzing the presence of an unknown stimulus based on only downstream cytophysiological change. Adult mammalian cells are usually highly differentiated and responsible for specific tasks in a multicellular organism. Some specialized mammalian cells such as taste-receptor cells, photoreceptor cells and gustatory-receptor cells can be used for highly sensitive detection of a specific external stimulus. Despite their great promise, mammalian cells are fragile to environment changes and require well-controlled culture environment (e.g., proper temperature, humidity, carbon dioxide concentration, and culture media) to maintain their normal cytophysiology, and thus it is a challenge to deploy mammalian cells in harsh environments. In addition, maintenance of mammalian cells is labor-intensive, which raises the cost of CBBs. Here, we review cell-based sensing units reported in CHBBs, from the perspective of sensing mechanisms. We discuss four types of sensing mechanisms (i.e., voltage gated channels, GPCR receptors, nuclear receptors and immunoglobulin receptors) that are widely used in CBBs (Fig. 3). More detailed information regarding natural receptors in sensors can be found in literature.[31,82,83]

Voltage gated ion channel — Voltage-gated calcium channel (VGCC) is one of the trans-membrane ion channels with permeability to calcium ions 1000 times greater than sodium ions. VGCCs are widely found in the membrane of excitable cells (e.g., neurons, cardiac cells, muscle cells, and secretory cells) and explored as a mechanism for biosensing ions and small molecules. Under normal cytophysiological conditions, VGCCs are closed, and the concentration of intracellular Ca^{2+} is several thousand times higher than extracellular Ca^{2+}. Exposed to a high concentration of extracellular Ca^{2+} ions, VDCCs are activated due to membrane depolarization. Extracellular Ca^{2+} rush into cytoplasm, resulting in series of cell physiological changes ranging from muscular contraction, excitation of neurons, gene expression, to exocytosis of hormones or neurotransmitters, which can be utilized for biosensing.[84,85] Detection of the heavy-metal ions was demonstrated using a cardiac

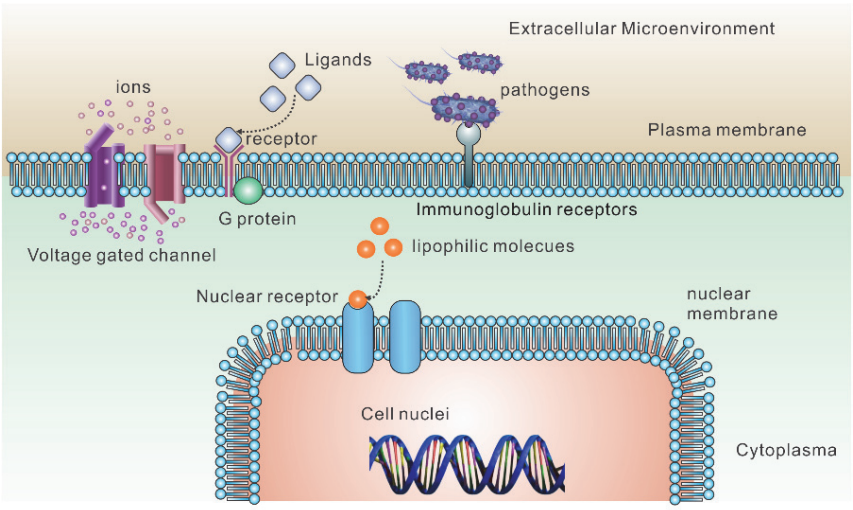

Fig. 3. Schematic of cell sensing mechanisms. Four types of typical sensing mechanisms are schematically illustrated, including voltage gated channels, GPCR receptors, nuclear receptors, and immunoglobulin receptors.

cell-based biosensor.[86] Beating cardiomyocytes generated periodic extracellular potential that was detected by a light-addressable potentiometric sensor. Exposure to heavy-metal ions, cardiomyocytes experienced characteristic changes in their beating parameters (i.e., frequency, duration, and amplitude) based on the physiological effects of metal ions. Fe^{3+} and Cu^{2+} induced very small changes in cardiomyocytes beating, while Hg^{2+} and Cd^{2+} resulted in significant changes in the beating pattern. By exploring the frequency-duration shifting in phase diagrams, different ions can be distinguished. The device provided an alternative way for heavy-metal monitoring and toxicity screening. Human neuroblastoma cells were incorporated into a 3D collagen type-I based biosensor.[87,88] Activation of VGCCs was demonstrated by supplying high concentration of K^+ ions (50 mM) that led to depolarization. Intracellular Ca^{2+} dynamics were transformed into fluorescence signals by calcium green-1, a calcium fluorescence indicator, and finally quantified using a fluorescence microscope. In addition, the membrane potential dynamics were fluorescently quantified using a potentiometric fluorescent dye.

G protein coupled receptor — G protein coupled receptors (GPCRs) are a super family of membrane receptors that enable a variety of cytophysiologically relevant processes, including visual sensing, gustatory sensing, olfactory sensing, and cell density sensing. GPCRs are activated by external signals such as electromagnetic radiation and exogenous molecules, including odors, pheromones, hormones, and neurotransmitters. The activation of GPCRs results in signal transduction and

ultimate cell physiological changes at cellular levels (e.g., cell growth and metastasis) and subcellular levels (e.g., increase in cytosolic calcium ion).[23,89] Thus, interaction between GPCRs and ligands or other signal mediators can be used as a sensing mechanism for CBBs. Kang and coworkers reported neuronal micro-circuit arrays for drug screening.[90] Primary hippocampal neurons were maintained in an agarose microwell with a microelectrode immobilized at the bottom to record drug's cytophysiological effect on neuronal circuit activity. Several drugs (AP, bicuculline, and NMDA) were tested using neurotransmitter γ-aminobutyric acid and glutamate as agonist or inhibitory receptors. This cell-based biosensor can potentially be a high-throughput drug-screening platform for excitable cells. Sun and coworkers developed a microfluidic cell-based biosensor that enables detection of transient chemical stimuli using GPCRs with a temporal resolution of milliseconds.[91,92] Spatiotemporally-controllable chemical stimulation of cells was achieved by hydrodynamic-gated sample injection.[93,94] Endogenous P2Y receptors on the membrane of NIH 3T3 fibroblast cells were activated using Adenosine Triphosphate, resulting in a series of subcellular signaling cascades, including activation of phospholipase C, hydrolysis of phosphatidylinositol bisphosphate into inositol triphosphate (IP3) and diacylglycerol by phospholipase C, binding between IP3 and IP3 receptors, and Ca^{2+} release from the internal Ca^{2+} stores to the cytoplasm. Intracellular Ca^{2}+ dynamics was indicated using Fluo-3, an intracellular calcium indicator and recorded by a fluorescence microscope. Feng *et al.*, bioengineered endogenous P2Y receptors (ATP–GPCRs) into a bullfrog fibroblast cell line and coupled these cells with microelectrode arrays for chemical sensing. Bullfrog fibroblast cells demonstrated advantages over mammalian cells for cell-based sensing, as they can be deployed under ambient atmospheric conditions and are suitable for long-term storage.[95]

Nuclear receptor — Nuclear receptors are a class of endogenous proteins found in metazoan cells. Nuclear receptors can sense lipophilic substances such as steroid, thyroid hormones and vitamins A and D and regulate the expression of specific genes.[73,96,97] Genetic engineered *Saccharomyces cerevisiae* cells with nuclear receptors were demonstrated for detection of estrogen.[98–100] *Saccharomyces cerevisiae* strains were genetically engineered with a human androgen receptor, estrogen receptor alpha, or estrogen receptor beta together with firefly luciferase initiated by a respective hormone responsive promoter. These cells are immobilized in 3D polyvinyl acetate and alginate hydrogel matrix. Detection of 17-β-estradiol, an estrogen, was demonstrated with a detection limit of 0.08 μg L^{-1} and a half maximal effective concentration of 0.64 μg L^{-1}. *Saccharomyces cerevisiae* encapsulated in alginate microbeads showed a high viability for luminescence measurements even after 1 month of storage at −80°C. The assay reproducibility for each test was illustrated by coefficient of variation ranging from 4.35 to 18.47%.

Immunoglobulin superfamily receptors — Immunoglobulin superfamily receptors are a class of soluble membrane proteins featured with immunoglobulin-like structures. Immunoglobulin receptors are found in immune cells (e.g., T and B lymphocytes), and they are involved in the recognition and binding of antigen. For example, B lymphocytes express B-cell receptors (an immunoglobulin receptor) that allow highly specific sensing of pathogens or toxin via antibody-antigen interaction.[5] Kim and coworkers developed a strategy to increase the detection sensitivity of lymphocyte based biosensor by exploring B cell as antigen presenting cells for T cell.[101] A confluent layer of B-cells was layered on T cells that were immobilized on hydrogel microwells. B cells captured and internalized exogenous proteins, proteolyzed these proteins into short peptides, and further presented these short peptides to the contacted T cells. Activation of T-cell-receptors by the presented peptides resulted in an increase in the cytosolic calcium level in T cells, which was monitored by fluorescence imaging of calcium via calcium sensitive fluorescence dye Fura-2. In addition, collagen-encapsulated Ped-2E9 cells (a B-cell hybridoma) were demonstrated for CBBs.[102] Rapid cytotoxicity assays of pathogens or their toxins were achieved by quantitatively measuring alkaline phosphatase (ALP) released from infected Ped-2E9 cells.

2.3 *Signal readout systems*

Basically, CHBBs have a signal readout system, which interfaces with cells and transduces cellular responses into quantitative or qualitative signals for detection of analyte. When cells are cultured on a 2D/3D surface, signal readout from individual cells can be obtained using multiple approaches, including electrochemical, mechanical, and optical platforms.[103–114] However, when cells are encapsulated in 3D hydrogels,[115,116] signal readout is geometrically limited, which makes it difficult for some sensing technologies (e.g., electrochemical, mechanical transduction) for single-cell analysis. Optical detection becomes a feasible way for single-cell analysis in CHBBs due to its advantages such as contactless detection, high spatial resolution and readability for naked eyes. Especially, bioluminescence provides an alternative strategy to deliver observable signals without transducers. In the future, microfluidic and lab-chip technologies will have considerable impact on CHBBs by monitoring cellular density profiles without the need for labelling. In this section, we focus on various sensing platforms from a CHBB perspective.

2.3.1. *Optical-based signal readout systems*

Optical detection is a widely-used approach to monitor absorbance, luminescence, or fluorescence signals that are functions of cytophysiological changes and cellular metabolite production.[7]

Fluorescence detection — In CHBBs, fluorescence dyes are employed convert physicochemical or biochemical events into fluorescence signals. Alternatively, fluorescence genes are fused into cells as a reporter to indicate expression of target genes. Diverse fluorescence detection technologies have proven to be invaluable gadgets for monitoring molecular changes in cellular mechanisms such as protein and gene expression.[86,117–119] Fluorescent-labeled gene and protein probes have also been employed to monitor cellular function in genetically engineered organisms for decades.[120] Recently, a fluorescence-based sensor has been developed to monitor continuous blood-glucose levels *in vivo* using glucose-responsive fluorescent hydrogel fibers.[121] The implanted fluorescent PEG-bonded polyacrylamide (PAM) hydrogel fibers transmitted fluorescent signals transdermally of the blood-glucose concentration.[121] In this platform, the fibrous structure of hydrogels further allowed this sensor to remain at the implantation site for a long time period (up to 140 days). Thus, a minimally invasive and transdermal hydrogel-based glucose sensor has been developed to increase the quality of life of diabetic patients.[121] On the other hand, the fluorometric imaging plate reader (FLIPR™) and highly sensitive fluorometric assays have been developed to assess membrane potential, intracellular calcium mobilization and cellular signaling processes.[82,122,123] In contrast to other labeling assays such as radiolabeling, fluorescence-based methods are safe-to-use, and do not cause any mutations and DNA damages as observed in the prolonged exposure of radioactive dyes.[124–126] However, auto-fluorescence of molecules can potentially interfere with fluorescent assays and cause false-positive results.[119]

Bioluminescence — Bioluminescence describes production and emission of light by a living organism. Bioluminescence naturally exists in some microorganisms, fishes, and fungi. Bioluminescent microorganisms include *Vibrio fischeri* and *Pyrodimium bahamense*. For example, *Vibrio fischeri* is utilized to detect mercury and selenium with a detection of limit of 1 ppb.[37] Compared to fluorescence, bioluminescence has advantages, including no need for light excitation source and long emission time. When transfected into cells, bioluminescence genes can be used to indicate the expression of a target gene in response to environmental stimuli. For example, Michelini and co-workers reported a yeast-based biosensor for bioluminescent detection of androgen-like compounds. Recombinant *Saccharomyces cerevisiae* cells were genetically modified with a human androgen receptor and a reporter gene *YIpLuc* to indicate activation of the human androgen receptor.[99]

Plasmonic detection — Plasmonic-based readout systems are one type of optical signal transduction methods that monitor minute changes at the close vicinity of a metallic substrate.[131–135] Surface plasmon-based approaches report vital information in terms of wavelength, extinction intensity and the angle of reflected light, which are particularly sensitive to binding and capture events as well

as the dielectric properties of medium on the metal substrate.[127–131,136] Surface plasmon-based detection strategies also provide a great opportunity to tune the sensitivity and specificity by altering the type of nanostructures at the sensor substrate.[131] Surface plasmon resonance (SPR) approach allows implementing hydrogels onto plasmonic substrates, and monitors physiological behavior of cell and cellular substances encapsulated in hydrogels. In these platforms, the presence of analyte causes hydrogels to expand in volume, and thus, the refractive index differs from that of the original state.[113] By monitoring local refractive index changes, SPR imaging technologies are employed to examine the hydrogel network and assist in improving the optimal conditions for large-scale production of biosensor arrays.[113,137] By changing coupling method (e.g., waveguide coupling) in SPR techniques, the other characteristics of hydrogel film such as thickness can also be examined.[138] Another plasmonic-based platform, localized surface plasmon resonance (LSPR), utilizes strong electromagnetic response of metal nanoparticles and assesses collective oscillation of nanoparticles.[139–141] LSPR biosensing platforms have been broadly used to detect nucleic acids, proteins, toxins, and viruses.[133,141–145] Hydrogels have recently been applied to LSPR-based platforms to monitor the physiological behavior of cells and to enhance the biosensing capacity of stimuli-responsive hydrogel-metal nanoparticles for medical applications.[146,147] For instance, a glucose oxidase was immobilized into the stimuli-responsive hydrogels to evaluate the effect of glucose binding.[146] The interparticle distance of silver nanoparticles in the hydrogel increased, when glucose was applied to the hydrogels. As a result of these interactions, the absorbance intensity of the LSPR peak wavelength decreased, and limit of detection was observed to be 10 pM for glucose sensing.[146]

Holographic sensing — By immobilizing nanoparticles within a hydrogel matrix, holographic sensors have been developed by incorporating ionizable monomers into hydrogel films.[148] In contrast to other optical pH sensors, the shrinkage and swelling behavior of hydrogels, were evaluated by monitoring changes in the holographic diffraction wavelength of holograms.[148] This holographic biosensor presented a sensitivity down to mili-pH changes, and this platform was also validated by quantifying the changes in proton concentrations in milk samples undergoing homolactic fermentation in the presence of *Lactobacillus casei*.[148] The major obstacle in this method is the fabrication of nanoparticle arrays and the need for a frequency doubled Nd-YAG laser.[113,148]

2.3.2. *Electrochemical-based signal readout systems*

Electrochemical sensing finds a variety of applications ranging from pH to conductivity sensing systems.[149,150] These systems can also be combined with other

biosensing platforms, including optical and mechanical sensors.[151] Hydrogels are used as a supporting material, where biomolecules such as DNA, peptides, enzymes are immobilized for biomolecular detection. Hydrogels are also utilized as a stabilizing material for the encapsulated bioactive component.[152–155] Further, hydrogels provide a large surface area, thus increasing the loading capacity and improving the detection sensitivity.[113,156] Conductometric measurements were used to quantify electrochemical signals from hydrogels.[157,158] These platforms utilized interdigitated electrodes and measured the electrical conductivity of hydrogels.[137] Recently, these signal transduction systems have been used to monitor cellular mechanisms such as viability, apoptosis and proliferation, and specific intracellular and extracellular cellular reactions for drug screening.[151,160–162]

3. Applications

CHBBs have recently gained a great deal of interest in drug screening, pathogen detection, and environmental monitoring. As a sensing unit, cells can quickly respond to delicate or drastic stimuli from their microenvironment, and the response can be transformed to qualitative and/or quantitative signals for biosensing. For example, cell morphological changes, altered protein expression profiles, and cell viability can be used to directly indicate the impact of environmental changes. Often, bioengineered cells are preferably used to detect specific proteins and emit fluorescence signals. Here, we summarize the applications of bioengineered cells for rapid pathogen detection and drug screening without reference to traditional methods such as enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR).

Rider *et al.* genetically engineered B lymphocytes for specific sensing a variety of pathogens at low concentrations.[83] First, B cell lines were engineered to stably express cytosolic aequorin, as a bioluminescent reporter. Second, these cell lines were transfected with plasmids that express antibodies specific for a pathogen of interest on the cell surface. The crosslinking of antibodies triggered by their corresponding antigens from a specific pathogen can elevate the level of intracellular calcium and thus lead to a fluorescence emission by aequorin. As reported, a number of B cell lines have been developed for a variety of bacteria and viruses including *Yersinia pestis*, orthopoxviruses, *Venezuelan equine encephalitis* (VEE) virus, *Escherichia coli* strain O157:H7, *B. anthracis* and foot-and-mouth virus. Most importantly, detection can be completed within 3–5 min, and no signal or nucleic acid amplification is required. Although the preparation of these cell lines seems to be complicated, it takes less than 1h hands-on time over two days, indicating that scaling-up of this platform technology can be made economically. Thus, this B cell sensing platform can be widely used for pathogen detection in clinical diagnosis,

bio-defense, and food safety. Similarly, another study demonstrated that fluorescent dye loaded mast cells detected *Escherichia coli* and *Listeria monocytogenes* at a picomolar level via a chimeric protein (fusion of Fc region of the IgE antibody and CD14).[163]

Recently, 3D culture has been developed on-chip to sense pathogens[101] or assess the toxicity of drug candidates.[164] For example, Kim *et al.* developed a T-cell/B-cell based microchip biosensor to detect intact pathogens.[101] In this biosensor, hydrogel microwells were first fabricated. The surface of microwells was then functionalized with antibodies for immobilizing T cells in an array format. On the top of the T cell layer, antigen-presenting B cells were seeded. When B cells presented specific antigens to T cells via T cell receptors, the level of intracellular calcium accordingly increases proportionally to the concentration antigenic peptides. The concentration of intracellular calcium can be measured by measuring the release of calcium-sensitive fluorescent dyes, which were preloaded. As demonstrated, this live cell array quantified a model peptide analyte ranging range 0.05 to 5 μM within minutes.[101]

Banerjee *et al.* reported a portable CHBBs platform using collagen-encapsulated B lymphocytes that can detect bacterial contamination and their toxin derivatives.[102] In this study, they fabricated a Type-I collagen gel matrix, in which lymphocyte origin murine Ped-2E9 cells were seeded at a density of 2.5×10^7 cells mL^{-1} in a 48 well plate. The cells were continuously cultured at 37°C for 24–96 h prior to addition of bacteria or toxin. It took 3–6 h for bacteria and toxin to diffuse through the collagen gel to Ped-2E9 cells and for the cells to secrete ALP. The supernatant was then transferred to another 48 well plate, which was preloaded with an ALP liquid substrate, leading to a color development. The color intensity was measured using a handheld spectrophotometer at a wavelength of 405/595 nm. Alternatively, the ALP liquid substrate can be added directly to the culture plate, and the color development can be measured using a standard spectrophotometer. Using this CHBBs platform, the authors achieved rapid detection of pathogenic *Listeria* and *Bacillus* species and the toxins from these organisms. Potentially, this portable platform can be used for improving food safety and environmental monitoring.

Currently, drug discovery, especially at the early stage, relies on cell-based assays to screen for therapeutic candidates and to assess toxicities. However, cells grown in a 2D format, either on a substrate or in a suspension, may respond differently to drug candidates compared to their 3D counterparts *in vivo*. Accumulating evidence has shown that 3D culture, in contrast to 2D planar culture on-dish, can better mimic the mechanical, chemical, and biological features of ECM.[165–167] Thus, 3D cell culture arrays have also been used to screen for drug candidates and to assess toxicity in a high-throughput manner. For example, Lee *et al.* fabricated a miniaturized 3D cell-culture array system, which consisted of one microchip,

named as DataChip, with 1,080 individual cell cultures in collagen or alginate gels spotted on a functionalized glass slide, and one microchip, termed as MetaChip, contained their cytochrome P450 — generated metabolites P450 in a complementary format.[164] When the DataChip was in contact with the MetaChip by stamping, the interaction between drug candidates and mammalian cells was initiated. The MetaChip was further rinsed, cultured, stained, and subsequently scanned using a fluorescence microarray scanner. The analysis of IC50 was used to assess the toxicity of drug candidates and their P450-generated metabolites. The IC50 values obtained from the DataChip were similar to that obtained on 2D and 3D microtiter plates despite nearly 2,000-fold miniaturization. However, due to the nature of cytotoxicity analysis, the assay time was fairly long (more than 3 days). Nevertheless, the DataChip allowed the high-throughput screening of drug candidates given the number of cell spots arrayed on the DataChip.

In another study, a 3D hydrogel-based microchip was developed for anticancer drug analysis, in which controlled morphology of microstructure was created without using photomasks.[168] For fabrication, cell suspension in hydrogel precursors was injected into a microchannel and local UV photopolymerization was applied to the photosensitive precursors with the aid a fluorescence microscope to form cell encapsulated hydrogel structures in the microchannel. The unreacted hydrogel precursors were then washed away using **Phosphate-buffered saline** and replaced with anti-cancer drug candidates. Via this system, the authors examined the apoptosis in HepG2 and A549 cells in the presence of two intracellular redox parameters such as glutathione (GHS) and reactive oxygen species (ROS). The cell viability and the levels of these intracellular redox parameters were monitored using a fluorescence microscope. Compared to the assessment on a 96-well plate, the 3D culture on-chip demonstrated similar levels of intracellular GSH and ROS contents. This method allows selective encapsulation of cells in hydrogels for 3D drug screening.

4. Conclusions and Perspectives

Encapsulating cells into hydrogels presents an emerging and promising strategy to develop cell-based biosensors. Hydrogels provide a cytocompatible 3D microenvironment for cell immobilization, maintenance and packaging. In addition, cell-encapsulating hydrogels mimic native tissues and provide an attractive drug screening platform. However, CHBBs are still limited by multiple challenges for real-world practice. First, new hydrogels need to be developed to enable a long-term cell preservation with high cell viability, especially for mammalian cells. Second, cells are randomly encapsulated in hydrogels, which brings a challenge in

standardizing CHBBs. Thus, fine cell manipulation technologies are needed to pattern individual cells in 3D hydrogels down to a single-cell resolution. Third, CHBBs inherit common issues of CBBs such as lack of selectivity and inability for quantitative analysis. To address these challenges, development of synthetic biology and genetic technologies is highly needed. Despite these challenges, enabling technologies such as 3D bioprinting, microfluidics, microscale assembly and microscale opto-electromechanical systems provide significant opportunities to advance CBBs toward practical applications. For example, microfluidic technologies can isolate rare cells from cell mixtures and manipulate individual cells to physiochemical stimuli for biosensing.[91–94,169–171] Microscale assembly of hydrogels significantly facilitates design and fabrication of CHBBs for both basic and clinical research.[32–36,44] With integration of miniaturized electronic/optical signal read out systems,[172] CHBBs can provide a powerful analytical toolbox for broad applications in environmental monitoring, drug screening, and clinical diagnostics in the near future.

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