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Rapid, Portable Detection of Endocrine Disrupting Chemicals Through Ligand-Nuclear Hormone Receptor Interactions

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Abstract

Endocrine disrupting chemicals (EDC) are structurally diverse compounds that can interact with nuclear hormone receptors, posing significant risk to human and ecological health. Unfortunately, many conventional biosensors have been too structure-specific, labor-intensive or laboratory-oriented to detect broad ranges of EDC effectively. Recently, several technological advances are providing more rapid, portable and affordable detection of general endocrine-disrupting activity through ligand-nuclear hormone receptor interactions. Here, we overview these recent advances applied to EDC biosensors – including lyophilization, cell-free systems, smartphones and improved competitive binding assays.

Introduction

Nuclear hormone receptors (NHR) are part of the endocrine system, involved in crucial physiological processes, including metabolism, homeostasis, development and reproduction. In the human body, such NHR include estrogen-, androgen- and thyroid receptors. A wide range of natural and synthesized molecules can act as endocrine disrupting chemicals (EDC) by binding to such NHR, posing significant risk to human health[1, 2] and ecosystems[3]. Thus, there is an increasing effort to detect EDC in various environments, including industrial and urban areas[4], remote ecosystems[5, 6], disaster areas[7], and household products[8]. Here we overview and compare recent advances in rapid and portable assaying of NHR-binding activity of EDC, towards decentralized, on-site detection.

A major challenge to detecting EDC is their structural diversity[9, 10]. Consequently, structure-based EDC detection methods are ill-suited for detecting a broad range of EDC, including previously unrecognized varieties. Alternatively, EDC can be detected by their NHR-binding activity using animal-based or cell-based bioassays. In particular, many cell-based approaches provide effective and robust detection of EDC and other environmental pollutants[11]. However, many of these established biosensors require days-long operations, specialized training and immobile laboratory equipment.[12] Due to these constraints, these methods have been limited in addressing an urgent need for exposure assessments[13].

Here we overview and compare recent improvements and alternatives to biosensors which address these limitations. These novel approaches (shown in Figure 1) are evaluated and compared according to several

key characteristics: (1) rapidity and portability, (2) ease-of-use and affordability, and (3) non-toxicity and non-infectivity.

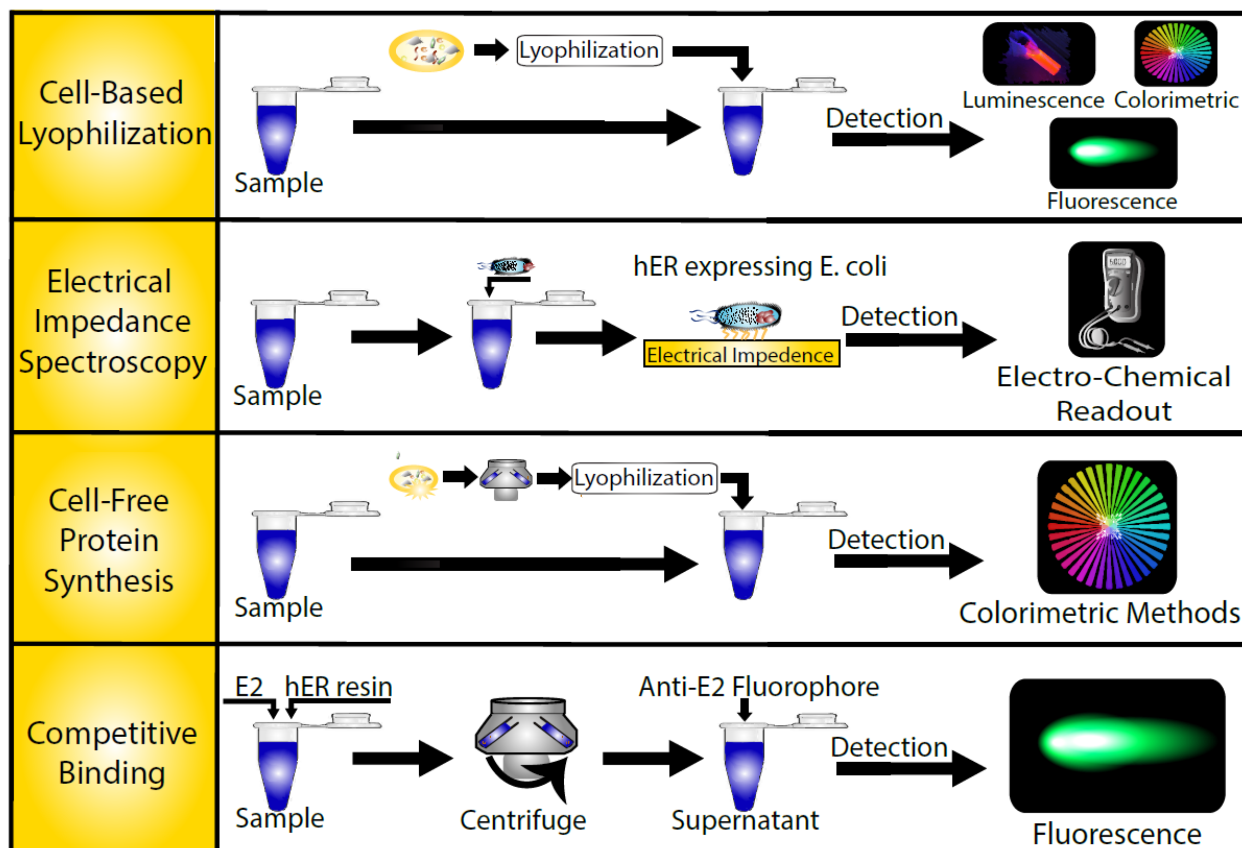

Figure 1. Illustration of the EDC biosensor methods discussed in this review including Cell-Based Lyophilization[14], Electrical Impedance Spectroscopy[15], Cell-Free Protein Synthesis[16], and Competitive Binding[17].

Rapidity and Portability

Biosensors require functional NHR to detect NHR-binding activity in samples. Such NHR are provided by animal models[18] or bioengineered mammalian[19, 20] and microbial cells. In these systems, NHR-binding can be reported by cell proliferation[21], transcriptional activation of reporter proteins[22], or allosteric activation of fusion proteins[23, 24]. The production of NHR via the above-mentioned organisms often involves time-consuming processes confined to the laboratory. Even yeast cells, which are cultured relatively quickly and easily, require lengthy sample processing and culture maintenance, as well as related specialized laboratory equipment. Several strategies have been explored to shorten and decentralize detection workflows, as follows.

Lyophilization

Cells and other biological materials can be carefully dehydrated, which preserves biological activity for storage, handling and transport purposes. Lyophilization – also known as freeze-drying or cryodesiccation – removes water from materials via sublimation at low temperature and pressure, and is the gold standard of long-term cell preservation.[25] Because lyophilization can cause stress to living cells and thus lower viability, strategic additions of cryoprotectants are often used to enhance viability.

Lyophilized cell systems have been successfully used to accelerate conventional yeast-based biosensors, where NHR-binding activity is quantified by reporter gene expression[14]. Alternative detection formats have also employed lyophilized cells. In a notable study, NHR-binding activity was measured with purified monobodies specific to ligand-bound estrogen receptors.[15] These monobodies were immobilized to the surface of a gold electrode, and reported binding by a change in the electrical impedance of the system. In this method, the estrogen receptors are provided by lyophilized E. coli cells that are engineered to surface-express the human estrogen receptor. The lyophilized cells are then simply incubated with the sample of interest, where any estrogen receptor ligand in the sample binds to the estrogen receptor on the surface of the E. coli cells. The electrical impedance of the electrode increases in proportion to the number of E. coli cells bound to its surface, and therefore with the concentration of hormone receptor ligands present in the sample.

The assay's workflow requires less than 1 hour, including duration for incubation and impedance readout. The approach depends on shelf-stable preservation of lyophilized E. coli cells and immobilized monobodies. Researchers have demonstrated the preservation of protein-functionalized surfaces[26], and some desiccation techniques have demonstrated preservation of active proteins at temperatures as high as 37 °C[27]. Future work may explore surface plasmon resonance as an alternative to electrical impedance spectroscopy in this lyophilized cell-based technique.

Due to the removal of cell culturing from the detection workflow, the method potentially accelerates and decentralizes detection of NHR-binding EDC. Lyophilization has also been utilized to create paper-based biosensors[28] for further portability, which may inspire future developments in EDC detection platforms. In addition, alternative dehydration technologies with increased energy efficiency and shortened dehydration duration[26, 27] could be evaluated for EDC detection applications.

Cell-free Expression of Nuclear Hormone Receptors

As an alternative to cell-based approaches, cell-free systems offer rapid in vitro protein expression for analytic purposes[29], including detection of EDC[30]. In such systems, clarified lysate is supplemented with energy-generating molecules and cofactors to produce proteins, including NHR, in a matter of hours[31]. Due to the lack of cellular metabolic processes and cell wall, cell-free biosensors are not limited by transmembrane transport or cytotoxicity[16, 32, 33]. A wide variety of proteins has been produced in cell-free systems including therapeutics[34, 35], proteins with disulfide-bonds[36], proteins with unnatural amino acids[37, 38], multimeric proteins[39, 40], ligand-binding receptor proteins[41].

Recently, cell-free biosensors have been used to detect EDC binding to the human thyroid receptor[30]. An allosterically activated chimeric protein[42, 43] – consisting of the ligand binding domain of the human thyroid receptor, a reporter enzyme, and stabilizing domains – was expressed in cell-free reactions at microliter-scale, directly in the presence of various human thyroid modulating ligands and environmental samples. A colorimetric substrate was added thereafter, leading to color change corresponding to receptor-binding activity. The entire workflow can be executed in less than an hour. Cell-free production was robust in the presence of a wide-range of samples, including raw sewage and human blood and urine[30]. While assay sensitivity was considerably lower than alternative methods, protein engineering of the chimeric receptor could improve sensitivity. Overall, cell-free detection of EDC is promising due to its versatility, speed and relatively low cost.

The above-described workflow was also demonstrated using lyophilized cell lysates, significantly improving storage and portability[44]. Such lyophilized cell-free systems have remained highly active after lyophilization and storage, retaining measureable activity after 90 days at room temperature[45]. Recently, lyophilized cell-free systems have been applied to aptamer-based biosensors as well[28]. The strengths of

lyophilization and cell-free technology complement each other well, potentially leading to rapid, shelf-stable and portable biosensors.

Competitive Binding with Nuclear Hormone Receptors

Numerous analytical methods, including biosensors, utilize pre-expressed and purified portions of the nuclear hormone receptor of interest as a crucial part of their mechanism[46]. Although the production and purification steps may add to the cost, the resultant biosensors can be especially rapid and very sensitive. One recent study achieved EDC detection in as little as two minutes.[17] This method uses competitive binding to purified estrogen receptors followed by an inhibition immunoassay against 17β-estradiol functionalized on an optical fiber. The fluorescent signal of the immunoassay's antibodies is measured real-time. The rinsed, regenerated, and reused optical sensors were active across 300 test cycles, although it appears that fluorescently labeled antibodies were added to each test sample, potentially limiting the affordability of this technique. Other competitive binding technologies may offer simpler mechanisms[46] and future developments, including lyophilization, may contribute toward their portability.

Ease-of-Use and Affordability

High costs and complexities of assays and equipment keep EDC detection largely to the laboratory setting. Several recent efforts have potential to make EDC detection affordable and easy to use, as highlighted here.

Smartphone-Based Signal Detection

Many EDC detection methods require signal transduction machineries which have been traditionally used in a centralized laboratory. In recent years, significant computing power has been miniaturized in the form of smartphones, which are widely available and affordable. Already, smartphones have been explored as commercial sensing in point-of-care diagnostics.

Smartphone cameras have been coupled with many miniaturized accessories – including dark boxes, filters, light sources, and microscopes[47] – to quantify absorbance[48, 49], luminescence[50], fluorescence[51, 52], electrical impedance[53], and surface plasmon resonance[54, 55] of various samples. Many of these studies are still in proof-of-concept stage requiring standardization and optimization. Nevertheless, smartphones show promise as portable signal detection tools with wide availability and simplified interface.

Reagents, Equipment, and Preparation

EDC biosensing technologies that lower development and reagent costs provide more benefit to consumers. Cell-based EDC biosensors can be affordably produced due to the relatively low cost of microbial culturing. In addition, cell-free EDC biosensing requires only micro-liter volumes of clarified cell lysate to detect EDC[30]. Thus cell-free protein synthesis and whole-cell biosensors are potentially more affordable than other assays which require expression and purification of NHR proteins or specialized antibodies.[17]

Non-toxicity and Non-infectivity

Future EDC detection devices for household and in-field use should be non-toxic and present a limited risk of introducing microbial infection or release of genetically modified organisms. Cell-based methods require microbial growth in order to produce a readout, and thus pose inherent risk to human health and the environment. This includes lyophilized cells, which have been demonstrated to retain viability[15] after reconstitution. Passive safety mechanisms such as genetic firewalls using engineered metabolism could be explored in future EDC biosensors[56].

Similarly, cell-free systems – when produced with common lysis and extract preparation procedures – can often contain low numbers of viable microbial cells. Accordingly, optimized lyophilization and lysis steps have been used to wholly remove residual cells from cell-free systems[57]. Cell-free systems for biosensor applications should utilize such protocols to mitigate toxicity and risk of infection.

Conclusion

Timely and accessible detection of EDC activity remains an important public health concern. Rapid and decentralized sensing of NHR-binding activity could enable early detection of such compounds outside of the laboratory. Several technological advances are promising towards this end, as summarized in Table 1. In particular, lyophilization complements existing cell-based methods by removing time-consuming cell culturing steps from their workflow, and increasing their portability and shelf-life. Competitive binding assays offer remarkably rapid detection. Smartphones are another widely available technology which makes such methods more portable and affordable. Notably, cell-free technology is a novel alternative to cell-based biosensor strategies. Cell-free biosensors are rapid and relatively low-cost, while also being robust to cytotoxic samples. The above discussed strategies are useful complements to existing laboratory methods, facilitating quick and affordable EDC detection in remote, non-laboratory settings.

Table 1. Comparison of the EDC detection technologies discussed with respect to characteristic fitness for decentralized use.

	Rapid	**Portable**	**Easy to use**	**Affordable**	**Nontoxic**
Lyophilized Cells	**	**	**	****	**
Electrochemical	****	**	**	*	**
Cell-free Expression	****	**	**	****	****
Competitive Binding	*****	*	*	*	****

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