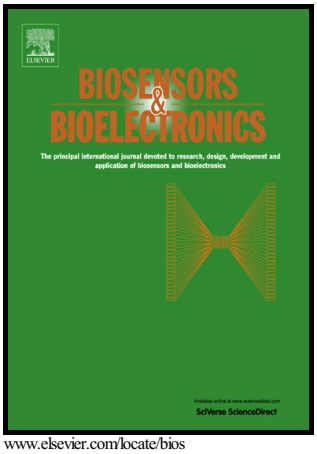
Facile screening of potential xenoestrogens by an estrogen receptor-based reusable optical biosensor

Lanhua Liu, Xiaohong Zhou, Yun Lu, Didi Shan, Bi Xu, Miao He, Hanchang Shi, Yi Qian



www.elsevier.com/locate/bios

PII: S0956-5663(17)30337-8
DOI: http://dx.doi.org/10.1016/j.bios.2017.05.026
Reference: BIOS9739

To appear in: *Biosensors and Bioelectronic*

Received date: 23 February 2017
Revised date: 8 May 2017
Accepted date: 11 May 2017

Cite this article as: Lanhua Liu, Xiaohong Zhou, Yun Lu, Didi Shan, Bi Xu Miao He, Hanchang Shi and Yi Qian, Facile screening of potential xenoestrogen by an estrogen receptor-based reusable optical biosensor, *Biosensors an Bioelectronic,* http://dx.doi.org/10.1016/j.bios.2017.05.026

Facile screening of potential xenoestrogens by an estrogen receptor-based reusable optical biosensor

Lanhua Liu, Xiaohong Zhou*, Yun Lu**, Didi Shan, Bi Xu, Miao He, Hanchang Shi and Yi Qian

State Key Joint Laboratory of ESPC, School of environment, Tsinghua University, Beijing 100084, China

Corresponding author: *xhzhou@mail.tsinghua.edu.cn; **luyun@mail.tsinghua.edu.cn

Abstract: The apparent increase in hormone-induced cancers and disorders of the reproductive tract has led to a growing demand for new technologies capable of screening xenoestrogens. We reported an estrogen receptor (ER)-based reusable fiber biosensor for facile screening estrogenic compounds in environment. The bioassay is based on the competition of xenoestrogens with 17β-estradiol (E_2) for binding to the recombinant receptor of human estrogen receptor α (hERα) protein, leaving E_2 free to bind to fluorophore-labeled anti–E_2 monoclonal antibody. Unbound anti–E_2 antibody then binds to the immobilized E_2–protein conjugate on the fiber surface, and is detected by fluorescence emission induced by evanescent field. As expected, the stronger estrogenic activity of xenoestrogen would result in the weaker fluorescent signal. Three estrogen-agonist compounds, diethylstilbestrol (DES), 4-n-nonylphenol (NP) and 4-n-octylphenol (OP), were chosen as a paradigm for validation of this assay. The rank order of estrogenic potency determined by this biosensor was DES>OP>NP, which were consistent with the published results in numerous studies. Moreover, the

E_2–protein conjugate modified optical fiber was robust enough for over 300 sensing cycles with the signal recoveries ranging from 90% to 100%. In conclusion, the biosensor is reusable, reliable, portable and amenable to on-line operation, providing a facile, efficient and economical alternative to screen potential xenoestrogens in environment.

Keywords: Estrogen receptor; Optical biosensor; Estrogenic activity; Xenoestrogen; Screening system

1 INTRODUCTION

The increasing concern worldwide over the adverse effects of endocrine disruptors on human health has created a need for screening systems to detect xenoestrogens, a diverse group of environmental chemicals that mimic estrogenic actions and are hypothesized to decrease mail fertility (Xia et al. 2010). Screening xenoestrogens in water environment can be implemented by either in vitro or in vivo methods. In vivo estrogenicity testings are measured in the whole animal by a number of possible endpoints such as an increase in uterus weight, with the great advantage of taking into account in vivo functioning, such as absorption, metabolic conversion and breakdown (Legler et al. 2000). Although these assays are widely used, they are unsuitable for large-scale screening, and their utility is further limited due to cost, relatively poor sensitivity and modest responsiveness, and labor-intensive end point measurements (Legler et al. 2000). In order to fulfill the 3R principle of Refinement, Reduction and Replacement of animal testing and reduce the costs, there is a clear need to develop in

vitro methods to efficiently screen chemicals and prioritize limited testing resources (Wang et al. 2014b).

A variety of in vitro molecular and cell-based assays have been developed to determine the potential estrogenic activities of compounds based on different steps in the estrogen receptor (ER) dependent signal transduction pathway, e.g. ER binding, ER controlled reporter gene expression or more downstream events such as ER-mediated cell proliferation (Tim 1997). These bioassays are sensitive, cost- and time-efficient, ethically more acceptable alternatives to whole animal testing (Escher and Leusch 2011), having been shown to produce relevant and consistent outcomes with in vivo testing (Wang et al. 2014b) and analytical chemistry results (Leusch et al. 2010). Among them, the ER binding assay examines the binding capacity of a compound to the estrogen receptor protein. Many endocrine disruptors are believed to bind to estrogen receptors either as agonists or as antagonists. Thus, the binding ability of the chemicals toward the ER is used to test their potential environmental toxicity (Rodriguez-Mozaz et al. 2004). Binding of a compound to the ER is only suggestive that it may behave as an xenoestrogens but does not provide sufficient evidence to conclude that the compound will adversely affect human health or environmental quality (Tim 1997). The advantage of receptor assays is that they are quite simple to perform and allow the identification of all endocrine disrupters that act through the estrogen receptor (Rodriguez-Mozaz et al. 2004).

In 1993, Schwartz and Skafar first reported an ER competitive ligand binding assay based on the competition of estrogenic compounds with radioactively labeled

17β-estradiol (E_2) for binding to the ER (Schwartz and Skafar 1993). The radiolabeling is highly sensitive and specific; however, it relies on expensive and potentially hazardous reagents with limited shelf life whose application requires special handling and disposal, as well as expensive instrumentation (Nicoli et al. 1980). Hence, a considerable amount of effort has been devoted to develop the so-called nonisotopic assays. The conformational change, folding or dimerization of ER were utilized for screening ligands by using enzyme-labeled secondary antibody (Garrett et al. 1999), fluorescent phytoestrogen (Wang et al. 2014a) or fluorescent proteins (Awais et al. 2004) as the reporters. Although more facile and popular, most of these reported bioassays are limited to homogeneous reactions, which, to a certain extent, are still controversial to be called as true biosensors due to lack of solid phases (Wang et al. 2013). With less conceptual dispute, the surface-based biosensors have been highlighted in practical uses because they are able to work as independent portable devices, amenable to use in the field, providing an efficient and economic tool for measuring the estrogenic potency of compounds in environment. Besides, integrated surface based biosensors usually contain regenerable sensing regions, which are beneficial to achieve cheap, automatic, on-line and facile detection (Wang et al. 2016). Up to now, numerous optical (Fechner et al. 2009; Hock et al. 2002; Seifert et al. 1999; Usami et al. 2002), electrochemical (Granek and Rishpon 2002; Xia et al. 2010) and other ER-based (Carmon et al. 2005) biosensors have been developed. However, judging from most ER-based sensing strategies in the past, binding of ER to 17β-estradiol–protein conjugates requires long spacer arms difficult to produce

(Garrett et al. 1999; Hock et al. 2002; Seifert et al. 1999; Usami et al. 2002) and immobilizing of ER protein on the sensor surface fades the reversible regeneration due to the damage of harsh elution conditions (Carmon et al. 2005; Fechner et al. 2009). Therefore, establishing a facile regenerable sensing surface when designing the ER-based biosensors is still challenging and worthy to be addressed.

Inspired by the immunosurface undergoing the antibody–antigen recognition which feature with highly reusability of up to 300 times of regenerated test cycles (Rodriguez-Mozaz et al., 2005; Zhou et al., 2014a), we describe here a novel ER-based reusable fiber biosensor strategy for screening xenoestrogens. This strategy is based on a recombinant receptor of human estrogen receptor α (hERα) protein, fluorophore-labeled anti–E_2 monoclonal antibody and an E_2-protein conjugate functionalized optical fiber which functions as the transducer of the biosensor system. The competition of the estrogenic compounds with E_2 for binding to the ER will leave unbound E_2, which can be detected by an indirect inhibition immunoassay on the fiber-based biosensor. The fluorophore-labeled anti–E_2 antibody will be captured via a facial antibody–antigen surface recognition and detected through fluorescence emission induced by evanescent field on the fiber surface. Apart from the inherent advantages of surface-based evanescent wave biosensors (e.g. sensitivity, selectivity, portable) (Wang et al. 2015a; Wang et al. 2015b; Wang et al. 2013) high reusability over other ER-based biosensors is expected to achieve benefiting from the reversible regeneration of solid surface-based antibody-antigen interaction (Hao et al. 2014; Hao et al. 2015; Long et al. 2009). To validate the bioassay, three estrogen-agonist

compounds, diethylstilbestrol (DES), 4-n-nonylphenol (NP) and 4-n-octylphenol (OP), were chosen as a paradigm.

2 MATERIALS AND METHODS

2.1 Construction and characterization of hERα protein as the recognition element

The recombinant receptor production of human estrogen receptor α (hERα) protein was conducted as described in a previous study with some modifications (Shang et al., 2014). Briefly, the hERα LBD (Ligand Binding Domain) of aa302-552 with three mutations (aa381, 417, 530; Cys to Ser) encoding plasmid (pET 28a) was transfected into *E. coli* (BL21 DE3). Bacteria first pre-grew in lysogeny broth medium containing 15% sucrose until a 1.0 optical density (OD600) was reached. Then temperature was slowly decreased to 16℃ in 3 h, synthesis of the recombinant protein was induced by 0.5 mM isopropylthiogalactopyranoside (IPTG, Merck, Germany) for 16 h. Cells were collected by centrifugation at 5000 g for 15 min, and frozen at -20℃ until use. Purification of the hERa was done at 4℃ or on ice. Cells from 100 mL culture were resuspended in 10 mL lysis buffer (20 mM Tris, pH = 7.5, 300 mM NaCl, 40 mM imidazole, 1 mM phenylmethanesulfonylfluoride (PMSF, Sigma, USA), 5 mM β-mercaptoethanol, 0.1% Nonidet P-40 pre-cooled on ice quickly with a vortex, and lysed with sonication. Lysate was centrifuged at 10,000 g for 30 min and filtered through 0.45 μm membrane. Supernatant was incubated with empty Ni–NTA-agarose resin with the average diameter of 90 μm (1 mL/20 μL: v/v) pre-washed in the sequence of deionized water, 0.1 M $NiSO_4$, deionized water and washing buffer (20

mM Tris, pH = 7.5, 300 mM NaCl, 40 mM imidazole and 0.1% Nonidet P-40) for 2 h. By adding six His tag to its N-terminal, the recombinant hERα LBD was gathered via binding to the Ni-charged resin. The resin was washed twice with washing buffer in order to remove the impurities. The purified resin–hERα complex was dispersed in 10 mL preservation buffer (50 mM Tris, 500 mM KCl, 2 mM dithiothreitol, 1 mM EDTA, 1 mM sodium orthovanadate and 10% glycerol, pH 8.0) and stored at -80℃ in small aliquots before use for the following biosensing process. Moreover, the hERα was eluted by elution buffer (20 mM Tris, pH = 7.5, 300 mM NaCl, 400 mM imidazole, 0.1% Nonidet P-40). The protein purity was checked by SDS-PAGE, and protein concentration was measured by the coomassie brilliant blue assay (see supplementary information, Page 2).

2.2 Fiber-based evanescent wave biosensor platform

Figure 1a shows the schematic diagram of the optical fiber-based biosensor used in this work. Our previous studies have reported this platform for the trace contaminant detection based on the immunoassay (Hao et al. 2014; Hao et al. 2015) and nucleic acid-based recognition (Liu et al. 2015; Wang et al. 2015a; Wang et al. 2015b). Briefly speaking, a 635 nm, 10 mW pulse diode laser with a pigtail was coupled into a multi-mode fiber probe through an optical fiber bundle. Due to the total internal reflection of incident light in the fiber transducer, the evanescent wave was generated on the surface and interacted with surface-captured fluorophores, thus inducing fluorescence excitation. After that, the emitted fluorescence collected by the fiber probe was passed through the same optical fiber bundle, and subsequently filtered by

means of a bandpass filter and detected by photodiodes through lock-in detection. The probe fiber was embedded in a flow cell with size of 55 mm in length and 2 mm in diameter. All reagents were pumped by a flow delivery system using a peristaltic pump. The controls of fluid delivery system and data acquisition and processing were automatically performed by a built-in computer.

2.3 Sensing mechanism

Figure 1b depicts the design of this estrogen receptor-based fiber biosensor for facile screening of estrogenic compounds in water samples. Briefly, the bioassay is based on competition of the estrogenic compounds with 17β-estradiol (E_2) for binding to the recombinant estrogen receptor in the form of resin–hERα complex, leaving the unbound E_2 separated by centrifugation to bind to fluorophore-labeled anti–E_2 monoclonal antibody. The remaining free anti–E_2 antibody then binds to the immobilized E_2–protein conjugate on the fiber surface, and is detected by fluorescence emission induced by evanescent field. The emitted fluorescent intensity inversely relates with the estrogen potency and concentration of compounds in samples, therefore providing the basis of in vitro screening potential estrogenic compounds. In the presence of estrogenic compounds, the hERα will be occupied due to the ER-ligand interaction, resulting in more unbound E_2 in the supernatant after centrifugation, which is accompanied by an increase in the formation of E_2–antibody complex. This will be reflected by a fluorescent signal decrease when detected on the fiber optical biosensor. Normally the stronger estrogenic activity exists, the lower signal will appear, exhibiting a typical "turn-off" sensing mode. Finally, a

regeneration process is performed by rinsing the fiber surface with sodium dodecyl sulfate (SDS) solution (0.5% w/v, pH 1.9) for 200 s and subsequently 0.01 M PBS buffer for 100 s at a flow rate of about 1 mL/min at room temperature, leaving it ready for a new test cycle.

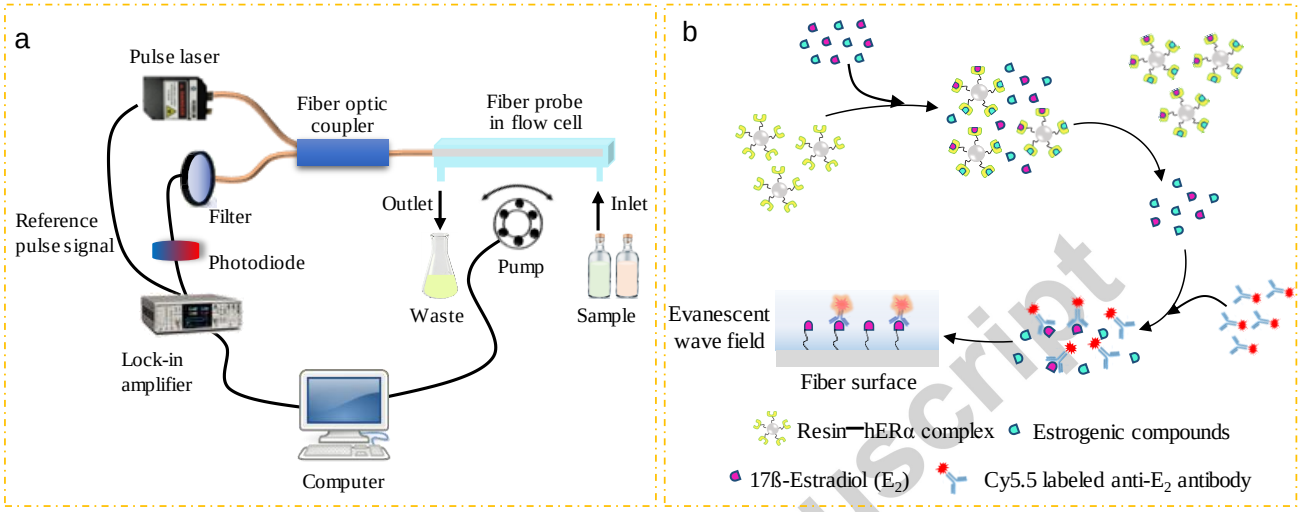


Figure 1 (a) Fiber-based evanescent wave biosensor platform; (b) Sensing mechanism of the estrogen receptor-based screening design for xenoestrogens: Briefly, the surface of the optical fiber was functionalized with the 17β-estradiol(E_2)–BSA conjugate, to which the unbound fluorophore-labeled anti–E_2 monoclonal antibody, which inversely related to the estrogen potency in water samples, could be captured via affinity reactions, thus exposing the fluorophores in the evanescent wave field.

2.4 Others

See supplementary information for chemicals, anti-E_2 monoclonal antibody, E_2–BSA conjugate and buffers used in this work (Page 3). Basic preparation works are also listed in the supplementary information, including preparation of antibody–BSA

conjugate-modified optical fiber by using the MTS/GMBS covalent crosslinking method, labeling of anti-E_2 antibody with Cy5.5 mono NHS ester (Pages 4-5).

3 RESULTS AND DISCUSSION

3.1 Establishment of hERα-based fluorescent bioassay on fiber optical biosensor

Routine screening xenoestrogens demands for cost-effective, reliable and sensitive detection technologies (Wang et al. 2014a; Yildirim et al. 2012), which could be accomplished by using the biosensor technology. The optical fiber biosensor used in this study generates the exponential decay of evanescent field strength, confining excited signals to a narrow distance from the fiber surface (usually one half of the excitation wavelength). Shielding possible background interferences from other components in bulk phase makes the sensitivity of this system dramatically increased (Zhou et al. 2014a). Immobilization is another key step in the development of biosensors. It not only helps to achieve the required close proximity between the biomaterial and the transducer, but also to stabilize the biomaterial for reuse (D'souza 2001). In our sensing design (**Figure 1b**), the hERα binds to the estrogenic compounds in homogenous phase. The biological recognition event could be converted into a readable signal through the indirect competitive immunoassay on the reusable optical biosensor, which can be realized by immobilization of antigen derivative on the fiber surface (Guo et al. 2016).

The bioassay relies on a two-step recognition process: (i) the competition of E_2 and xenoestrogens binding to the hERα; and (ii) the inhibition of Cy5.5-labeled anti-E_2 antibody binding to the antigen immobilized on the solid surface (existing as the

antigen–protein conjugate) by the free E_2 in the sample. Therefore, the ratio between the hERα concentration and E_2 concentration, and the Cy5.5-labeled anti-E_2 antibody are both critical factors to determine the bioassay performance. The Cy5.5-labeled anti-E_2 antibody concentration was optimized to be 1.0 μg/mL using the method in our reported work (Hao et al. 2014; Zhou et al. 2014a) (see supplementary information, Pages 6-7).

The hERα concentration and E_2 concentration was further optimized via the hERα recognition and signal transduction on optical fiber biosensor (see supplementary information, Pages 8-9). **Figure 2a** shows the measured variations of relative signals of optical fiber fluorescent biosensor with the E_2 concentration for different hERα concentration. The relative signal (B/B_0) was calculated by dividing the response of the analyte-containing solutions (B) by the response of the blank solution (B_0). Through curve-fitting with the logistic function embedded in Origin Software, the target concentration providing a 10% decrease of the blank signal is generally used to define the detection limit; whilst signals ranging from 80% to 20% of the maximal signal are used to define the linear working range (Zhou et al. 2014b). With this rule, the relative signals represented by the curve with no added hERα displayed a linear detection range of 0.07~0.41 ng/mL, as highlighted by shadow in **Figure 2a**. As expected, adding the hERα to the immunoassay made the calibration curves moved to the right-hand side, giving rise to the increased detection limit of E_2. This occurs because the hERα is binding to the E_2, making it unavailable for binding to the anti-E_2 antibody and, consequently, increasing the binding of the antibody to the E_2-protein

conjugate immobilized on the fiber surface, which will be excited by the evanescent wave field. To ensure the maximum sensitivity of this bioassay, the added hERα concentration binding to the E_2 should leave the unbound E_2 concentration close to the upper limit of linear range (~0.41 ng/mL) without adding hERα. Bearing this rule, the hERα concentration of 58 pmol was chose where the signal started to fall linearly with E_2 >0.41 ng/mL. Half maximal inhibitory concentration (IC_{50}) was interpolated as 1.6 µg/L at the curve with addition of 58 pmol hERα, achieving the most sensitivity caused by variation of E_2. Therefore, 1.6 µg/L was set as the fixed E_2 concentration for the competition with xenoestrogens. The ratio between the hERα and E_2 was 10 under the above-mentioned optimization.

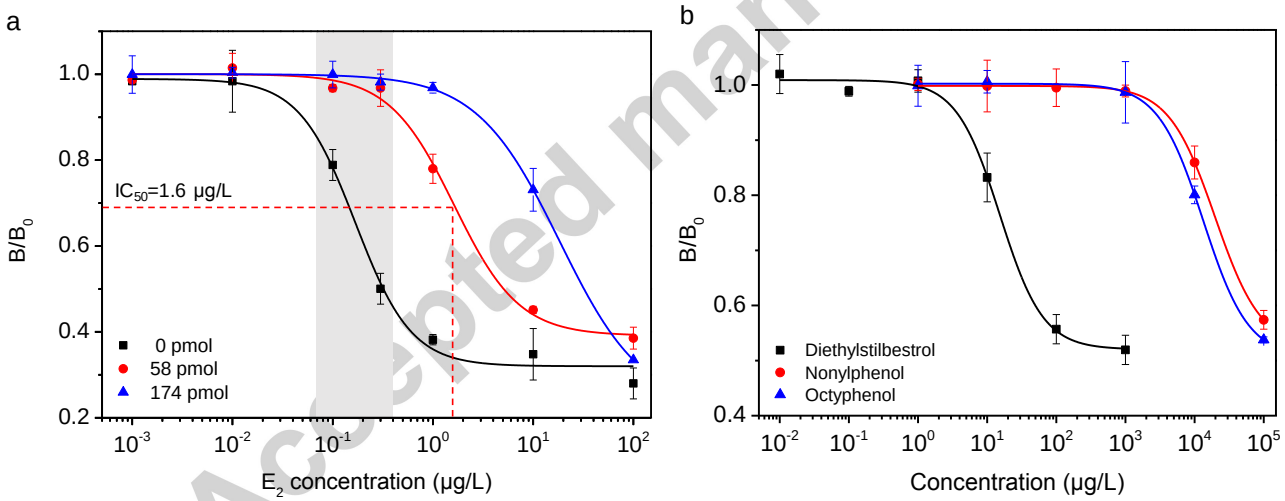


Figure 2 (a) Measured variations of relative signals with the E_2 concentration for different hERα concentrations: Optimization of the ratio between the hERα concentration and E_2 concentration used; (b) Competitive binding curves of three estrogenic compounds based on the fiber-based evanescent wave biosensor platform. The curves were generated in the presence of 58 pmol of hERα and 5.87 pmol (1.6

μg/L, 1 mL) of 17β-estradiol for diethylstilbestrol (DES), nonylphenol (NP) and Octyphenol (OP)

It is a prerequisite of this bioassay that the tested xenoestrogens do not bind to the Cy5.5-labeled anti-E_2 antibody; otherwise, the fluorescence would decrease because of competition between the xenoestrogens and the antibody for binding to the E_2-protein conjugate immobilized on the solid phase. Therefore, the cross-reactivities of various xenoestrogens were tested. The results, provided in **Table S1** (see supplementary information, Page 10), show that of the compounds tested, only estrone, estriol and bisphenol A exhibited any cross-reactivity (44%, 121% and 0.007%, respectively). Furthermore, the nonspecific binding capacity of E_2-protein conjugate functionalized fiber were investigated by treating with 1.0 μg/mL Cy5.5-labeled anti-E_2 monoclonal antibody and 1.0 μg/mL Cy5.5-labeled anti-microcystin-LR monoclonal antibody, respectively. As shown in **Figure S4** (see supplementary information, Page 11), the nonspecific signals induced by 1.0 μg/mL Cy5.5-labeled anti-microcystin-LR monoclonal antibody was negligible compared with that by the specific binding process. The signal noise ratio, which was defined as the peak value of the specific signal divided by that of nonspecific, was higher than 10.

3.2 Validation of ER-based fluorescent bioassay on fiber optical biosensor

Under the optimized conditions (see supplementary information, Pages 6-9), three estrogen-agonist compounds, diethylstilbestrol (DES), 4-n-nonylphenol (NP) and 4-n-octylphenol (OP), were chosen as a paradigm for validation of this assay. DES is a

well-known artificial estrogen (Palmer and Palmer 1995). NP and OP are the degradation products of one group of nonionic surfactants, the alkylphenol polyethoxylates, and exposure to them has been demonstrated to induce estrogenic effects both *in vitro* and *in vivo* (Harris et al. 1997). By using 5.87 pmol (1.6 μg/L, 1 mL) 17β-estradiol and 58 pmol hERα receptor, the effect of competition of other compounds with the E_2 was observed as a variation in the relative signals of fluorescence (**Figure 2b**). At the same concentration, the stronger estrogenic potency of xenoestrogen would result in the weaker fluorescent signal, consequently with a calibration curve moving towards left hand-side. Judging by this rule, the estrogen-agonist effect of DES was greater than NP and OP, which were consistent with the published results in numerous studies (Hong et al. 2015; Shang et al. 2014). Meanwhile, the estrogenic potency of OP was slightly greater than that of NP determined by this biosensor. The same finding has been confirmed by other in vivo and in vitro bioassays (de Voogt et al. 1997; Legler et al. 1999; Murk et al. 2002). Results indicate the reliability of this biosensor platform used for screening xenoestrogens.

3.3 Regeneration

Compared with their disposable counterparts, the reusable biosensors need less material and reagent costs and fewer probe preparation time. Besides, the obtained calibration curve can be applied for a series of subsequent measurements (Abel et al. 1996), which will allow an increase in the detection speed and signal reproducibility compared to traditional methods. Most importantly, the reusability of biosensor enable

large quantities of samples screened semi-continuously. It paves the way towards automatic on-line monitoring of contaminants, which is critical for certain circumstances, such as environmental monitoring (Wang et al. 2015b). In the light of easily reversible binding between antibody and antigen (existing as the immobilized antigen-protein conjugate), the sensing surface of fiber could be regenerated by a facile acid buffer washing step using 0.5% SDS (pH 1.9) solution. To evaluate the regeneration ability of this E_2-protein conjugate functionalized sensing surface, about every other 100 detections, standard tests were conducted using the same sample. Four representative standard signal traces are shown in **Figure 3**, the signal recoveries of all measured samples, which was defined as the signal divided by the initial one, ranged from 90% to 100%. In light of the reversible regeneration of antigen derivative functionalized fiber surface, this biosensor is applicable for xenoestrogen screening with enough accuracy and reusability.

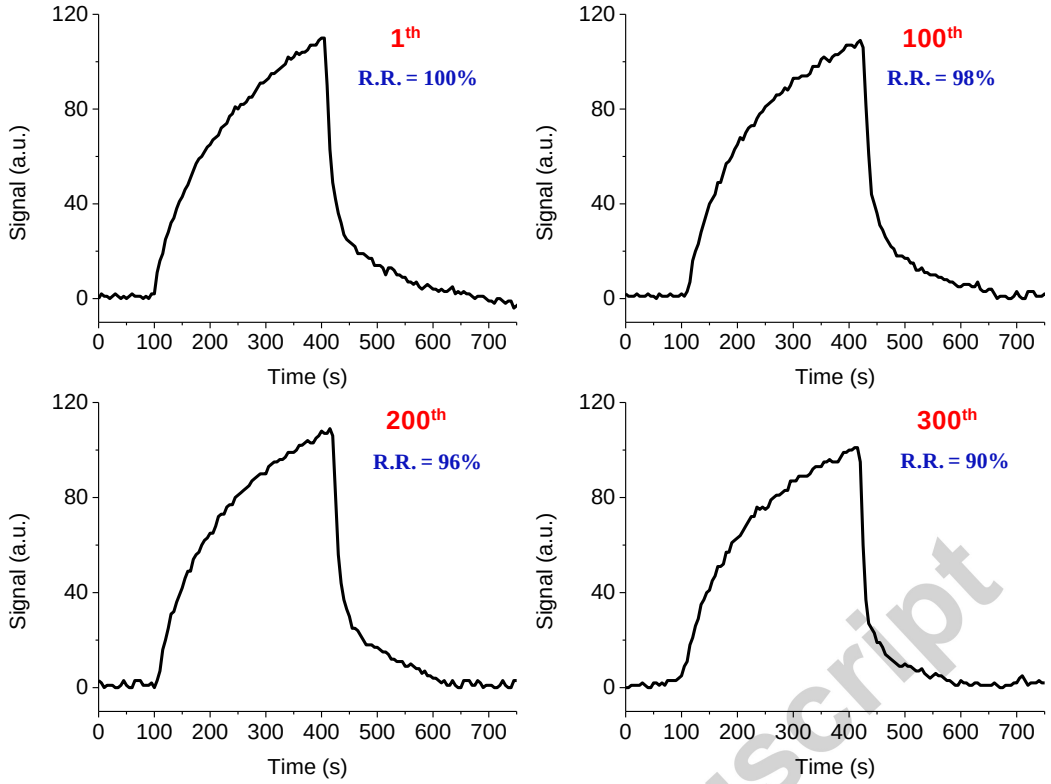


Figure 3 Representative standard signal traces obtained at different times using one E_2-protein conjugate functionalized fiber (all being treated with 1.0 μg/mL Cy5.5-labeled anti-E_2 antibody). The number of regeneration cycles (in red) and signal recoveries rates (R.R., in blue) of all measured samples were labeled in the figure, respectively.

4 CONCLUSIONS

We reported a facile method of screening potential estrogen compounds based on an ER-based fiber optical biosensor. Under optimized conditions, the addition of xenoestrogens promotes the ligand–ER complex formation in competition with E_2, thus releasing E_2 free to bind with the fluorophore-labeled anti-E_2 antibody. Unbound anti-E_2 antibody is captured by the antigen derivative on the fiber surface, reflected by

the increase in the fluorescent signals of this biosensor. Through this bioassay, three known estrogen-agonist chemicals are validated with the potency order of DES>OP>NP, which accords with the published results in numerous studies. The E_2 derivation functionalized fiber surface could be reversible regenerated for over 300 times. In conclusion, the biosensor is reusable, reliable, portable and amenable to on-line operation, providing a facile, efficient and economical alternative to screen potential xenoestrogens in environment.

ACKNOWLEDGEMENT

This research is supported by the National Nature Science Foundation (21677082).

REFERENCES

Abel, A.P., Weller, M.G., Duveneck, G.L., Ehrat, M., Widmer, H.M., 1996. Anal. Chem. 68(17), 2905-2912.

Awais, M., Sato, M., Sasaki, K., Umezawa, Y., 2004. Anal. Chem. 76(8), 2181-2186.

Carmon, K.S., Baltus, R.E., Luck, L.A., 2005. Anal. Biochem. 345(2), 277-283.

D'souza, S.F., 2001. Biosens. Bioelectron. 16(6), 337-353.

de Voogt, P., de Beer, K., van der Wielen, F., 1997. TrAC-Trend. Anal. Chem. 16(10), 584-595.

Escher, B., Leusch, F., 2011. Bioanalytical Tools in Water Quality Assessment, IWA publishing, London, UK, 27-30.

Fechner, P., Proll, F., Carlquist, M., Proll, G., 2009. Anal. Bioanal. Chem. 393(6-7), 1579-1585.

Garrett, S.D., Lee, H.A., Morgan, M.R.A., 1999. Nat. Biotechnol. 17(12), 1219-1222.

Granek, V., Rishpon, J., 2002. Environ. Sci. Technol. 36(7), 1574-1578.

Guo, H.L., Zhou, X.H., Zhang, Y., Gu, C.M., Song, B.D., Shi, H.C., 2016. RSC Adv. 6(17), 13837-13845.

Hao, X.J., Zhou, X.H., Zhang, Y., Liu, L.H., Long, F., Song, L., Shi, H.C., 2014. Sensor. Actuat. B-Chem. 204, 682-687.

Hao, X.J., Zhou, X.H., Zhang, Y., Long, F., Song, L., Shi, H.C., 2015. Sensors-Basel. 15(4), 8302-8313.

Harris, C.A., Henttu, P., Parker, M.G., Sumpter, J.P., 1997. Environ. Health Persp. 105(8), 802-811.

Hock, B., Seifert, M., Kramer, K., 2002. Biosens. Bioelectron. 17(3), 239-249.

Hong, H., Branham, W.S., Ng, H.W., Moland, C.L., Dial, S.L., Fang, H., Tong, W., 2015. Toxicol. Sci. 143(2), 333-348.

Legler, J., Broekhof, J.L., Brouwer, A., Lanser, P.H., Murk, A.J., van der Saag, P.T., Vethaak, A.D., Wester, P., Zivkovic, D., van der Burg, B., 2000. Environ. Sci. Technol. 34(20), 4439-4444.

Legler, J., van den Brink, C.E., Brouwer, A., Murk, A.J., van der Saag, P.T., Vethaak, A.D., van der Burg, B., 1999. Toxicol. Sci. 48(1), 55-66.

Leusch, F.D., De Jager, C., Levi, Y., Lim, R., Puijker, L., Sacher, F., Tremblay, L.A., Wilson, V.S., Chapman, H.F., 2010. Environ. Sci. Technol. 44(10), 3853–3860.

Liu, L.H., Zhou, X.H., Shi, H.C., 2015. Biosens. Bioelectron. 72, 300-305.

Long, F., He, M., Zhu, A.N., Shi, H.C., 2009. Biosens. Bioelectron. 24(8), 2346-2351.

Murk, A.J., Legler, J., Van Lipzig, M.M., Meerman, J.H., Belfroid, A.C., Spenkelink, A., Vethaak, D., 2002. Environ. Toxicol. Chem. 21(1), 16-23.

Nicoli, D.F., Briggs, J., Elings, V.B., 1980. P. Natl. Acad. Sci. USA 77(8), 4904-4908.

Palmer, B.D., Palmer, S.K., 1995. Environ. Health Persp. 103(4), 19-25.

Rodriguez-Mozaz, S., Lopez de Alda, M., Barceló, D., 2005. Water Res. 39, 5071-5079.

Rodriguez-Mozaz, S., Marco, M.P., Lopez de Alda, M.J., Barcelo, D., 2004. Anal. Bioanal. Chem. 378(3), 588-598.

Schwartz, J.A., Skafar, D.F., 1993. Biochem. 32(38), 10109-10101 10115.

Seifert, M., Haindl, S., Hock, B., 1999. Anal. Chim. Acta 386(3), 191-199.

Shang, G.D., Xue, J., Li, M., Hu, H.Y., Lu, Y., 2014. Chemosphere 104, 251-257.

Zacharewski, T., 1997. Environ. Sci. Technol. 31(3), 613–623.

Usami, M., Mitsunaga, K., Ohno, Y., 2002. J. Steroid Biochem. 81(1), 47-55.

Wang, D., Xie, J., Zhu, X., Li, J., Zhao, D., Zhao, M., 2014a. Biosens. Bioelectron. 55, 391-395.

Wang, R., Xiang, Y., Zhou, X., Liu, L.H., Shi, H., 2015a. Biosens. Bioelectron. 66, 11-18.

Wang, R., Zhou, X., Shi, H., Luo, Y., 2016. Biosens. Bioelectron. 78, 418-422.

Wang, R.Y., Zhou, X.H., Shi, H.C., 2015b. Biosens. Bioelectron. 74, 78-84.

Wang, S., Aarts, J.M., de Haan, L.H., Argyriou, D., Peijnenburg, A.A., Rietjens, I.M., Bovee, T.F.,

2014b. J. Appl. Toxicol. 34(9), 1031-1040.

Wang, X.-d., Wolfbeis, O.S., Meier, R.J., 2013. Chem. Soc. Rev. 42(19), 7834.

Xia, W., Li, Y., Wan, Y., Chen, T., Wei, J., Lin, Y., Xu, S., 2010. Biosens. Bioelectron. 25(10), 2253-2258.

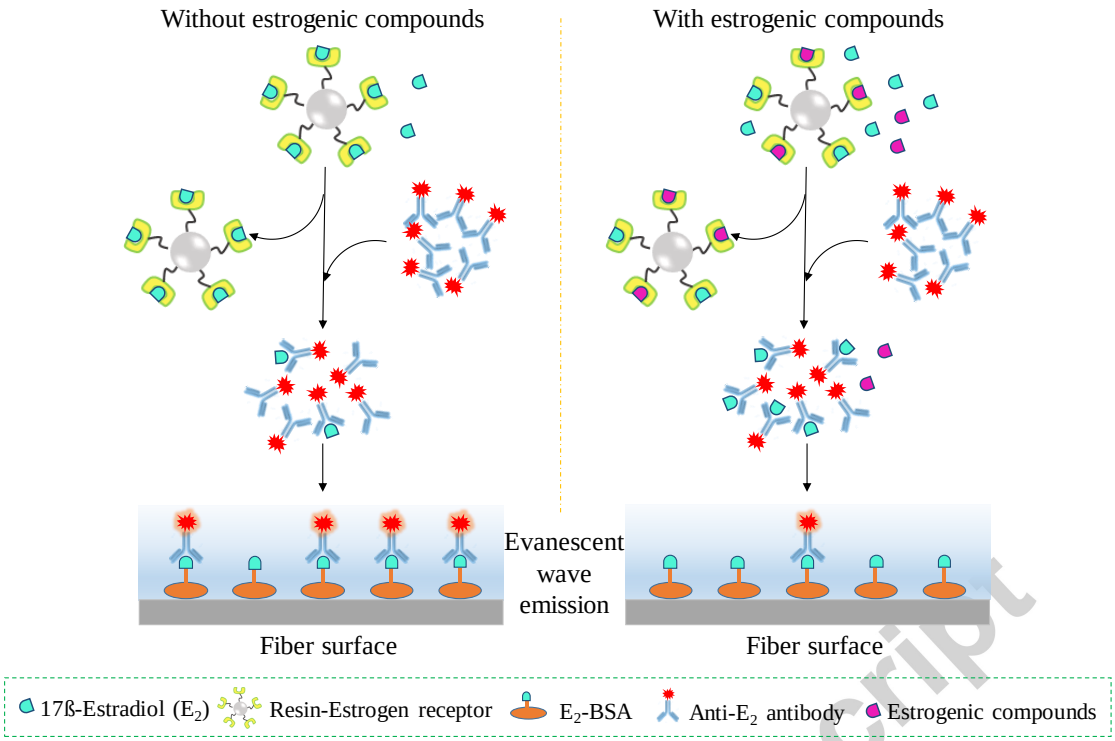
Yildirim, N., Long, F., Gao, C., He, M., Shi, H.C., Gu, A.Z., 2012. Environ. Sci. Technol. 46(6), 3288-3294.

Zhou, X.H., Liu, L.H., Xu, W.Q., Song, B.D., Sheng, J.W., He, M., Shi, H.C., 2014a. Sci. Rep-UK. 4, 4572.

Zhou, X.H., Song, B.D., Shi, H.C., Liu, L.H., Guo, H.L., He, M., 2014b. Sensor. Actuat. B-Chem. 198, 150-156.

Highlights

- An estrogen receptor-based reusable optical biosensor was established by using fiber as transducer
- Facile screening of potential xenoestrogens was realized on this biosensor
- The sensor was reusable for 300 testing cycles
- Estrogen receptor recognition of various xenoestrogens and advantages featured by evanescently excited fluorescence make the system attractive



For TOC only