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FIBER OPTIC SOS-TYPE BIOSENSOR FOR THE CONTROL OF THE GENOTOXICITY OF SOME ENVIRONMENTAL OBJECTS

N.F. Starodub*, M.V. Taran, N.F. Shpirka, K.E. Shavanova

National University of Life and Environmental Sciences of Ukraine, Heroyiv Oborony St., 15, Kyiv-03041, Ukraine.

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***Corresponding Author**
Dr. N. F. Starodub
National University of Life and Environmental Sciences of Ukraine, Heroyiv Oborony St., 15, Kyiv-03041, Ukraine.

ABSTRACT

It was proposed simple SOS-type biosensor based on the fiber optics worked in differential regime and allowed the control of such environmental objects which are chemical nature. This biosensor was tested at the determination of the genotoxicity of the number substances as: ethanol, dimethylsulfate and mitomycin C. The sensitivity of the proposed biosensor corresponds to the approaches based on the application based on the traditional, complicate and expensive devices. The developed biosensor may be used for the express analysis, namely during 20 min if the optrodes with the appropriate immobilised cells will be prepared in advance. It was informed that according to the preliminary obtained results the functional activity of such prepared optrodes may be served up to one day. Moreover, it was concluded that the proposed biosensor may have perspective in future for the using in field conditions.

KEYWORDS: biosensor, SOS-type, fibre optics, chemical substances, genotoxicity, control.

INTRODUCTION

The toxicity of the different ecological objects on the living organisms may be occurred on the level of cells in respect of their metabolic changes, or full deeds, or some reconstruction of genetically programmed nucleic acids. That is way, the specific effects may have a different implications for cells: (a) the repairing damage without any further consequences;

(b) the remained non-repaired defects lead to death, as well as (c) induction of mutagenesis or cancerogenesis as result of realizing error-prone repair pathways.

For the control of the last two effects it is necessary to accomplish the testing of genotoxicity of the different environmental factors. Today are more than 100 different methods to assess genotoxicity but really no more than 20 test systems are practically used. In last time according to practice demand to have information about not only total toxicity but and genotoxic effect of the environmental factors. In this case it is necessary obtaining test results in a online regime. The practical realization of such task may be done through the application of the new generation of the instrumental approaches based on the biosensor technologies. The start in the development of them intended for the determination of genotoxicity are was done not long ago.[1]

Today we have the panel of the bacterial tests based on the DNA damage depended induction of the SOS repair system: SOS-Chromo[2], Umu[3], Lux-Fluoro[4], VitoTOX®[5] and some biosensors variants.[6] The Lux-Fluoro test is a unique combination of two bioassays[7], which coincidentally measure genotoxicity (SOS-Lux test) and cytotoxicity (Lac-Fluoro test) of substances and mixtures of substances. The SOS-Lux assay, like the SOS-Chromo test or the Umu test, is based on the measurement of DNA damage-dependent induction of the bacterial SOS system in genetically modified *Salmonella typhimurium*. TA1535 bacteria[8], which have been transformed with the plasmid pPLS-1 carrying the promoter less lux genes of *Photobacterium leiognathi* as reporter element under the control of a DNA damage-dependent SOS promoter from ColD as sensing element.[9]

This system reacts to agents, which induce DNA damages inside these bacterial cells with the dose-dependent production of bioluminescence. The bioluminescence as a signal for DNA damage is an enzymatic reaction of a photolyase with its specific substrate, both encoded by the luxCDABFE genes of *Photobacterium leiognathi*, in the presence of oxygen. Since the bioluminescent light can be registered by an appropriate detector like a photomultiplier without destroying the cells, the kinetics of the processing of the DNA damage by the SOS system can be followed in living cells. The SOS-Lux test as a bioassay for genotoxicity can be used partially or fully automatically for routine measurements and can be employed for high throughput screening.

Of course, the biosensor approaches are need in continuous development and improvement in diagnostics, namely for the simultaneous determination of the content of the lidocaine and phenytoin. Late[12] this construction on the basis of the fiber optic biosensor was proposed for the direct control of the luminescence level of *Daphnia's* living medium at the determination of the toxicity some chemical substances, in particular, mycotoxins. The main aim of this article is the experimental demonstration of the created biosensor based on the fiber optics at the determination of the genotoxicity effects of the number of the toxic agents.

MATERIALS AND METHODS

Biosensor was constructed by the combining the SOS system, indicative of DNA-damaging agents, as a receptor component with the bioluminescence system as a rapid reporter one. This process was fulfilled according to the procedure described early in.[13] Recombinant *E. coli* C600(pPLS-1) carries a plasmid with the promoterless lux operon (luxCDABFE) of *Photobacterium leiognathi* under control of a strong SOS promoter that originates from part of the cda gene of the plasmid ColD. All procedure with the DNA manipulations, including plasmid isolation and molecular cloning, were performed by standard methods.[14] The needed plasmids were obtained from (Pharmacia). At first it was constructed a promoterless lux operon. For that the EcoRI-SphI fragment of pBR322 was replaced by the EcoRI-SphI polylinker fragment of pUC18 in order to remove the appropriate promoter from pBR322 and to add suitable sites for cloning. Then the 6.7-kbp SphI-SphI fragment of plasmid pPL-2 carrying the luxCDABFE genes but not the lux promoter respect of the transducers and as sensitive elements too as well as working out a special algorithm for the analysis fulfillment and adjustment of it for the individual test objects. Early[10, 11] we have developed the fiber optic immune biosensor based on the principle of enhanced chemiluminescence for the medical was inserted into this new plasmid at the SphI site located at the end of the polylinker fragment.

On the next step it was used the sequence data of the bioluminescence operon of *Ph. leiognathi* from ponyfish (ATCC 25521) to localize the starting codon of the luxC gene.[15] As an SOS-dependent promoter for the lux operon it was chosen the SmaI-SmaI fragment of ColD carrying a truncated cda gene with a strong SOS promoter. This SmaI-SmaI fragment of ColD contains a 1.7-kbp fragment of the coding region of the cda gene (less than half of the gene). It was inserted into pBRPL-1 at the Small site of the polylinker fragment. The resulting plasmid was used to transform *E. coli* C600 cells. They were grown overnight at

37°C in LB medium[16] supplemented with 50 mg/mL of ampicillin for the positive selection of cells carrying plasmid pPLS-1. After dilution (1:50) in fresh LB medium, the culture was incubated at 37°C until the optical density at 560 nm (OD560) reached up to 0.3. Aliquots of this culture were used for the genotoxicity assay.

For the immobilization of the prepared cell culture it was fulfilled according to[17] which was based on the application of the special sol–gel mixture which was obtained by the mixing 2 ml of tetramethylorthosilicate (TMOS, Aldrich) with 1 ml of distilled water and 0,25 ml of 0,1 M HCl. The mixture was sonicated for 10 min to ensure uniformity and left on the 24 h at 4 ^{o}C. Suspension (1 ml with concentration of 10^8 cells/ml) in LB was thoroughly mixed with 0.5 ml of the sol–gel solution and then it was kept in the presence of optrodes during 10 min. After that optrodes were removed and dried for 5 min under room temperature. At last optrodes were washed with phosphate buffer and then by LB medium, both at pH 7, and kept in the special measuring cell in phosphate buffer.

After incubation of the optrodes in mixture of LB and the solution to be analysed during some time (from 10 to 90 min) at the room temperature the light emission was measured. The signal was presented in the units relative to the control value.

As a toxic element for the testing system it was used dimethylsulfate (DMS), mitomycin C (MC) and ethanol (Et) at the concentrations in range of 5 µM to 1.0 mM, 1 nM to 10 µM and 0,5-4%, respectively. All these reagents were from Sigma-Aldridge (USA).

RESULTS AND DISCUSSION.

The overall scheme of the developed fibre optic biosensors is shown in Figure 1.

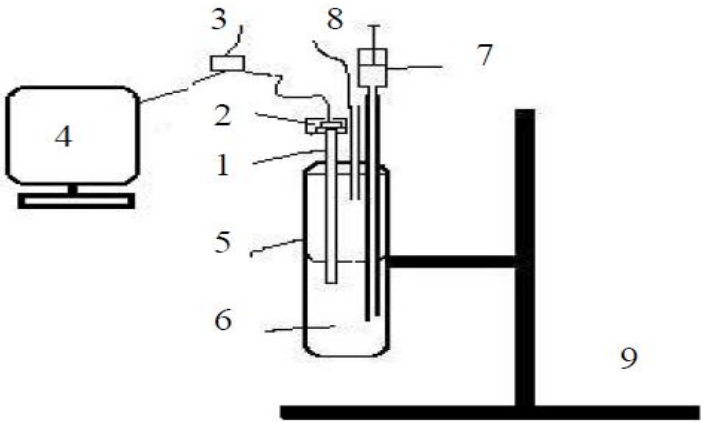

Figure 1: Overall scheme of the one of block of the optical biosensor based on the optrodes as transducer surface, where: 1- fiber optics with immobilized cells; 2 –

photodiode, 3 – system for signal registration; 4 –computer; 5 – glass container; 6 – sample; 7 – syringe for reagent introducing; 8 – pressure compensator and 9 – support.

It is compact device content two blocks with the replacement optrodes and the special system for the introducing analysed samples. Both blocks are able to work in the differential regime according to which it is possible to control the relative luminescence level when one of the above mentioned substances was added to the measuring cell of one block and aliquot of the distilled water was introduced into the other one.

At first it was carried out experiments about kinetic of the induced luminescence in case of the application of the maximal concentration of the used chemical substances. The obtained results are presented in Figure 2. For the DMS and MC the registered luminescence was appeared trough about 15-30 min and achieved maximal level during 150-180 min. After that time the level of the luminescent signal was stay on the some level or become to decrease though some time (no faster then 180-240 min and its depended on analysed substance. In spite of the relatively long time of the achievement of the maximal level of the signal, there is possible to currying express control at the exposition during 20 min. In case of the application of Et the appearance of the luminescence was revealed through 30 min and its level was decreased after the exposition about 200 min.

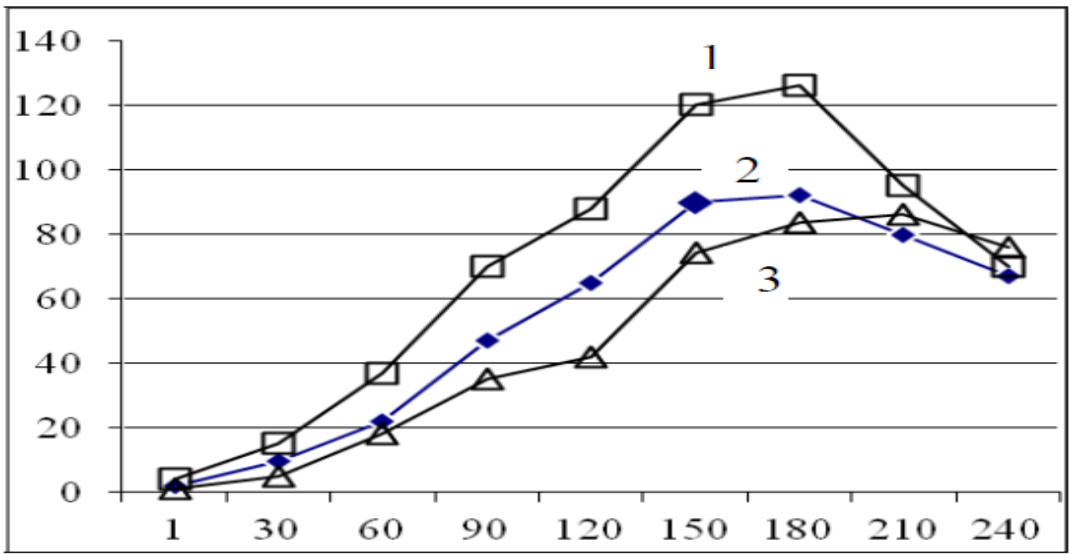

Figure 2: Dynamics of changes of the chemiluminescence level of biosensor after adding the DMS (1) MC (2) and Et (3) to the measuring cell. Ordinate - relative units of the chemiluminescent level and Abscise - time of measuring in min.

During the next experiments it was analysed the dependence of the chemiluminescent level on the concentration of the used substances. As result of the investigation it was stated that Et aroused maximal level of the chemiluminescence at the concentration in 3% (Figure 3A).
In case of the using DMS and MC such effect was appeared at their concentration of 0.5 mM and 1 µM, respectively (Figure 3B and 3C). Simultaneous, there is necessary to mention that the minimum of the concentrations which could be able to stimulate a marked increase in the level of the chemiluminescence are: 10 µM, 20 nM and 1% for DMS, MC and Et, respectively. In all cases the linear responses were between the minimal and maximal levels (Figure 3).

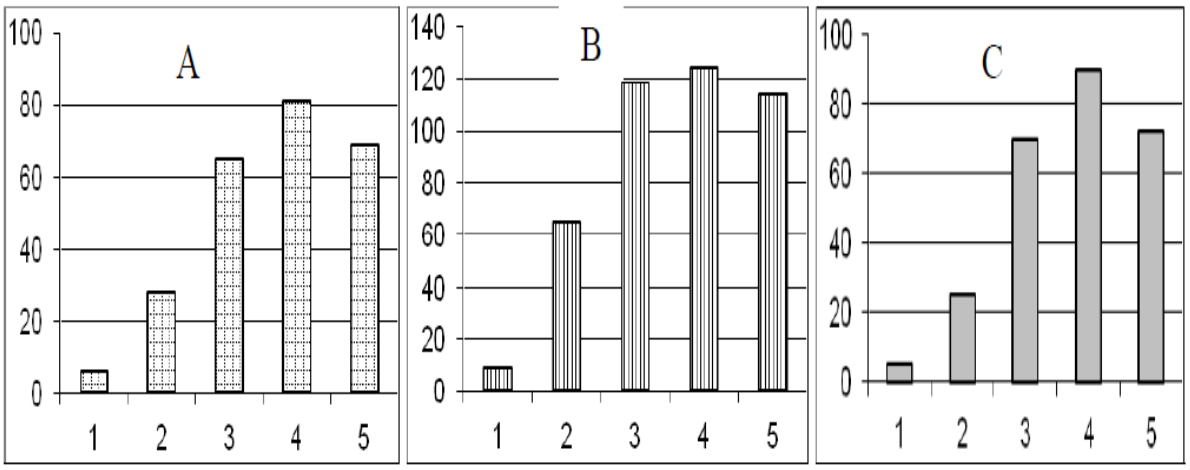

Figure 3: Induction of the level chemiluminescence (relative units) of biosensor at the different concentrations of Et (A), DMS (B), MC (C). 1-5: 0,5; 1,0; 2,0; 3,0 and 4,0% for ET; 0,005; 0,01; 0,1; 0,5 and 1,0 mM for DMS; 0,001; 0,01; 0,1; 1,0 and 10 µM for MC.

The above presented results testify that the development of the fibre optic SOS biosensor is able to control of the genotoxity of the number of chemical substances. There is necessary to underline that the obtained results are in good agreement with that received by others authors with the using analogues SOS systems but at the registration of different traditional registration approaches, namely, Luminescence Spectrometer (model LS 50 B of Perkin Elmer, UK), LKB Luminometer 1250; Pharmacia Biotech, Uppsala, Sweden) and others which are very complicate and expensive.[8,9,15,16] The proposed SOS biosensor is very simple, may be modified for the application in field condition even. As it was demonstrated above this biosensor gives possibility to reveal genotoxicity of the chemical substances in on line regime, in particular during 20 min only if the optrodes with the immobilised appropriate cells will be prepared previously. In our preliminary studies it was shown that the optrodes

with the immobilised SOS system may preserve functional activity during 1 day at the preservation of them in the LB medium.

CONCLUSION

So, it was proposed a very simple construction of biosensor for the registration of the genotoxicity of the number of environmental factors of chemical nature. This biosensor was tested at the determination of the genotoxicity of such components as with the very low concentration. The developed biosensor demonstrates sensitivity which is similar to the approaches with the application of the complicate and expensive traditional devices.

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REFERENCES

1. Starodub N. F. Genotoxiticy: modern instrumental approaches for its control in Environmental objects. J. Biosens Bioelectron, 2015; 6: 2,http://dx.doi.org/10.4172/2155-6210.1000169.
2. Quillardet P., Hofnung M. The SOS chromotest: a review. Mutat. Res., 1993; 297: 235-279.
3. Oda Y., Nakamura S., Oki I., et al. Evaluation of the new system (umu-test) for the detection of environmental mutagens and carcinogens. Mutat. Res., 1985; 147: 219-229.
4. Baumstark-Khan C., Khan R. A., Rettberg P., Horneck G. Bacterial Lux-Fluoro test for biological assessment of pollutants in water samples from urban and rural origin. Anal. Chim. Acta, 2003; 487: 51-60.
5. Verschaeve L., Van Gompel J., Thilemans L., et al. VITOTOX bacterial genotoxicity and toxicity test for the rapid screening of chemicals. Environ. Mol. Mutagen., 1999; 33: 240-248.
6. Polyak B., Bassis E., Novodvorets A., et al. Optical fiber bioluminescent whole-cell microbial biosensors to genotoxicants. Water Sci. Technol., 2000; 42: 305–311.
7. Rettberg P., Bandel K., Baumstark-Khan C., Horneck G. Increased sensitivity of the SOS-LUX-Test for the detection of hydrophobic genotoxic substances with *Salmonella typhimurium* TA1535 as host strain. Anal. Chim. Acta, 2001; 426: 167-173.

8. Ptitsyn L.R., Horneck G., Komova O., et al. A biosensor for environmental genotoxin screening based on an SOS lux assay in recombinant *Escherichia coli* cells. Appl. Environ. Microbiol., 1997; 63: 4377-4384.
9. Baumstark-Khan Ch., Rabbow E., Rettberg P., Horneck G. The combined bacterial Lux-Fluoro test for the detection and quantification of genotoxic and cytotoxic agents in surface water: Results from the "Technical Workshop on Genotoxicity Biosensing". Aquatic Toxicol., 2007; 85: 209-218.
10. Starodub N.F., Arenkov P.J., Starodub A.N., Berezin V.A. Fiber optic immunosensors based on enhanced chemiluminescence and their application to determine different antigens. Sensors and Actuators B, 1994; 18-19, 161-165.
11. Starodub N. F. Biosensors for the Control of Biochemical Parameters in the Diagnostics of Diseases. Book of SERIES IN SENSORS, Portable Biosensing of Food Toxicants and Environmental Pollutants, CRC Press, Taylor & Francis Croup Boca Raton London, New York, 2013; 743-775.
12. Starodub N. F. Efficiency of Biosensors in Environmental Monitoring. Book of Series in sensors, Portable Biosensing of Food Toxicants and Environmental Pollutants, CRC Press, Taylor & Francis Croup Boca Raton London, New York, 2013; 515-560.
13. Ptitsyn l. R., Horneck G., Komova O., et al. A Biosensor for Environmental Genotoxin Screening Based on an SOS lux Assay in Recombinant Escherichia coli Cells. Applied and Environmental Microbiology., 1997; 63(11): 4377–4384.
14. Maniatis, T., Fritsch E. F., and Sambrook J. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982.
15. Lee C. Y., Szittner R. B., and Meighen E. A. The lux genes of luminous bacterial symbiont Photobacterium leiognathi of the ponyfish. Eur. J. Biochem., 1991; 201: 161–167.
16. Miller, J. H. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1972.
17. Premkumar J. R., Rosen R., Belkin Sh., Lev O. Sol–gel luminescence biosensors: Encapsulation of recombinant *E. coli* reporters in thick silicate films. Anal. Chim. Acta, 2002; 462(1): 11–23.