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Development of a Microbiosensor Based on Fish Chromatophores Immobilized on Ferromagnetic Gelatin Beads

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Summary

Development of a microbiosensor based on immobilized living chromatophores of Siamese fighting fish, *Betta splendens*, for the detection of microbial and environmental toxins is described in this paper. Chromatophores were immobilized on ferromagnetic gelatin microbeads (d=250 μm). Kinetics of cell attachment, immobilization efficiency, population density, and an optimum content of ferromagnetic powder (iron(II,III) oxide, d_p<5 μm) with respect to preservation of the viability of cells was studied. The rate of cell attachment to the gelatin microbeads followed first-order kinetics with attachment efficiency of more than 95 %. Pretreatment of beads with fibronectin, known as a cell attachment promoting agent, resulted in a 10 % increase of the attachment rate constant compared to the attachment rate constant obtained without fibronectin. A detrimental effect on cell viability was observed when more than 10 % of ferromagnetic material was added to the beads. Operation of microbiosensor was tested with the neurotoxin analog clonidine as a model toxin. A double-exponential model is proposed to describe the toxin-induced change of cell area covered with pigment. Experimental data fitted well the proposed model.

Key words: microbiosensor, immobilization, chromatophore, ferromagnetic gelatin beads, model toxin

Introduction

Animal cell cultures may be used for a variety of toxicity and pollutant tests (*1–3*). In this study, we made use of living chromatophores of Siamese fighting fish, *Betta splendens*, immobilized on gelatin microbeads containing ferromagnetic powder, to design a microbiosensor for detection of environmental and bacterial toxins.

Chromatophores are neuron-like cells containing pigment granules which are responsible for the vivid colors of fish, amphibians, reptiles and cephalopods. These animals are capable of changing their color as an adaptive behavioral response under the control of the nervous and endocrine systems mediated by receptors on the cell surface. The change of pigment distribution in *Betta splendens* chromatophores may also be induced by various pathogenic bacterial toxins and pollutants, thus making these cells very appropriate for use as biosensor elements (*3–5*). The reaction mechanism of chromatophore response to toxins, *e.g.* the movement of pigment organelles along microtubules, is rather complex, and is mediated through G–protein-linked receptors (*6,7*).

The microscale biosensor, which is in the process of development by a multidisciplinary research group, is a

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microchannel bioreactor with channels 300 × 700 μm in cross section. This microscale system was selected because it can provide precisely controlled microenvironment, faster response time, its design flexibility is increased, and has the potential for parallel operation. These attributes are uniquely suited for industrial, military and research applications requiring high-throughput analysis, including testing devices for environmental sampling, medical screening, proteomics, combinatorial pharmaceutical development, and detection of microbial pathogens.

The schematic of a single microchannel biosensor is presented in Fig. 1. Basic sensor elements are microbeads with fish chromatophores immobilized on the bead surface, which also contains embedded ferromagnetic material. Transport and positioning of microbeads within the microchannel is facilitated by fluid flow and by the interaction between the ferromagnetic material and the magnetic field generated by a »capture-dot« device. Capture-dots are microsolenoids deposited in the microchannel walls, described previously (*4*).

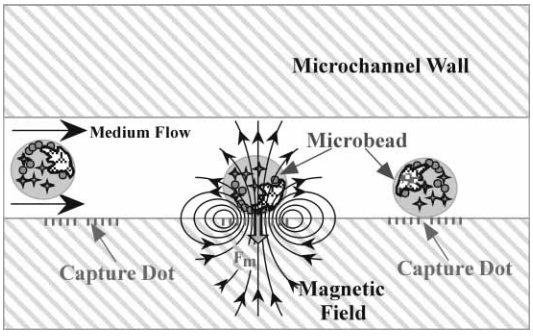

Fig. 1. Microchannel type biosensor. Transport and capturing of microcapsules containing immobilized chromatophores within the magnetic field generated by »capture dot«

Immobilization of chromatophores on the surface of the microbeads represents a very important step in biosensor development. Fish chromatophores are anchorage dependent cells that require compatible surface for attachment, and subsequent spreading and growth (*3*). Our previous study demonstrated high compatibility of gelatin-based material for chromatophore attachment, as well as for preservation of their sensitivity towards pathogens (*8*). Gelatin microcarriers have also been reported suitable for various types of animal cell immobilizations (*9–12*). In this study, we investigated inclusion of ferromagnetic material into gelatin microbeads and we determined the optimum amount of ferromagnetic powder that would allow good cell viability. We also tested the functionality of the immobilized chromatophores while monitoring and modeling their response to a neurotoxin.

Non-immobilized fish chromatophores are sensitive to a broad range of bioactive compounds such as: heavy metals, organophosphate pesticides, live microbes or microbial toxins, chlorinated aromatic hydrocarbons, *etc.* (*3,4*). The mode of their response may vary for different classes of agents, with some causing hyperdispersion of pigment granules, other causing only partial aggregation, and some showing no visible effects (*3*). A very pronounced aggregation of pigment granules occurs when fish chromatophores are exposed to neurotoxin, noradrenaline, or to adrenergic agonist clonidine, which is chemically more stable.

Here, experimental results for the immobilization of chromatophores on gelatin-microbeads containing ferromagnetic powder and their response to neurotoxin clonidine that causes rapid cell aggregation and a decrease of the pigmented cell area are presented. A double-exponential model is proposed to fit the toxin-induced change in the pigmented cell area.

Material and Methods

Isolation of primary cell culture

Fish chromatophores were isolated from the tails and fins of *Betta splendens* fish. Red *Betta splendens* fish containing red pigmented cell-erytrophores were used in this study. Tissue was washed a minimum of six times with skinning solution and then treated with an enzyme solution for 20 min, with gentle agitation (v=80 rpm). Skinning solution consisted of 1 mM Na_2EDTA (Sigma), 5.6 mM glucose (Sigma), penicillin/streptomycin mixture in m/V ratio 1/100 (Sigma), in calcium and magnesium free phosphate-buffered saline, Gibco. Enzyme solution consisted of 20 mg of collagenase type 1, Worthington Biochemical Co, 178 U/mg, and 1 mg of hyaluronidase, Worthington Biochemical Co. 348 USP/NF, in 7 mL of PBS, Gibco. After 20 min, the enzyme solution with digested tissue was separated from undigested tissue pieces, and subsequently centrifuged for 2 min at 3250 × g in the clinical centrifuge. Supernatant was removed using a sterile transfer pipette and reapplied to the tissue. The first pellet, which contained mostly epithelial cells, was discarded. After additional 30 min of shaking, the above procedure was repeated, but the cell pellet, containing fish chromatophores, was suspended in L-15 (Leibovitz, Hyclone Lab) medium, and centrifuged again. After removing the supernatant, fish chromatophores were resuspended in a desired volume of L-15 medium and used as an inoculum for microcarriers.

Preparation of microcarriers and immobilization of fish chromatophores

Macroporous ferromagnetic gelatin beads containing various amounts of ferromagnetic powder (iron(II,III) oxide, powder d_p<5 μm, Aldrich) were prepared according to the procedure described by Nilsson and Mosbach (*13,14*). An appropriate amount of iron(II,III) oxide (mass fraction of 5, 10, 15, 20 and 25 % on gelatin powder) was added to the water-gelatin solution (type I gelatin from porcine skin, Sigma) before further processing. At the end of processing, dry beads were sieved, and those with diameters between 180 and 300 μm were collected and cross-linked with glutaraldehyde (grade I, 50 %, Sigma). Before use, microcarriers were hydrated in phosphate-buffered saline (PBS) without calcium and magnesium ions, washed extensively, and then resuspended in PBS at concentration of 5 g/L. Ferromagnetic gelatin microcarriers were autoclaved for 20 min at 121 °C.

In preparation of microcarriers, an appropriate amount of microcarrier stock suspension was transferred to a 50-mL sterile conical centrifuge tube and the beads were allowed to settle. After removing the supernatant, the microcarriers were washed twice with growth medium (L-15) and transferred to Erlenmeyer flasks, where the cells were attached to beads. When the effect of cell attachment-promoting agents such as fibronectin was studied, the appropriate amount of microcarrier was kept for 2 h prior to its use in PBS (20 mL) with 100 μL of fibronectin stock solution (Sigma), and then washed with growth medium before transferring it into Erlenmeyer flasks. The number of microbeads per gram of beads was determined in order to optimize cell/bead ratio (λ). Beads were counted in a standard volume on a haemocytometer grid. An average value of $0.82 \cdot 10^6$ beads/g was found in repeated measurements for beads with 10 % of ferromagnetic powder.

The attachment of the cells to the beads was performed in siliconized (by Sigmacote, Sigma) Erlenmeyer flasks in L-15 medium with very gentle stirring (30–50 rpm). L-15 medium was enriched with 5 % of fetal bovine serum, FBS (Hyclone, Lab). The rate of attachment of the cells from inoculated microcarrier cultures was determined by counting the cells remaining in the culture. Culture samples (200 μL) were taken at intervals of 20 min and allowed to settle for 1 min in an Ependorf tube. The microcarrier-free supernatant was introduced into a haemocytometer for cell counting.

Culture samples were also examined microscopically to determine cell viability and toxin-sensitivity. Chromatophores that responded to the addition of neurotoxin were considered alive and toxin-sensitive.

Testing of microcapsules of immobilized chromatophores with clonidine

The response of immobilized cells to different concentrations of clonidine was monitored by a change in pigmented cell area induced by the neurotoxin. Suspension of immobilized cells (1.5 mL) in L-15 medium, usually containing 15–20 beads, was transferred in individual wells of 24-well plate. The 24-well plate was placed on a Leica DMIL inverted microscope. Image capture of immobilized chromatophores was performed with a digital Pulnix TMD-7DSP CCD camera connected to a Matrox computer, at constant preset time intervals controlled by image-capturing software. The first acquired image frame at t=0 presented pigment area of immobilized cells before the addition of toxin. Then, 0.5 mL of an appropriate concentration of the clonidine solution in L-15 medium was added with a microsyringe and image capture was continued for 6 min total time. The final concentration of toxin in the well was one fourth of the injected concentration. Clonidine (2-[2,6-dichloraniline]-2-imidazoline), Sigma C-7897, was resuspended at 10 mM concentration in distilled water and stored at 4 °C. Just prior to use it was diluted in L-15 medium to 4× final test concentrations. Captured images were analysed to determine the change in the pigmented cell area induced by the model toxin using proprietary software (»Cell cruncher«) described elsewhere (*3*,*15*,*16*).

Results and Discussion

Immobilization of cells

Attachment of fish chromatophores to gelatin beads containing various mass fractions of 0–25 % of iron(II,III) oxide was observed under the conditions described earlier (*8*). The kinetics of the attachment to gelatin beads containing 10 % of ferromagnetic material is presented in Fig. 2. To promote cell adhesion, gelatin beads were pre-treated with fibronectin.

After 140 min, 95 % of all cells present in the solution were attached to the microcarrier. Semi-logarithmic plots of unattached cell concentration as a function of time yielded straight lines, indicating first order kinetics (Fig. 2). The first order attachment kinetics had been reported previously for immobilization of anchorage-dependent cells on DEAE-derivatized sephadex (*17*,*18*). However, the authors used charged microcarriers and also reported an increase in the attachment rate with increasing exchange capacity of the microcarriers. Apparently, the kinetics of cell binding to the charged microcarriers and the attachment rate constant are at least one order of magnitude higher than the one reported for the attachment to the biospecific macroporous gelatin carrier (*10*,*11*). However, for both types of carriers the final attachment efficiency was reported as high as 90–100 %.

Fig. 2 shows that the attachment rate constant for fibronectin-pretreated beads (k=0.94·10^{-2} min^{-1}) is approximately 10 % higher than for beads without pretreatment (k=0.85·10^{-2} min^{-1}). This result could be expected since proteins like fibronectin, vibronectin, laminin and collagen make up the extracellular matrix between cells or between cells and substratum, and mediate cell attachment and spreading (*19*–*21*).

No significant effect of ferromagnetic material on the cell attachment rate constant was noticed in the range from 0 to 25 % of iron(II,III) oxide concentrations used in this study (data not presented). Attachment rates for samples containing different amount of ferromagnetic material were found to be statistically indistinguishable from the rates reported for gelatin beads without ferro-

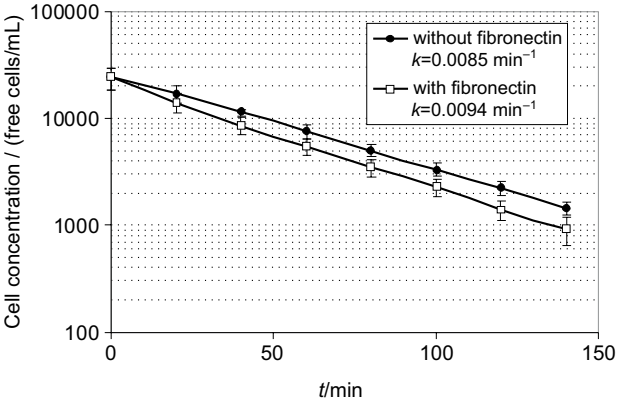

Fig. 2. The kinetics of the attachment of fish chromatophores on gelatin microcarriers with 10 % of iron(II,III) oxide. Reaction conditions: L-15 medium supplemented with 5 % serum, pH= 7.4, time t=140 min, stirring rate ν=40 rpm, cell/bead ratio λ=70. Data presented are mean values of 3 experiments±standard deviation

magnetic material (*8*). However, as shown in Fig. 3, a significant effect of the concentration of ferromagnetic material on the cell viability was found. Cell viability was seriously affected on beads with 25 % iron(II,III) oxide (Fig. 3). The 10 % mass fraction of iron(II,III) oxide may be considered appropriate for use in this biosensor study because it does not compromise cell viability and it supports complete cell functionality and toxin sensitivity. Our current studies (unpublished data) are determining the range of magnetic field intensities needed to provide capturing and releasing of gelatin ferromagnetic beads within biosensor microflow channels and the effect of this dynamic phenomenon on the biosensing sensitivity and reproducibility.

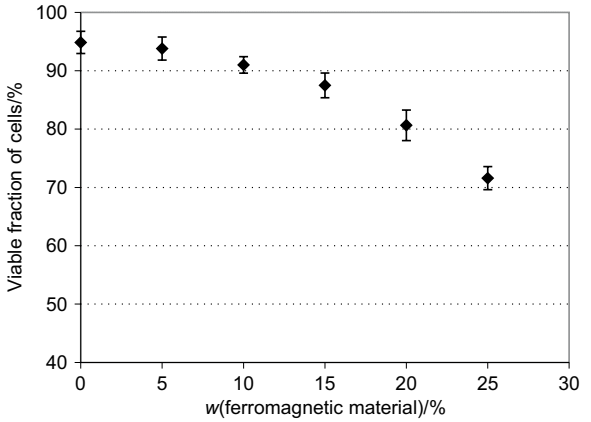

Fig. 3. Effect of the amount of ferromagnetic material in gelatin beads on chromatophore viability. Reaction conditions: L-15 medium supplemented with 5 % serum, pH=7.4, time t=140 min, stirring rate v=40 rpm, cell/bead ratio λ=70. Data presented are mean values of 3 experiments±standard deviation

Effect of cell to bead ratio

The effect of the cell/bead ratio (λ=number of cells/beads) on the cell attachment rate constant and on the viability of immobilized cells was determined for gelatin beads with 10 % of ferromagnetic material (Table 1). It is important to insure a large initial cell to bead ratio, which would not affect cell viability and also to minimize the proportion of unoccupied beads during the immobilization process. Fish chromatophores are terminally differentiated cells and do not replicate in tissue culture, thus the initial cell/bead ratio will not increase with time, as reported for some other proliferating animal cells like Vero cells (*10*). By microscopic examination, we observed that immobilized fish chromatophores stay functional *e.g.* responsive to clonidine for 2 to 4 weeks, although a small decrease in cell/bead ratio occurred due to apoptosis or cell death.

Table 1. Effect of cell/bead ratio on fish chromatophore attachment rate constant k and on cell viability of immobilized chromatophores*

Initial cell/bead ratio (λ)	Attachment rate constant $\frac{k \cdot 10^2}{\text{min}}$	Fraction of viable immobilized cells %	Viable immobilized cell/bead ratio
15	0.74	92	12
30	0.78	91	25
50	0.84	88	41
70	0.85	83	55
80	0.85	70	52

*Reaction conditions: Attachment was performed on gelatin microcarrier with 10 % iron(II,III) oxide, d=250 μm, in L-15 medium supplemented with 5 % serum at pH=7.4 during t=140 min with stirring rate of v=40 rpm. Viability of immobilized cells was measured after 24 h. The data presented are mean values of triplicate experiments

For each initial cell/bead ratio observed, viable immobilized cell/bead ratio was calculated from the total number of cells immobilized and measured after 24 h. Typical results are presented in Table 1. These results indicate that λ=70 is an optimum value to use for immobilization of fish chromatophores. Higher initial cell/bead ratio causes lower cell viability, and therefore lower viable immobilized cell/bead ratio is achieved. Lower viability is most probably due to the shortage of living space and to higher competition of cells for nutrients. At λ=70, microscopic examination did not show the presence of unoccupied beads.

Testing of immobilized chromatophores with clonidine

Fig. 4 presents experimental video image output at time intervals t=0, 50, 180 and 360 s after the addition of

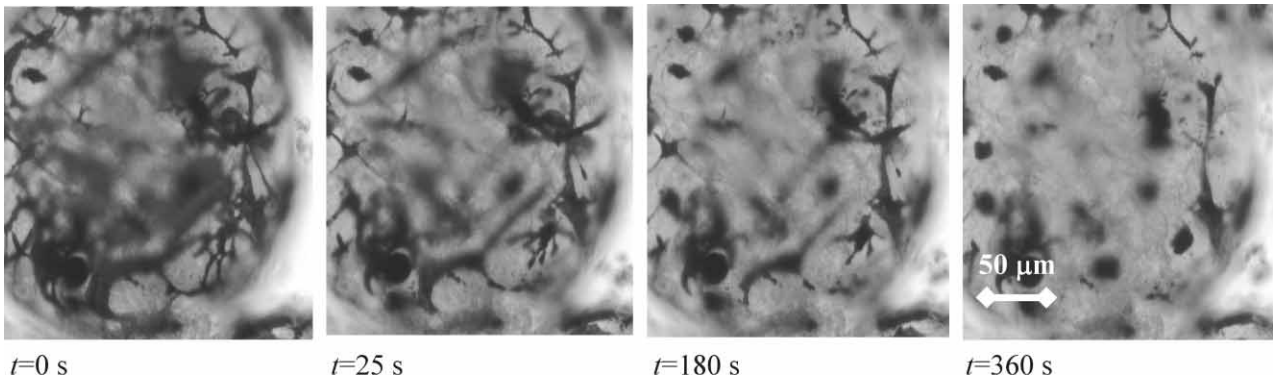

Fig. 4. Video image frames of immobilized chromatophore beads with 10 % of ferromagnetic powder at intervals t=0, 50, 180 and 360 s after exposure to clonidine (c=50 nM)

toxin to fish chromatophores immobilized on gelatin microcarrier with 10 % of ferromagnetic material. The aggregation of pigment granules within cells induced by toxin is obvious.

The response to clonidine is mediated by cell-surface receptors of the classic G-protein-linked type (*6*). The mechanism of signal transduction is rather complex. Receptors that cause aggregation are linked to the G_i proteins, whose activation results in a decrease in cyclic adenosine monophosphate content (cAMP) in cells. On the other hand, receptors that cause dispersion are linked to G_s proteins, whose activation results in an increase in cAMP. This cAMP increase activates cAMP-dependent protein kinase (protein kinase A), which phosphorylates and activates other proteins, initiating a cascade of events resulting in pigment granule dispersion. The long-range movements of pigment granules depend on polar microtubules and specific motor proteins bound to the pigment granules. There are two major families of motor proteins, kinesins that move their cargo outward, and dyneins that move the granules inward, towards the centrosome. The ability of these motor proteins to bind microtubules, and thus transport pigment granules, is regulated by the phosphorylations resulting from the signal cascade initiated by G-protein linked receptor binding (*6*,*7*,*22*).

Fitting experimental data with a double exponential model for cell area decrease

The graph presented in Fig. 5 shows an exponential decrease in the cell area covered by pigment after the addition of 50 nM clonidine. A double exponential model is proposed for this response. The model implies the existence of a heterogeneous population of cells, which can be roughly divided into two subpopulations, differing in the rate of cell reaction to the toxin exposure. It is interesting to note that the visual appearance of fast responding cells ('star-like' cells) and slow responding cells ('sheet-like' cells) can be easily discriminated under the microscope and with the aid of the shape recognition software.

$$A_t = A_0 - B\cdot(1-e^{-C\cdot t}) - D\cdot(1-e^{-E\cdot t}) \qquad /1/$$

At steady state ($t \to \infty$), the above equation reduces to:

$$A_\infty = A_0 - B - D \qquad /2/$$

where A_0 is the initial area, A_t is the cell area at time t, A_∞ is the cell area at $t=\infty$, B is the total area change of the first cell subpopulation (faster responding cells), C is the constant rate for the first subpopulation, D is the total area change of the second cell subpopulation (slower responding cells), and E is the constant rate for the second subpopulation.

Total percentage of area change (PAC) may be calculated as:

$$(A_0 - A_\infty)/A_0\cdot 100 = (A_0 - A_0 + B + D)/A_0\cdot 100 = (B+D)/A_0\cdot 100 \qquad /3/$$

How well the proposed model fits with experimental data is represented by the mean relative percentage deviation modulus σ (%) which is defined as:

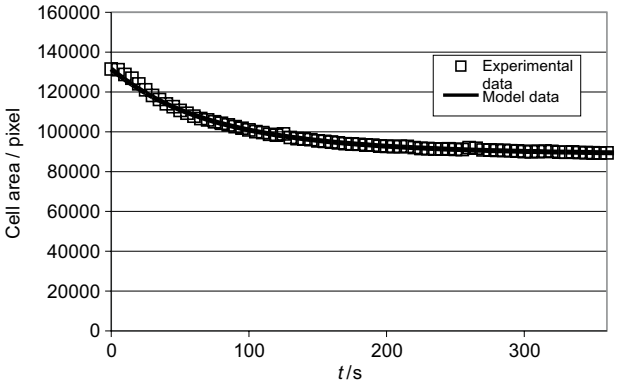

Fig. 5. Decrease of the cell area of immobilized chromatophores with time, as a response to clonidine (c=50 nM). Experimental data (–□–), and model data (–), σ=0.009 %

$$\sigma = (100/N)\sum \frac{\varepsilon_i}{x_i} \qquad /4/$$

where N is number of trials, ε_i experimental error, and x_i is model predicted value.

As shown in Fig. 5, the proposed model and the experimental data are in very good agreement, with σ= 0.009 %.

Double exponential model equation that represents experimental data from the graph in Fig. 5 is presented bellow:

$$A_t = 131565 - 26744.9\cdot(1-e^{-0.0168\cdot t}) - 16368.9\cdot(1-e^{-0.0078\cdot t}) \qquad /5/$$

From the values of the coefficients B and D (26744.9 and 16368.9, respectively), it is obvious that the majority of cells are fast responding cells. The subpopulation of fast responding cells caused 20.3 % of area change, while the population of slow responding cells caused 12.4 % of area change. Total percentage of area change (PAC) for applied clonidine concentration (50 nM) is 32.7 % (Eq. 3). Coefficients C and E (0.0168 and 0.0078, respectively) are rate constants, which are specific for the toxin used and prevailing experimental conditions. Higher rate constant C characterizes the subpopulation of faster responders, while the smaller constant E is a characteristic of the slower cell subpopulation.

Different reactive agents (environmental toxins, heavy metals, bacterial toxins, chlorinated hydrocarbons, drugs, *etc*.) may produce quite different cell reactions. The main differences could be in (*i*) mode of response: aggregation, dispersion, no reaction, or »freezing« of chromatophore; (*ii*) magnitude of response: partial aggregation, full aggregation, partial dispersion, full dispersion; (*iii*) kinetics of responses, and (*iv*) differences in responses of particular cell subtypes. These four main response features may be quantified with the parameters of the presented model. Toxins and agents could be classified into different categories according to chromatophore responses. It is already shown (*3*) that agents belonging to the same category show similar effect on chromatophore. For example, the category of adrenergic neurotoxins is characterized by rapid and full aggregation, quick response time (< 5 min), high sensitivity (> several nM) and high reproducibility. On the other hand, the category of agents which elevate cAMP levels

in cells causing dispersion, such as the melanocyte stimulating hormone (MSH) and some bacterial toxins, exhibited slower response time (~20–80 min), but also high sensitivity and high reproducibility (data is not presented). However, the reaction to some chlorinated hydrocarbons is much slower with lower sensitivity (data not presented). An important issue that should also be addressed is testing and categorizing of substances or environmental factors that act as false positive agents. These agents should be identified and the cell responses should be analyzed and included into the biosensor database. The reduction of false positive and false negative responses is one of the objectives of our ongoing study.

Modeling of various toxin responses includes specifying and defining characteristic model parameters for different toxins. Linking these parameters to physiological and metabolic processes in cells may help elucidate very complex mechanisms of cell reactions to toxins. Creation of a database of toxin responses may also assist in toxin identification. If, for example, two agents give similar exponential cell area decrease, specific parameters from Eq. 1 are expected to be characteristic for the two toxins, thus providing a degree of discrimination. It is important to note that parameters A_0 and A_∞ may be evaluated independently from experimental observations, thus reducing the five-parameter model to only three degrees of freedom.

Effect of toxin concentration

Effect of toxin concentration on the percentage of overall area change (PAC) from Eq. 3 is presented in Fig. 6. Percentage of the area change increased for the clonidine concentration range from 20 to 100 nM, achieving a plateau value for higher toxin concentrations. As shown in Fig. 6, concentration of 20 nM caused a significant response of fish chromatophores, 15.2 % of area change. However, the detection of lower concentrations is accompanied with higher error than the detection of higher concentrations.

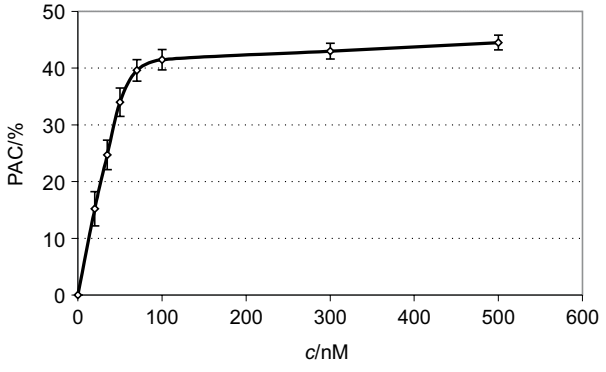

Fig. 6. Effect of clonidine concentration on PAC of immobilized chromatophores. Data presented are mean values of 3 measurements±standard deviation

Conclusion

Development of a microbiosensor based on immobilized living chromatophores of Siamese fighting fish, *Betta splendens*, for detection of microbial and environmental toxins was investigated in this study. Fish chromatophores were immobilized on ferromagnetic gelatin microbeads (d=250 μm). Optimum conditions for the immobilization of fish chromatophores and optimum amount of ferromagnetic material incorporated in gelatin microbeads allowing good cell viability and toxin sensitivity were observed.

The response of immobilized chromatophores to neurotoxin clonidine was monitored by measuring cell area covered by pigment. Percentage of area change is dose-dependent for this model toxin in a range of concentrations from 20 to 100 nM, achieving a plateau value for higher concentrations. The cell area decrease is shown to fit very well the proposed double exponential model and the rate of cell reaction to various toxins may be described with model coefficients.

Currently, we are testing biosensors for a number of microbial toxins, biological agents and pollutants, creating a large library of responses. Classification of agents according to specific features of cell responses, quantified by model parameters, is in progress. Further system development, miniaturization and integration are expected.

Acknowledgment

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References

1. M. Butler: *Animal Cell Culture and Technology: The Basis,* IRL Press, Oxford (1996) p. 104.
2. R. Shoji, A. Sakoda, Y. Sakai, M. Suzuki, H. Ustumi, Rapid bioassay of toxicity in environmental water by LDL-uptaking activity of human cell, *Water Sci. Technol. 38* (1998) 271–278.
3. F. Chaplen, R. Upson, P. McFadden, W. Kolodziej, Fish chromatophores as cytosensors in a microscale device: Detection of environmental toxins and microbial pathogens, *Pigm. Cell Res. 15* (2002) 19–26.
4. P. McFadden, F. Chaplen, W. Kolodziej, G. Jovanovic, T. Plant, J. Liburdy, B. Paul, J. Trempy, W. Gerwick, C. Willard. *Methods of detecting bioactive compounds: USA Utility Patent No: 245–59404* (2001).
5. K. Dierksen, L. Mojovic, B. Caldwell, R. Preston, R. Upson, J. Lawrence, P. McFadden, J. Trempy, Responses of fish chromatophore-based cytosensor to a broad range of biological agents, *J. Appl. Toxicol. 5* (2004) 363–369.
6. R. Fujii, The regulation of motile activity in fish chromatophores, *Pigm. Cell Res. 13* (2000) 300–319.
7. E. Reese, L. Haimo, Dynein, dynactin, and kinesin II's interaction with microtubules is regulated during bi-directional organelle transport, *J. Cell. Biol. 151* (2000) 155–166.
8. L. Mojovic, R. Upson, C. Willard, F. Chaplen, G. Jovanovic, Immobilization of fish chromatophores onto gelatin-based microcarriers, *Proceedings of the 6th World Congress of Chemical Engineering* (CD Edition), Melbourne (2001) 1–7.
9. W.J. Malaisse, E. Olivares, A. Belcourt, K. Nilsson, Immobilization of pancreatic islet cells with preserved secretory potential, *Appl. Microbiol. Biotechnol. 52* (1999) 652–653.

10. Y.C. Ng, J.M. Berry, M. Butler, Optimization of physical parameters for cell attachment and growth on macroporous microcarriers, *Biotechnol. Bioeng. 50* (1996) 627–635.

11. T.J. Nikolai, W.S. Hu, Cultivation of mammalian cells on microporous microcarriers, *Enzyme Microb. Technol. 14* (1992) 203–208.

12. A. Warner, S. Duvare, J. Muthing, H. Buntemeyer, H. Lunodorf, M. Straus, J. Lehman, Cultivation of immortalized human hepatocytes HepZ on macroporous Cultispher G microcarriers, *Biotechnol. Bioeng. 68* (2000) 59–70.

13. K. Nilsson, K. Mosbach, Preparation of immobilized animal cells, *FEBS Lett. 118* (1980) 145–150.

14. K. Nilsson, K. Mosbach, *Macroporous particles for cell cultivation or chromatography. US Patent 5015576* (1991).

15. A. Pacut: *Stochastic Modeling of Diverse Scales: From Poison to Network Neurons*, Oficyna Wydawnicza PW, Warsaw (2000).

16. A. Pacut, W. Kolodziej, F. Chaplen, Cytosensors for early detection of biological and chemical threats-statistical approach, *Proceedings of the 4th International Conference »Neuronal Networks and Expert Systems in Medicine and Healthcare«–NNESMED*, Milos Island (2001) 437–442.

17. V.B. Himes, W.S. Hu, Attachment and growth of mammalian cells on microcarriers with different ion exchange capacities, *Biotechnol. Bioeng. 29* (1987) 1155–1163.

18. W.S. Hu, J. Meier, D. Wang, A mechanistic analysis of the inoculum requirement for the cultivation of mammalian cells on microcarierrs, *Biotechnol. Bioeng. 27* (1985) 585–595.

19. M.R. Koller, E.T. Paputsakis: Cell Adhesion in Animal Cell Culture: Physiological and Fluid-Mechanical Implications. In: *Cell Adhesion Fundamentals and Biotechnological Applications*, M.A. Hjortso, J.W. Roos (Eds.) Marcel Dekker, Inc. (1995) pp. 61–111.

20. G.F. Panino: Monolayer Growth Systems: Multiple Processes. In: *Animal Cell Biotechnology*, R.E Spier, J.B. Griffiths (Eds.) Academic Press, London (1985) pp. 211–287.

21. E. Ruoslahti, M.D. Pierschdocher, New perspective in cell adhesion, *Science, 238* (1987) 491–495.

22. H. Nilsson, Melanosome and erythrosome positioning regulates cAMP-induced movement in chromatophores from spotted triplefin *Grahamina capito, J. Exp. Zool. 287* (2000) 191–198.

Razvoj mikrobiosenzora na bazi kromatofora ribe imobiliziranih na feromagnetskim želatinskim nosačima

Sažetak

U radu je opisan razvoj mikrobiosenzora za detekciju mikrobnih toksina i onečišćavača okoline na bazi imobiliziranih kromatofora ribe sijamskoga borca, *Betta splendens*. Kromatofori su imobilizirani na feromagnetskom želatinskom mikronosaču (d=250 μm). Ispitivani su kinetika vezivanja stanica, učinkovitost imobilizacije, gustoća stanica i optimalni udjel feromagnetskoga praha (željezov(II,III) oksid, d_p<5 μm) da bi se osiguralo preživljavanje stanica. Utvrđeno je da je kinetika vezivanja kromatofora za feromagnetski želatinski mikronosač reakcija prvoga reda s djelotvornošću vezivanja od 95 %. Prethodna obrada nosača s fibronektinom pridonijela je povećanju konstante vezivanja stanica za 10 % u usporedbi s vrijednošću dobivenom bez dodatka fibronektina. Kada se upotrijebi iznad 10 % feromagnetskoga praha u želatinskom nosaču, primjećuje se negativan učinak na preživljavanje kromatofora. Funkcioniranje mikrosenzora testirano je s neurotoksinom klonidinom (analog adrenalina). Predložen je eksponencijalni matematički model koji opisuje promjenu stanične površine pokrivene pigmentom, uzrokovanu dodatkom toksina. Uočeno je dobro slaganje eksperimentalnih rezultata s predloženim modelom.