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CHROMATOPHORE BASED CYTOSENSOR: RESPONSES TO VARIOUS BIOLOGICALLY ACTIVE AGENTS

The design, operation and performances of a cytosensor based on immobilized living chromatophores isolated from Betta splendens Siamese fighting fish are presented in this paper. Development of the cell immobilization technique as an important cytosensor enabling technology, and integration of the immobilized cells in the cytosensor system is described. The cytosensor was used to test several classes of biologically active agents including neurotransmitters, adenyl cyclase activators, cytoskeleton effectors, cell membrane effectors, and protein synthesis inhibitors and the obtained responses were analyzed. Particular response features such as mode of response, its magnitude, kinetics, sensitivity and dose dependence were monitored and may be used as a basis for mathematical modeling of the responses. The results indicated that the cytosensor has great potential for use in food and water testing and in pharmacy.

Key words: Fish chromatophores, Cytosensor, Biologically active agents.

Chromatophores are terminally differentiated, neuron–like cells containing pigmented granules which are responsible for the beautiful colours of fish, amphibians, reptiles and cephalopods, and their adjustable camouflage capabilities [1,2]. They are biochemically related to nerve cells and share many of their important sensory properties and responses to biologically active chemical agents. Various biologically active and/or toxic substances can act on chromatophores as signalling molecules. Chromatophore responses to these agents are mediated through a complex array of cell surface receptors, signal transduction pathways and/or metabolic processes resulting in the movement of pigment granules along microtubules [3,4]. Figure 1 presents a characteristic response of chromatophores to biologically active agents. Changes in pigment color and/or location within cells can be monitored microscopically. In addition to various environmental toxins such as heavy metals, organophosphate pesticides, polynuclear aromatic hydrocarbons [5], herbicides, fungicides, and some genotoxins [6], the cytosensor has the capability to detect a wide variety of potential toxicants from various classes of bacterial toxins to numerous cell receptor agonists [7].

Because chromatophores are as complex and rich in physiological targets of drug and toxin action, they are very promising for use as broad–ranging cytosensors of utility in medical screening, pharmaceutical research, food toxicology, and environmental monitoring. The design of the prototype of the micro–cytosensor studied in this paper is presented in Fig. 2. It is a microchannel reactor with the size 500 x 700 μm in cross–section, and a length of 5
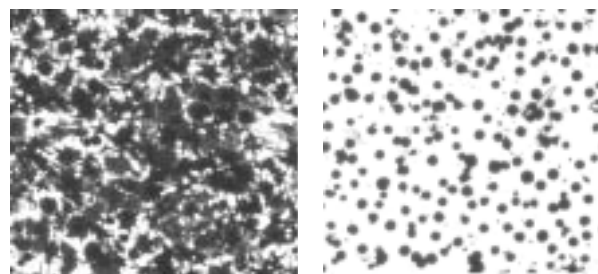
a) ____ 50 μm T = 0 min.   b) T = 8 min.

Figure 1. Aggregation of pigment in response to 500 nM clonidine

cm. The microscale system was chosen because it could provide precisely controlled microenvironments, faster response times, increased design flexibility, and had the potential for massively parallel operations. These attributes are uniquely suited for industrial, military and research applications of high–throughput analysis, including testing devices for environmental sampling, medical screening, proteomics, combinatorial pharmaceutical development, and the detection of microbial pathogens.

A basic micro–biosensor element is a microbead or a microcapsule (d=250 μm) containing two essential rudiments: i) immobilized and live chromatophores isolated from Siamese fighting fish, Betta splendens, and ii) inert ferromagnetic powder embedded in a microcarrier matrix. The transport and positioning of microbeads within the microchannel is facilitated by fluid flow, and by the interaction between the ferromagnetic material and a magnetic field. A specially designed "capture dot" device, presented in Figures 2b) and 2c), is a micro solenoid deposited in the microchannel walls [8–11]. Its main function is to generate the magnetic field needed for capturing the microbeads.

The primary objective of this study was to investigate the design of a micro–biosensor based on immobilized chromatophores of Siamese fighting fish, Betta splendens and to develop enabling technologies
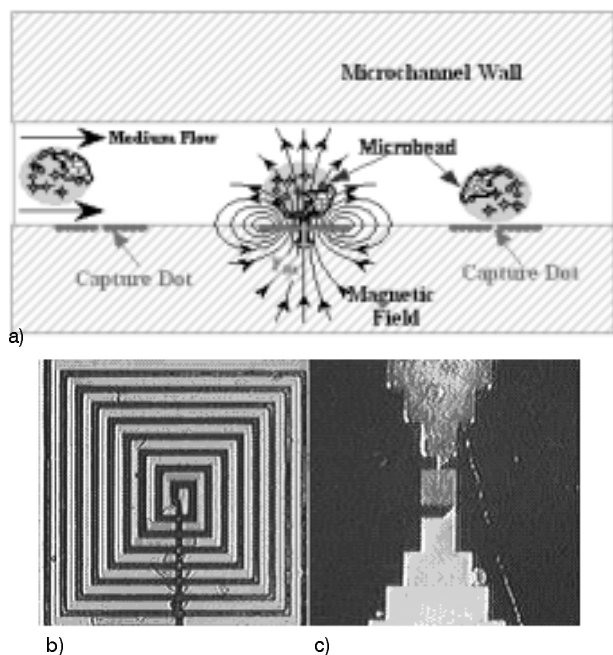
a)

b)   c)

Figure 2. Microchannel type biosensor a) Transport and capture of microcapsules containing immobilized chromatophores within the magnetic field generated by "capture dot". b) High magnification photomicrograph to show coil configuration. c) Lower magnification photomicrograph to show electrical leads.

such as the immobilization of chromatophores on an appropriate microcarrier which would enable their movement through the biosensor device, whilst preserving their biosensing capabilities. For these purposes three different types of microcarriers such as glass, polystyrene and gelatine beads were tested for their efficiency in binding fish chromatophores. The kinetics of cell attachment and the optimal conditions for cell binding were determined. In addition, the incorporation of ferromagnetic powder of iron (II, III) oxide into gelatin beads was studied both from the point of preserving the cell viability and the functionality of the micro–cytosensor. The cytosensor was used to test several classes of biologically active agents and their responses were monitored, analyzed and categorized. These results may suggest the specific areas of cytosensor utilizations.

EXPERIMENTAL

Isolation of the Primary Cell Culture

Fish chromatophores were isolated from the tails and fins of *Betta splendens* fish according to a procedure described previously [6, 11]. Only red *Betta splendens* fish, which consisted of erytrophores (red pigmented cells), were used in this study.

Microcarriers

Three types of microcarriers were used in the study: glass (Sigma, d=150–212 (μm), polystyrene

(BangsLabs, Inc., d=186 (μm), and macroporous ferromagnetic gelatine. Macroporous ferromagnetic gelatine 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 [12, 13]. An appropriate amount of iron (II, III) oxide (mass fraction of 5%, 10%, 15%, 20% and 25% on gelatin powder) was added to the aqueous–gelatin solution (type I gelatine 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). All three types of microcarriers were hydrated in phosphate–buffered saline (PBS) without calcium and magnesium ions, washed extensively, and then resuspended in PBS at a concentration of 5 g/l. Glass and gelatine microcarriers were autoclaved for 20–min at 121°C, while the polystyrene microcarriers were sterilized by incubating at 70°C for 2 h as recommended by the manufacturer. After sterilization, the microcarriers were kept in PBS solution at room temperature.

In the 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 attachment of cells to beads was performed. When the effect of cell attachment–promoting agents such as fibronectin was studied, the appropriate amount of microcarrier was kept for 2 hours prior to its use in PBS (20 ml) with 100 μl of fibronectin stock solution (Sigma), and then washed with growth medium before transfer into Erlenmeyer flasks. The number of microbeads per gram of beads was determined in order to optimize the cell/bead ratio (λ). The 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.

Cell Immobilization

The attachment of the cells to the beads was performed in siliconized (Sigmacote, Sigma) Erlenmeyer flasks in L–15 medium with very gentle stirring (30–50 rpm). The 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 20 minute intervals and allowed to settle for 1 min in an Eppendorf tube. The microcarrier–free supernatant was introduced into a haemocytometer for cell counting.

The culture samples were examined microscopically to determine cell viability and toxin–sensitivity. The chromatophores that responded to the addition of neurotoxin were considered alive and toxin–sensitive.

Capturing of Microcapsules Using a Magnetic Field

For these experiments, in order to protect the cells from environmental shear stress, a ~5 μm thin membrane made of alginate and poly–L–lysine was created around the immobilized microcarrier [14]. The movement of microcapsules with immobilized chromatophores was observed microscopically in a glass microtube (d=700 μm; l=5 cm) with the magnetic field conduit embedded in the wall. The experimental setup is presented in Figure 6 in the Results section. Fluid (L–15 medium) velocities applied were in the range from 1.6 mm/s to 6.4 mm/s and corresponded to the predicted operational fluid velocities of the biosensor [9]. The fluid flow was provided by a micro syringe pump (LabTronix, USA).

The magnetic field intensity needed to stop and capture microcapsules was determined for various fluid velocities and for different amounts of added ferromagnetic material. The magnetic field intensity was measured by a gaussmeter, Model 410 (Lake Shore Cryotronics, Inc).

Testing the Cytosensor with Various Biologically Active Agents

The testing of the cytosensor was performed with various chemicals and bacterial toxins. The agents used are presented in Table 1 in the Results section. Purified bacterial toxins were purchased from List Biologicals Laborotories (USA). All the chemicals used as test agents were of high purity and were purchased from Sigma.

The response of the immobilized cells to different concentrations of agents was monitored by the image analysis technique. The change in pigmented cell area induced by the agents was measured as described previously [8].

RESULTS AND DISCUSSION

Attachment of Cells to Microcarriers

Figure 3 shows the percent of attached cells to gelatin, glass, and polystyrene microcarriers, 3 hours
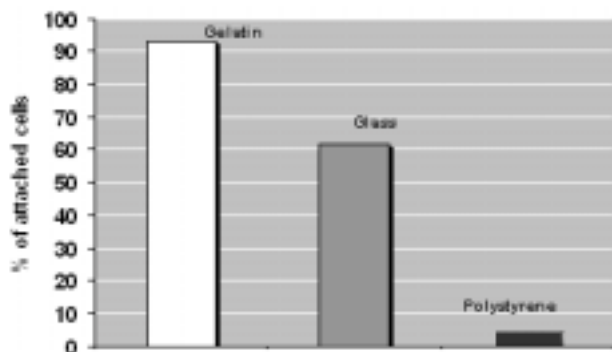

Figure 3. Attachment of fish chromatophores to three types of mi-crocarriers. The % of attachment was measured 3 hours after cell inoculation. The data presented are mean values of triplicate measurements.

after cell inoculation. The best results were obtained with gelatin beads (95% of the attached cells). Attachment to glass microcarrier resulted in a significantly lower percent of cells attached (62%), while fish chromatophores showed the lowest affinity towards polystyrene microcarrier (17% of the attached cells).

Gelatin beads have already been reported to be appropriate for various cell types, such as human fibroblast cells [15], pancreatic islet cells [16], Chinese hamster ovary (CHO) cells [17], green monkey kidney cells (Vero) [18], and human hepatocytes [19]. Cell attachments close to 100%, as well as high cell densities have been reported for gelatin microcarriers. These were attributed to a microcarrier chemical structure that enabled biospecific binding of the cells, as well as to a high surface area originating from a porous structure. Thus, cells might also populate the interior of the cavities and could withstand higher agitation rates than those on solid microcarriers [17].

Although glass carriers are widely used in cell culture studies [20], the fish chromatophores showed only moderate affinity, lower than some mammalian cell lines reported in the literature. Improvements in cell attachment might be achieved by either pretreatment with cell attachment promoting agents, or by using special types of aluminium borosilicate glass with controlled pore size [21]. Despite the fact that many cell types adhere better to polystyrene than to glass [20], the fish chromatophores demonstrated poor attachment. This result may be a consequence of inappropriate surface charges present on the commercial support that was used. The gelatin beads gave the best results and were selected for further studies.

The attachment of fish chromatophores to gelatin beads containing various amounts (0–25% mass fraction of iron (II, III) oxide was observed under conditions described previously [9]. The kinetics of the attachment to gelatin beads containing 10% of ferromagnetic material is presented in Figure 4. The gelatin beads were pre–treated with fibronectin to promote cell adhesion.

After 140 minutes 95% of all the cells present in the solution were attached to the microcarrier. Semi–logarithmic plots of the unattached cell concentration as a function of time yielded straight lines, indicating first order kinetics (Figure 4). First order attachment kinetics have previously been reported for the immobilization of anchorage–dependent cells on DEAE–derivatized sephadex [21,22]. 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 charged microcarriers and the attachment rate constant are at least one order of magnitude higher than the one reported for the attachment to a biospecific macroporous gelatin carrier [16,18]. However, for both types of carriers the final attachment efficiency was reported to be as high as 90–100%. Figure 4 shows that the attachment rate constant for fibronectin–pretreated beads ($k=0.94 \cdot 10^{-2} \text{min}^{-1}$) is approximately 10 % higher than
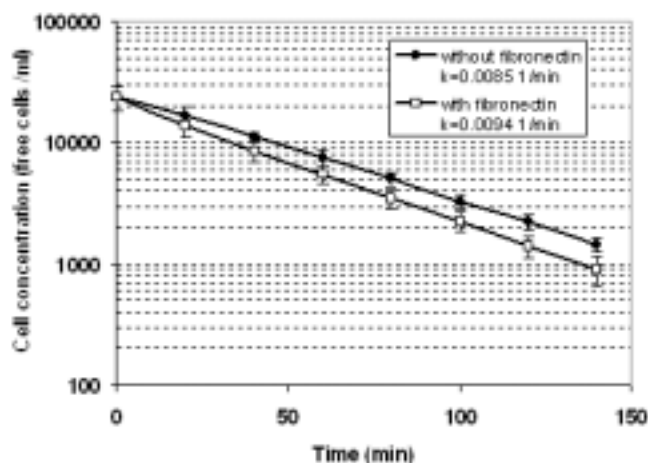
Figure 4. The kinetics of attachment of fish chromatophores to gelatin microcarrier with 10% (w/w) of iron (II, III) oxide. Reaction conditions: L–15 media supplemented with 5% of serum, pH=7.4 time τ=140 min; stirring rate ν=40 rpm; cell/bead ratio, λ=70.The data presented are mean values of triplicate measurements.

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 the cells and the substrate, and mediate cell attachment and spreading [20,23].

No significant effect of the 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). The attachment rates for samples containing different amounts of ferromagnetic material were found to be statistically indistinguishable from rates reported for gelatin beads without ferromagnetic material [11]. However, as shown in Figure 5, a significant effect of the concentration of ferromagnetic material on the cell viability was found. The cell viability was seriously affected on beads with 25% iron (II, III) oxide (Figure 5). 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.

Capture of Microcapsules Using a Magnetic Field

In order to integrate the chromatophores immobilized to ferromagnetic gelatin beads, we studied the effect of the fluid flow velocity and the amount of ferromagnetic material on the magnetic field needed to stop and capture the beads while moving through the microchannels. The experimental set-up for these measurments is presented in Figure 6 and the results are presented in Figure 7. Since the applied fluid flow velocities and magnetic fields could damage the immobilized cells, an aditional protective membrane, 5 μm thick, based on alginate and poly–l–lysine was made around the chromatophores immobilized on
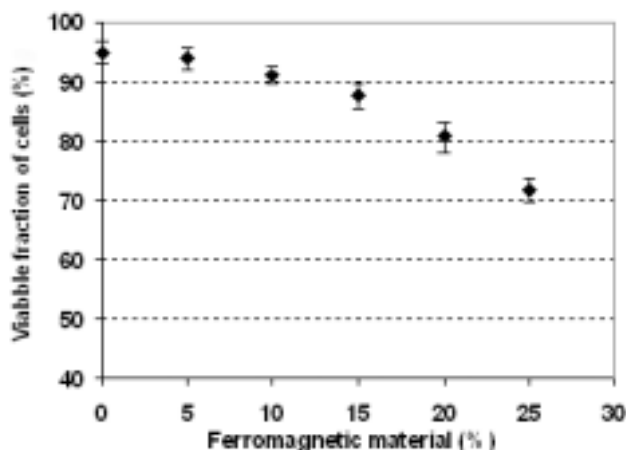

Figure 5. Effect of the amount of ferromagnetic material present in the gelatin beads on chromatophore viability. Reaction conditions as in Fig. 4. The data presented are mean values of triplicate measurements.
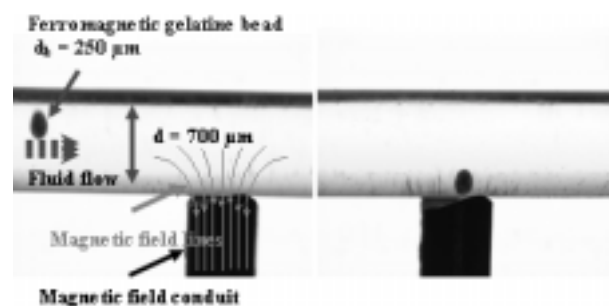

Figure 6. Capture and release of gelatin beads with iron (II, III) oxide with magnetic field under fluid flow. Ferromagnetic gelatine bead in a micro–channel approaching magnetic field (a), and captured with magnetic field (b).
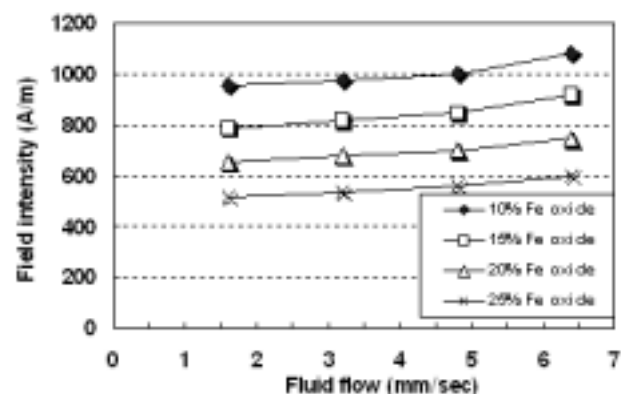

Figure 7. Effect of fluid flow velocity on the intensity of the magnetic field needed to capture microcapsules with different contents of ferromagnetic material.

ferromagnetic gelatine beads. A micrograph of immobilized fish chromatophores on ferromagnetic gelatin beads is presented in Figure 8. As can be seen in Figure 7, higher magnetic field intensities are needed to capture microbeads containing lower amounts of
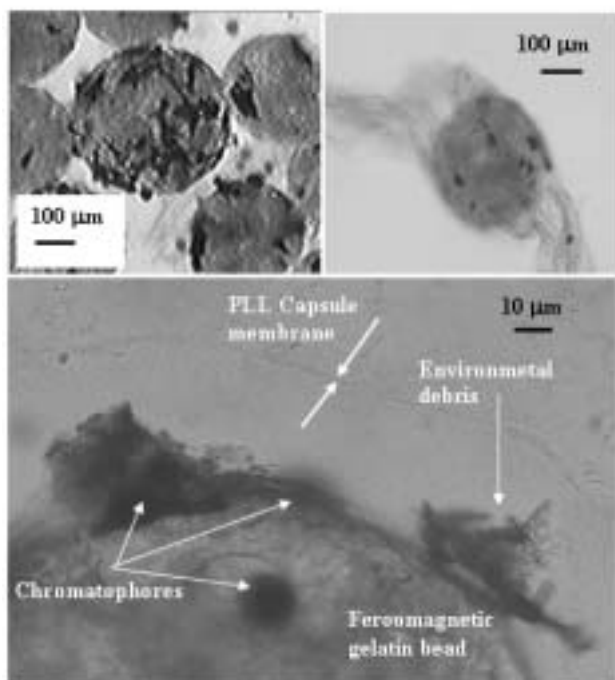
Figure 8. Micrographs of immobilized chromatophores on ferro-magnetic gelatin beads

ferromagnetic material. These results are in agreement with the basic principles of magnetism [24,25], and also with the experimental results obtained with ferromagnetic particles fluidized in a magnetic field [26]. Generally, magnetic forces acting on ferromagnetic particles increase with increase of the following parameters: magnetic field intensity, magnetic susceptibility (χ) of the ferromagnetic material, and volume fraction of the ferromagnetic material contained in the microbeads.

The field intensities needed to capture microcapsules of immobilized chromatophores with 10% of ferromagnetic material, which were considered the most appropriate for use in the biosensor, were found to be in the range from 954 A/m to 1092 A/m for the applied fluid velocities from 1.6 mm/s to 6.4 mm/s. These magnetic field intensities may be easily provided by a solenoid or some other type of magnetic field conduit.

Responses to Various Biologically Active Agents

The agents discussed in this manuscript were categorized by cellular target into five groups: neurotransmitters, adenyl cyclase activators, cytoskeleton effectors, protein synthesis effectors, and cell membrane effectors. The agent list, effective concentration ranges, and chromatophore reactions are summarized in Table 1.

Generally, the chromatophores showed very strong reactivity to agents from the group of neurotransmitters. The reaction to adrenergic, dopaminergic and serotonergic agents was
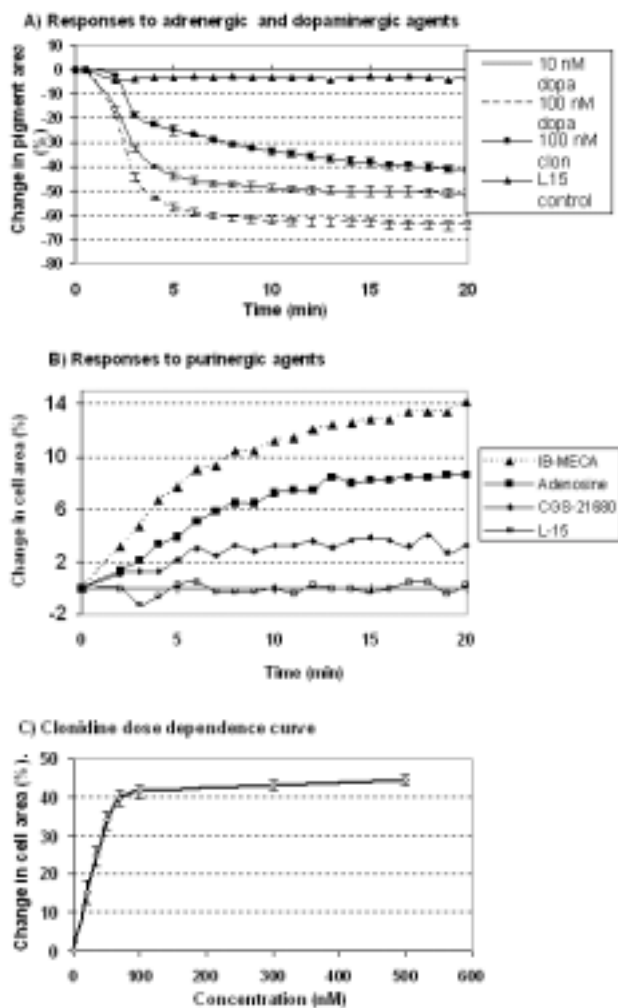

Figure 9. (A, B, C) Responses of immobilized chromatophores to agents belonging to the group of neurotransmitters

characterized by rapid aggregation in an dose dependant manner, quick response time (<10 min), and fairly high sensitivity and high reproducibility. The responses are shown in Figure 9A, while the dose–dependance curve to clonidine neurotransmitter is presented in Figure 9B. Among these agents chromatophores were the most sensitive to dopamine, which caused a reaction at a concentration of 1 nM. In contrast to the pigment aggregating activity of the previous neurotransmitters, purinergic agents caused pigment granule dispersion, also in a dose dependent manner (Figure 9C).

The adenyl cyclase activators tested (Table 1), among which there were three bacterial adenyl cyclase toxins, one peptide hormone (MSH) and a diterpenoid (forskolin), induced dispersion in the pigment granules with differences in the kinetics of the response and the sensitivity. MSH induced rapid dispersion at a picomolar concentration, whereas forskolin required thousand fold higher concentration. This is probably due to differences in their mode of action; MSH acts through receptors while

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Table 1. Representative agents tested in the cytosensor

Cellular pathway affected	Effector	Reaction[1]	Effective Concentration range
I. Neurotrans–mitters			
A. Adrenergic	Arterenol (norepinephrine)	Aggregation	1 nM–1 µM
	Cirazoline	Aggregation	1 µM–100 µM
	Clonidine	Aggregation	10 nM–1 µM
B. Dopaminergic	Dopamine	Aggregation	1 nM–100 µM
	Bromocriptine	Aggregation	10 nM– 1 µM
C. Serotonergic	Serotonin	Aggregation	10 µM–1 mM
	Alpha–methyl serotonin	Aggregation	10 µM–1 mM
	RU24969	Aggregation	100 µM–1 mM
	Metergoline	Aggregation	10 µM
D. Purinergics	Adenosine	Dispersion	1 µM–1 mM
	IB–MECA	Dispersion	100 nM–10 µM
	CGS–21680	Dispersion	1 µM–10 µM
II. Adenyl cyclase activators			
	B. pertussis adenyl cyclase	Dispersion	1 µg/ml–5 µg/ml
	B. anthracis adenyl cyclase + protective antigen	Slight dispersion	1–2 µg/ml +1–2 µg/ml
	Forskolin	Dispersion	10 µM–500 µM
	Melanocyte stimulating hormone (MSH)	Dispersion	10 pM–100 nM
	V. cholerae cholera toxin	Slow dispersion	2.5 ng/ml–25 µg/ml
III. Cytoskeleton effectors			
	C. difficile Toxin B	None	50 ng/ml–200 ng/ml tested*
IV. Protein synthesis effectors			
	C. diphtheriae diphtheria toxin	None	10 ng/ml–25 µg/ml*
	P. aeruginosa exotoxin A	None	0.01 µg/ml–10 µg/ml*
V. Cell membrane effectors			
A. Peptide hemolysins	*S.pyogenes* SLS	Aggregation	0.1 –2.5 mg/ml; 12–290 HU
		Dispersion	≥2.5 mg/ml; 290 HU
B. Complex hemolysins	*C. tetani* tetanolysin	Dispersion	>20 ng/ml; >10 HU/ml
	S. aureus alpha–toxin	Partial aggregation	0.1 µg/ml –10 µg/ml; 3.7 –370 HU
	S.pyogenes SLO	Slight dispersion; pigment movement	0.1 µg/ml–10 µg/ml
	V. parahemolyticus hemolysin	Moderate aggregation and dispersion	8.5–85 µg/ml; 5–50 HU

[1] – Indicates a direct effect (dispersion/aggregation) on chromatphores within 2 hours post agent application.
* – Indicates the concentrations tested

forskolin must penetrate through the cell membrane [27]. The responses to bacterial adenyl cyclase toxins (*B. pertussis* adenyl cyclase, *B. anthracis* adenyl cyclase and cholera toxin) were much slower in kinetics compared to the other adenyl cyclase effectors tested. Figure 10 illustrates the dispersion of pigment granules caused by the addition of MSH and adenylate cyclase from *Bordetella pertussis*. A particularly slow response was observed for cholera toxin, even requiring an induction period of 1–2 hours, depending on the dose (Table 1).

The chromatophores did not show any reactivity to agents belonging to the group of cytoskeleton effectors among which some very potent cytotoxins such as *Clostridium difficile* toxin B with proven strong activity against mammalian cells in cell culture were tested [28]. Similarly, the chromatophores did not respond to *Diphtheriae* toxin and *Pseudomonas aeruginosa*

exotoxin A, which both function as protein synthesis inhibitors. Hypothetically, the chromatophore cells may lack receptors necessary to allow the entry of the toxins. Alternatively, since the chromatophore cells are not actively growing, and thus presumably the level of protein synthesis is too low, the impact of protein synthesis inhibitors may be marginal.

The last group of agents tested, cell membrane effectors (Table 1) caused very pronounced and fairly unique reactions of the chromatophores. They reacted by aggreagtion or dispersion, or by complex response patterns including both dispersion and aggregation. The chromatophores were very sensitive to peptide hemolysin *S. pyogenes* streptolysin S (SLS) at concentrations greater than 0.1 mg/ml (11.6 HU). At concentrations from 0.1 mg/ml to 2 mg/ml (11.6 – 232 HU), SLS caused an initial weak dispersion followed (after 20 min) by weak aggregation, while at higher
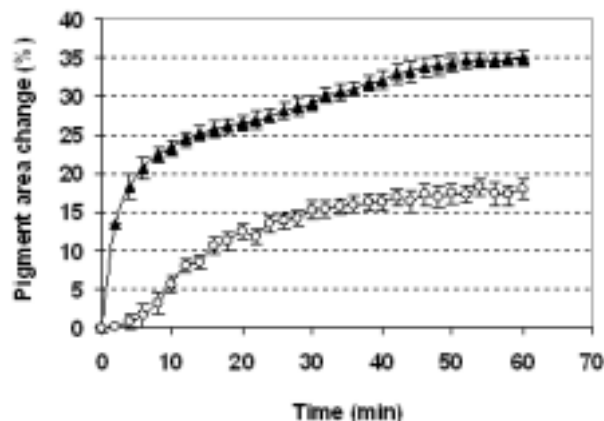
Figure 10. Change in pigment area (%) over time for adenylate cyclase from Bordetella pertussis. and MSH. (–▲–) MSH 10 nM; (–o–) 5 µg/ml adenylate cyclase. The data are mean values of three measurements ± standard deviation.

concentrations (2.5 mg/ml; 290 HU) it caused weak dispersion. Consequently, the dose dependence curve (Figure 11), the concentration versus cell area change, does not have a straightforward shape. Rather, it indicates aggregation up to a concentration of ~ 0.5 mg/ml, but dispersion at higher concentration. A similar dose–dependence pattern was observed with S. aureus α–toxin and could be related to the number of pores in the cell membrane, e.g., with a small number of pores the aggregation trend is predominant, while more pores cause dispersion [7].

Generally considered, the response patterns observed in the immobilized chromatophores showed the main differences in i) the mode of response: aggregation, dispersion, no reaction, or "freezing" of the chromatophore; ii) the magnitude of response: partial aggregation, full aggregation, partial dispersion, full dispersion; iii) the kinetics of responses, and iv) the differences in responses of particular cell subtypes. These four main response features may be used as a basis for modelling the responses [8,9]. The modelling of various toxin responses included 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 and also assist in agent identification [9,29].

CONCLUSION

the development of a micro–cytosensor based on immobilized living chromatophores of Siamese fighting fish, Betta splendens, for the detection of microbial and environmental toxins was investigated in this study. Fish chromatophores were immobilized on ferromagnetic gelatin microbeads (d=250 µm). The optimum conditions for the immobilization of fish chromatophores and optimum amount of ferromagnetic material
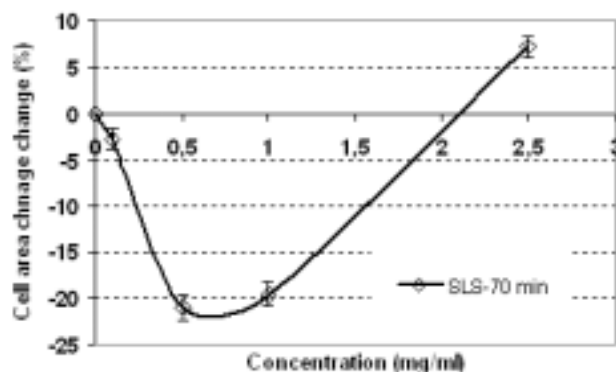

Figure 11. Dose dependence curve for S. pyogenes streptolysin S (SLS)

incorporated in the gelatin microbeads allowing good cell viability and toxin sensitivity were determined.

The movement of microcapsules with immobilized chromatophores within microchannels, and their capture with a magnetic field were studied in the range of predicted operational fluid velocities of a biosensor from 1.6–6.4 mm/s. For these velocities, the magnetic field needed for capturing and positioning microcapsules depended mostly on the amount of ferromagnetic material, and was in the range from 512 A/m–1080 A/m.

The cytosensor was efficient at responding to numerous biological agents and purified bacterial toxins suggesting a possible application of this biosensor in food and water testing as well as for pharmaceutical and biomedical purposes. Particular response features such as the mode of response, its magnitude, kinetics, sensitivity and dose dependence for different classes of agents tested were discussed. The chromatophores showed remarkably strong reactivity to neurotransmitters, adenyl cyclase activators and cell membrane effectors. Conversely, protein synthesis inhibitors did not cause a significant effect on the chromatophores.

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IZVOD

CITOSENZOR NA BAZI HROMATOFORA: ISPITIVANJE ODGOVORA RAZLIČITIH BIOLOŠKI AKTIVNIH AGENASA

(Naučni rad)

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U radu je prikazan dizajn, način rada i karakteristike citosenzora na bazi imobilisanih hromatofora izolovanih iz vrste ribe Betta splendens, Sijamski borac. Ispitana je i razvijena tehnika imobilizacije ovih ćelija, koja predstavlja jednu od ključnih pomoćnih tehnologija na kojima se bazira funkcionisanje citosenzora, kao i uslovi potrebni da bi se imobilisane ćelije in-tegrisale u mikroreaktor senzora. Ispitani su odgovori citosenzora na širok spektar biološki aktivnih agenasa koji uključu-ju: neurotransmitere, aktivatore adenil ciklaze, agense koji deluju na ćelijski skeleton, inhibitore sinteze proteina i agense koji deluju na ćelijsku membranu. Praćene su, analizirane i uporedjene sledeće karakterike odgovora hromatofora na ove agense: način odgovora, jačina odgovora, kinetika, senzitivnost i zavisnost odgovora od koncentracije agensa. Na osnovu ovih karakteristika moguće je je vršiti njihovo modelovanje i klasifikaciju. Rezultati su pokazali da je citosenzor pogodan za korišćenje u kontroli vode i hrane, zagadjenja okoline, kao i u farmaceutske svrhe.

    Ključne reči: Hromatofore riba, Citosenzor, Biološki aktivni agensi.