BIOSENSORS: MICROSCALE DESIGN, TESTING, AND ORTHOGONALITY

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High specificity to variety of chemicals and biological agents makes biosensors unreliable for the detection of unknown agents. Numerous analytical techniques are prone to systematic errors originating from the mechanism of detection, thus often leading to false negative and false positive results. One way to 'combat' these problems is to confirm the results of these analyses with independent data and information from other 'orthogonal' methods of detection. Sensor methods are considered orthogonal if system responses to bioactive compounds are mediated through independent mechanisms of action. Orthogonal methods with well–characterized modes of detection may also more effectively capture the mechanism of action of a bioactive compound and are less subject to systematic errors than individual detection methods.

In this work we present a method to improve the reliability of detecting potentially harmful agents by using two different cell–based sensing systems, fish (*Betta splendens*) chromatophores and algal (*Mesotaenium caldariorum*) cells. The response of fish chromatophores is based on intracellular movement of pigment–like organelles that was quantified using optical density of cells' images. The algal sensor readout is based on well–known principles of fluorescence of living photosynthetic cell. The cells were exposed to four different elicitors; paraquat, mercuric chloride, sodium arsenite and clonidine.

Fish chromatophores were effective in detecting nanomolar concentrations of clonidine and micro molar concentrations of mercuric chloride and sodium arsenite. An increase in fluorescence was observed for the algal cells when exposed to paraquat due to inhibition of photosynthesis. Clonidine did not elicit substantial response from the algal cells. Reduction of fluorescence was observed for the remaining two toxins owing to disruption of the chlorophyll pigment.

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The response curves were fit using simple exponential models of the form, $F(t) = A \exp[-Bt]+C$, where A is the intercept, B refers to the decay constant, C denotes the steady state response and 't' refers to the elapsed time. The parameters A, B, C were employed in the subsequent statistical analyses. The two systems were independently investigated for classification of the toxin set by performing discriminant analyses. The fish chromatophore system was able to classify 91% of the toxins into their actual group whereas the algal system classification efficiency was only 72%. The combination of parameters for the two systems yielded a 100% correct toxin classification. Therefore the statistical analyses prove the hypothesis that a combination of two orthogonal sensing systems facilitates better classification of the toxins by reducing the probability of false positives and false negatives readouts.

METHODS

Many fish, amphibian, reptile species and some invertebrates are capable of changing their color in response to a wide variety of environmental stimuli and this adaptation is useful for purposes like camouflage, intra–species communication, thermoregulation etc. This ability of fish to change their color makes them a natural biosensor. The macroscopically perceived color of the fish is due to its chromatophores. Typically, they are isolated from the scales and fins of these fishes. Obika (1986) studied the morphology of the chromatophores and found that it varies from highly dendritic to discoid shape, depending on the location in the animal and on animal species. He also suggested that pigment movement in chromatophores ultimately changes the overall color of the fish. At the extremes, the pigment granules can be found in the center of the cell (aggregated) or throughout the cell (dispersed). In the aggregated state the fish is lighted in color and when the pigment granules are dispersed the fish appears darker. Kumazawa and Fuji (1984) showed that melanophores could be indirectly manipulated by stimulating the nerves when the skin is exposed to elevated potassium ion, due to the release of endogenous stores of a neurotransmitter from nerve

endings. The migration of pigments in the chromatophores offers a considerable scope for their use as Biosensors. With the use of chromatophores, several bioactive agents that elicit a response can be detected using microscopy and image analysis. These cells have also been used to monitor man–made or naturally occurring bioactive agents. Karlsson et al (1991) used chromatophores to detect catecholamine in the medical diagnosis of petrussis toxin, which is responsible for whooping cough. Elwing et al (1990) monitored catecholamine levels in human blood plasma using chromatophores. Frank Chaplen et al. (2002) developed a portable microscale device capable of detecting certain environmental toxins and bacterial pathogens by monitoring changes in pigment granule distribution. Mojovic and Jovanovic (2003) developed and tested a microscale carrier for immobilization of fish cells, thus further reducing to practice biosensor detection based on fish chromatophores.

Many Diatoms and Desmids are known to be sensitive to changes in metal concentrations, pollution or environmental conditions. The following attributes make desmids and diatoms an ideal choice for toxin detection: abundance, ease of isolation, biological relevance, and sensitivity to changes in environmental conditions. In algae, and cyanobacteria, pigments are the means by which the energy of sunlight is captured for photosynthesis. Because the electrons move freely, the porphyrin ring has the potential to gain or lose electrons easily, and thus the potential to provide energized electrons to other molecules. This is the fundamental process by which chlorophyll "captures" the energy of sunlight. In our study, the algal cell response to toxins has been quantified by fluorescence measurements. Algal cells contain chlorophyll pigments that are essential for their energy metabolism, that is, conversion of light energy into sugars. When these cells are exposed to toxins, it can result in either the disruption of the structural integrity of chlorophyll (upstream effect) or inhibition of electron transport in photosynthesis (downstream effect). Both these effects can be quantified through fluorescence measurements and it is of interest to note that, while the upstream effect results in reduction of fluorescence, the downstream effect leads to an increase in fluorescence. The energy metabolism in algal cells is totally independent of the G–protein mediated signaling observed in fish chromatophores. Hence, these two systems are orthogonal to each other.

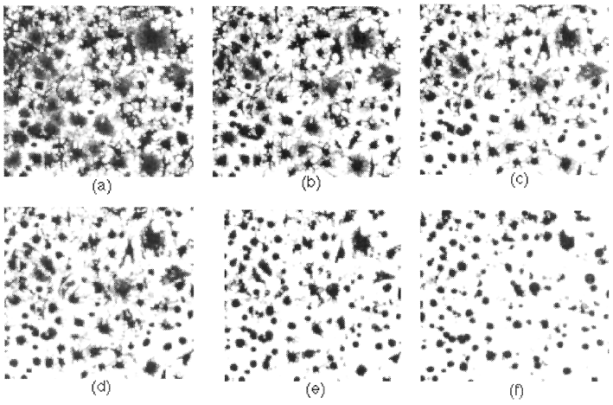

Figure 1. Time sequence of images (a= 0 sec, b=30 sec, c=60 sec, d=120 sec, e=240 sec, f=360 sec) characteristic for the reaction of fish chromatophores to clonidine 200 nmol.

RESULTS AND DISCUSSION

As an example we show two characteristic reactions of fish cells and algae on the two elicitors (clonidine and paraquat). Figure 1 shows a time sequence of images characteristic for the reaction of fish chromatophores when exposed to clonidine. Figure 2 shows experimental data obtained when paraquat is used as the elicitor.

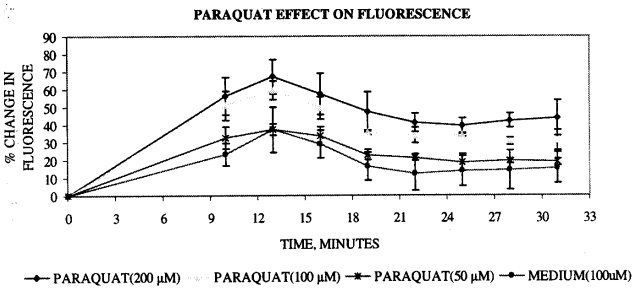

Figure 2. Paraquat effect on algal cells. The concentrations refer to the total dose administered to the cells at 23–24°C).

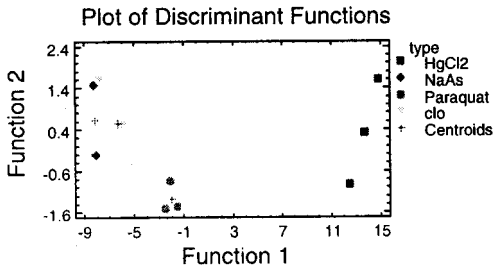

Figure 3. Classification plot of the discriminant functions for the fish system.

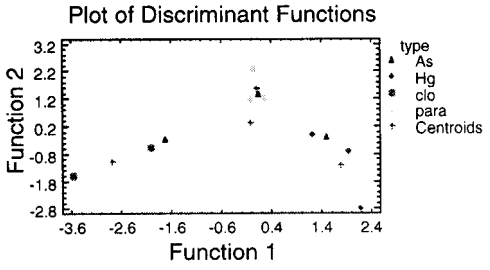

Figure 4. Classification plot of the discriminant functions for the algal system.

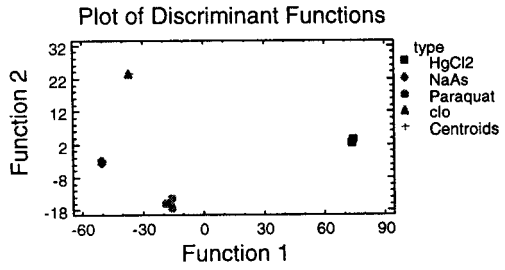

Figure 5. Classification plot of the discriminant functions for combined systems.

Table 1. Classification table of the combined systems.

Actual type	Group Size	Predicted type			
		$NaAsO_2$	$HgCl_2$	Clonidine	Paraquat
$NaAsO_2$	2	2 100.00%	0 0.00%	0 0.00%	0 0.00%
$HgCl_2$	3	0 0.00%	3 100.00%	0 0.00%	0 0.00%
Clonidine	2	0 0.00%	0 0.00%	2 100.00%	0 0.00%
Paraquat	3	0 0.00%	0 0.00%	0 0.00%	3 100.00%

CONCLUSION

We infer from the results of this work that the combined sensing systems are able to provide a reliable classification of the toxin set considered in this study. This confirms our hypothesis that a parallel sensing system increases the classification of chemical and biological agents. The fish chromatophore system was able to classify 90% of the toxins into their respective groups. The response pattern of sodium arsenite and clonidine seemed to overlap with each other resulting in the inappropriate classification of 500 nM clonidine. Although Paraquat did not evoke any significant response form the fish cells at high concentrations, its response indices did not interfere with any of the other toxins. On the other hand, the algal system was unable to detect clonidine whereas paraquat elicited a clear dose response. Also, the algal sensing system could not classify all the toxins into their actual group due to its low sensitivity to clonidine and sodium arsenite. Therefore we conclude that the fish chromatophore sensing technique was a better detection tool to predict the presence of the toxins used in this study.

Further research in the area of parallel sensing techniques can be extended to a wide range of chemical toxins and biological agents. Data from several experiments could bolster our hypothesis and also work towards minimizing the errors on the statistical estimates. Three or more orthogonal sensing techniques would always enhance the reliability of detection of an unknown agent.

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