Whole-cell biochips for online water monitoring

Tal Elad* and Shimshon Belkin
The Alexander Silberman Institute of Life Sciences; Department of Plant and Environmental Sciences; The Hebrew University of Jerusalem; Jerusalem, Israel

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*Correspondence to: Tal Elad;
Email: talelad@mail.huji.ac.il

Chip-integrated luminescent recombinant reporter bacteria were combined with fluidics and light detection systems to form a real-time water biomonitor. The biomonitor was exposed to a continuous water flow for up to ten days, in the course of which it was challenged with spikes of both model toxic compounds and toxic environmental samples. All simulated contamination events were reported within 0.5–2.5 h. Furthermore, the response pattern of the reporter bacteria was indicative of the nature of the contaminating chemicals. Efforts were aimed at improving signal quality and at the development of an alarm management software. Following further research, a device of the proposed design could be implemented in monitoring networks as an early warning system against water pollution by toxic chemicals.

Real-time continuous monitoring systems are important for the protection of water resources against either accidental or intentional releases of toxic chemicals. Analytical instrumentation for detecting chemicals in water, though highly accurate and sensitive, is hard to integrate in early warning systems against unknown chemical threats. More suitable for this purpose is the bioassay-based approach, which utilizes live organisms to test water quality. The test organisms are continuously exposed to the water, and changes in their properties indicate the presence of toxic substances. Among available test organisms, bacteria are advantageous in terms of costs, maintenance requirements and response time.

An additional attractive characteristic of bacteria is their susceptibility to genetic manipulation, which allows one to engineer them to respond by a detectable dose-dependent signal to pre-specified changes in their environment. One well-established technique to achieve this is to transform bacteria with a plasmid that contains a fusion of a stress- or chemical-responsive gene promoter to the bacterial bioluminescence genes luxCDABE. When the designated environmental conditions are met, the transcription of the lux operon is promoted and a light signal is produced that is proportional in intensity to the magnitude of the stimulus.

To date, the use of bioluminescent recombinant reporter bacteria has been mostly restricted to laboratory environments. Other reports have suggested innovative designs for their integration in deployable systems, including systems for continuous water monitoring. We describe a new chip-based design for online water monitoring with integrated luminescent recombinant reporter bacteria. A panel of bacterial reporter strains, characterized by different toxicants response spectra, is immobilized in agar hydrogel in 12-well polydimethylsiloxane (PDMS) biochips placed in specialized flow-through chambers. Each well has a volume of 60 μL and contains ca. 10⁸ cells, harvested at mid-exponential growth phase. Single photon avalanche diode (SPAD) devices detect and quantify the light signal. These detectors are connected to a single-axis stepper motor and move along the flow-through chambers as the monitored water continuously flows above the immobilized bacteria. The system is connected to a computer station, which, by a dedicated program, controls the movement of the detectors and records the intensity of the light signal.
Figure 1. A schematic description of the biomonitoring device. The device contains four flow-through chambers, each harboring a polydimethylsiloxane (PDMS) chip perforated with 12 wells in which the reporter cells are immobilized. The chambers are separately connected to four feeding tubes, while four other tubes navigate the discharged fluids to a waste container. Three aligned single photon avalanche diode (SPAD) detectors, connected to a single-axis linear stepper motor, measure the light signal emitted by the bacterial reporters. A computer station controls the movement of the detectors and records their readings.

Figure 2. A photograph of the biomonitoring device. The device was constructed by Dr. Ronen Almog and Prof. Yosi Shacham-Diamond from the Department of Physical Electronics, Tel Aviv University, Israel.
displays a schematic description of the biomonitoring device. A photograph of the apparatus can be seen in Figure 2.

Three inducible bacterial reporter strains were used in this study to demonstrate the functions of the water toxicity monitor. The strains incorporate fusions between the lux reporter genes and the recA, micF and arsR gene promoters, respectively activated by DNA damage, oxidative stress and heavy metals.20-22 Each of the three reporter strains was immobilized in an individual biochip and placed in a different flow-through chamber. Tap water was pumped continuously through the system for 10 days, in the course of which five simulations of pollution events were carried out. In each simulation, the biosensor was challenged with a 2-h pulse of tap water spiked with different toxicants. The system was challenged by arsenic (6 mg/L) on days 1 and 7, by the DNA damaging agent nalidixic acid (NA; 20 mg/L) on day 3, and by the herbicide paraquat (50 mg/L), an oxidative stressor, on day 5. The fifth toxic pulse, introduced on day 9, was of a mixture of arsenic, NA and paraquat. In each of these cases, a different response pattern was observed: the arsR reporter responded to arsenic, recA (and to a much smaller extent micF) reacted to NA, micF to paraquat and all three reporters were induced when exposed to the mixture. Figure 3 depicts, as an example, the signal emitted by the arsR reporter. Figures 3A and B respectively display the photon counts in their raw and processed forms. Figure 3C displays the signal in terms of the difference between consecutive readings, which allows for the calculation of the response times as explained below. All the responses were characterized by a relatively rapid increase in luminescence followed by a more gradual decrease of the signal back to its basal level. Response times ranged between 0.5 and 2.5 hours. Note that not only did the biosensor successfully detect all simulated contamination events, it was also capable of indicating the nature of the toxic chemical involved by the identity of the responsive reporters.

Two additional experiments have demonstrated the validity of the concept also when challenged by "real-world" samples, as well as the biosensor's sensitivity in relation to drinking water standards and guidelines. In the first experiment, the three abovementioned reporters were immobilized in separate biochips; the tap water which was pumped through the system was spiked for 2 hours with a diluted industrial wastewater, characterized by high concentrations of organic carbon, dissolved solids and adsorbable organic halogens. The spike was identified by the biosensor within 92 min. It elicited a response from the recA reporter, indicating a potential genotoxic hazard, possibly due to one of the halogenated organic constituents.23 In the second experiment, the biosensor was equipped with a biochip housing arsR reporter cells. It was challenged in the course of 8 days with spikes of arsenic and antimony at different concentrations, including at the range of EU and USEPA standards (0.01 and 0.005 mg/L for arsenic and antimony, respectively). The biosensor was able to detect the spikes within 92 min.
respectively.24,25 In all cases, a clear and distinctive response was measured, proportional in intensity to the semimetal dosage, with no change in response times. To accurately determine the system’s response time, a special procedure has been implemented, involving two main steps: (1) calculating the average reading of replicate wells and smoothing it by a simple moving median filter (Fig. 3A and B); (2) calculating the difference between consecutive average readings and smoothing it by a simple moving average filter (Fig. 3C). The response time was then determined as the interval between the time of exposure and the time when the smoothed difference exceeded its mean baseline by a number of standard deviations.

Before putting this procedure into practice, the size of the time window used by the filters and the number of standard deviations by which the difference was to exceed its mean baseline had to be determined. In order to assign values to these two parameters, we set a range of 20–100 min for the time window and a range of 3–5 for the standard score threshold. We then tested all possible combinations in search for the lowest values that would yield no false detections. The process is exemplified in Figure 4A, which shows the optimization results for the arsR reporter, for which the selected parameter values were a time window of 90 min and a standard score threshold of 5. When assigning values to the time window and the standard score threshold, one might also consider the relation between the two parameters and the response time. For example, our results show that the response times of the arsR reporter were more dependent on the size of the time window than on the standard score threshold (Fig. 4B). Similar observations were made with reference to the msrA reporter, whereas the response times of the micF reporter depended on both parameters equally.

The above procedure may serve as a signal analysis tool in a future stand-alone version of the proposed biomonitor. According to the suggested methodology, an alarm would be raised should the difference between consecutive readings exceed its mean baseline by a predetermined number of standard deviations. Importantly, the difference between consecutive readings was chosen under the assumption that the rate at which the signal changes with respect to time would serve as a better marker for a pollutant-induced signal increase than the signal’s absolute value. If proven, this assumption might facilitate the reduction of false detections, ideally, to zero.

Another feature of the system’s response characteristics are the occasional spikes in the light signal. Such spikes, clearly visible in Figure 3A, are correlated with the presence of air bubbles through the flow channels. As oxygen is essential for the bioluminescent reaction, this correlation may indicate a limitation in oxygen supply; nevertheless, oxygen concentration was evidently sufficient for the reporter cells to respond to the stimuli by an increased light signal. A simple moving average filter (Fig. 3B), unlike a simple moving average filter, is not susceptible to very large deviations from the trend. Given the abovementioned spikes, it was thus suitable for smoothing the signal. Ultimately, a combination of technical and digital means should be applied in order to achieve a high quality signal that will permit the best analysis of bioreporter behavior.

Scientific and technological advances now allow to couple reporter bacteria and diverse engineering platforms in the design of novel whole-cell biosensing devices.26 The present work provides a concrete proof of concept of a recombinant bacteria-based biosensor for
continuous water monitoring. The biosensor, which is easy to install and to handle, operated for at least ten days under continuous tap water flow, successfully detecting and classifying all chemicals it was challenged with. Further research efforts are necessary before such a device can be put to full-scale field tests, including establishment of detection limits for a broad range of toxic chemicals and optimization of biochip storage conditions.

Once such challenges are overcome, biosensor devices of this type can be integrated into an early warning scheme against accidental or intentional penetration of toxic chemicals into diverse types of water systems, from surface waters to municipal distribution networks.

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