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Miniaturized bacterial biosensor system for arsenic detection holds great promise for making integrated measurement device

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Combining bacterial bioreporters with microfluidics systems holds great promise for in-field detection of chemical or toxicity targets. Recently we showed how *Escherichia coli* cells engineered to produce a variant of green fluorescent protein after contact to arsenite and arsenate can be encapsulated in agarose beads and incorporated into a microfluidic chip to create a device for in-field detection of arsenic, a contaminant of well known toxicity and carcinogenicity in potable water both in industrialized and developing countries. Cell-beads stored in the microfluidics chip at -20°C retained inducibility up to one month and we were able to reproducibly discriminate concentrations of 10 and 50 μg arsenite per L (the drinking water standards for European countries and the United States, and for the developing countries, respectively) from the blank in less than 200 minutes. We discuss here the reasons for decreasing bioreporter signal development upon increased storage of cell beads but also show how this decrease can be reduced, leading to a faster detection and a longer lifetime of the device.

Bacterial bioreporters hold great promise as cheap bioanalytical alternatives for certain specific compounds or as first-line detectors of general sample toxicity.[1] Bioreporters couple target detection by a living cell to a standardized output in form of a reporter protein that can be measured in an easy and sensitive way. Multiple proofs of evidence for successful engineering of target compound detection by bacterial cells have been presented in laboratory settings, but actually very few studies have addressed how the 'living sensor part' (i.e., the bacterial reporter cells) might be embedded in a closed sample incubation and detector system.[2] Such a system could take optimal advantage of the small size of the bacterial reporter cells and their potential ease of maintenance in order to build a miniaturized sampling and detector unit.

To illustrate this potential we recently showed how *Escherichia coli* cells engineered to produce a variant of green fluorescent protein (eGFP) in response to contact with arsenite and arsenate, can be packed in 40–70 μm diameter agarose beads and concentrated in miniaturized filter cages on a microfluidics chip, where they are exposed to the aqueous sample.[3] Concentrating cells in beads in the filter holder also has the advantage that the total signal output is increased to a level where it becomes easily detectable with a low cost detector. The reason to focus on a bioreporter system for arsenic is that contamination of drinking water resources with arsenic is a world-wide problem occurring in both industrialized and developing countries.[4] Moreover, several geographical regions that currently suffer most from extensive arsenic contamination, such as Vietnam, Bangladesh, India and Cambodia,[5] are also the areas without the typical infrastructure needed to reliably measure arsenic down to μg per L concentrations to comply with typical drinking water standards. Given

their reported sensitivity in the low μg per L range, bacterial bioreporter assays for arsenic could thus provide a realistic alternative for expensive chemical analytical instruments,[6,7] and allow large-scale field testing, provided such a bioreporter assay would be sufficiently robust, easy to handle and have consistent and reproducible output.

The advance we proposed would form an essential step in the miniaturization, maintenance and closed operation of the bioreporter assay towards a fully operated integrated field detector. In order to miniaturize the assays we developed a PDMS microfluidic chip with one to four parallel flow lanes, in which reproducibly ~1,000 bead-embedded bacterial bioreporters are captured by the liquid flow in a 500 x 500 μm^2 cage. We demonstrated that the bioreporters can be loaded on the chip and stored within the cages by freezing in 15% glycerol solution at -20°C. Frozen chips with cells in beads can be thawed, connected to the sample flow lines and exposed to aqueous samples containing inorganic arsenic. The evolving eGFP reporter signal from the cells in the cage is detected over a period of 2–3 h or as long as is necessary to complete a reliable measurement. Most notably, we showed that both end-point measurements (at one fixed duration of the assay) and kinetic modes (integrating the rate of signal increase over time) provided accurate and sensitive ways to analyze the reporter output. In terms of practicality the most notable finding was that cell-beads frozen at -20°C in the microfluidic chip retained inducibility for up to a month and that arsenic samples with 10 or 50 μg per L could be reproducibly discriminated from each other and from samples without any arsenic.

One of the main weaknesses we still observed was a decrease in reporter signal development over time of induction with increased storage time. Indeed, the time for reliable and reproducible detection for 50 μg arsenite per L increased from 60 min the first day to 140 min after one month storage, and from 60 to 200 min for a concentration of 10 μg per L. Thus we concluded that the lifetime of the cells in beads within the PDMS chip would be limited to between 2 and 4 weeks. In particular we noticed that the time delay before the reporter signal development started increased whereas the rate of signal increase diminished over prolonged storage periods. This suggested that either the number of dead cells would increase over time of storage, or that cells remained alive but somehow lost their potential to become induced with arsenic. Microscopic imaging of beads stained for live (using SYTO9) or dead cells (using propidium iodide) did not indicate a significant increase in the number of dead cells during storage. We then tested whether certain nutrients would perhaps become limiting over time in the storage solution. In our procedure cells in beads are stored in a mineral salts buffered medium with 15% glycerol and with glucose to energize the cells. However, the medium lacks thiamine and further organic micronutrients in order to prevent reporter cell division within beads, since the *E. coli* host cell for the arsenic reporter construct is an auxotrophic mutant. To test, therefore, whether a simple pre-incubation of cells after thawing of a -20°C frozen PDMS chip with organic micronutrients would be sufficient to reactivate them, we flushed the flow lines and cages for 30 minutes with 10 μl of rich LB medium before exposing cells in beads to an aqueous arsenic-containing sample. Indeed, we found that this procedure effectively recovers the reactivation potential of the cells and, as a consequence, leads to high and rapid reporter signal development (**Fig. 1**). In fact, whereas with this particular batch of cell-beads we were barely able to distinguish 0, 10 and 50 μg arsenite per L after two weeks of -20°C storage, adding the pre-incubation step with LB resulted in full recovery and discrimination of the three concentrations even after one month -20°C storage. More precisely, LB pre-incubating resulted in a much shorter time delay in the onset of detectable eGFP signal development. This simple extra step can therefore at least double the lifetime of frozen reporter cell chips and allows faster detection (compare **Fig. 1C** with 1**D**). Maintenance of inducibility of the stored reporter cells for periods of a few months are important when considering production and product transport.

Still, even with pre-incubation a decrease in reporter signal development over time can probably not be completely avoided. One solution could be to work with dried or freeze-dried reporter cells that need to be rewetted by contact with the sample and with the advantage that storage at ambient temperature may be possible. Typically, however, freeze-drying results in considerable amounts of dead cells and re-activating dried cells may take much longer than 30 min.[8] Another solution could be a chip containing a microchemostat, which produces a continuous supply of fresh cells used for the reporter assay. However, such a system is challenging in terms of design and microfabrication, and would be more complicated to operate. In fact such microchemostat should contain a cultivation chamber in which the cells receive nutrients at a specified dilution rate, and the number of cells per time interval flowing out of this chamber would need to be reliably controlled, for example using valves.[9] Furthermore, in this case free cells will have to be captured for the assay instead of cells encapsulated in agarose beads. Such a cage will be more challenging to produce because of the smaller dimensions of cage pillars (<0.5 μm). The free cells will have to be introduced into a detection zone, which can be perfused with the aqueous sample to be tested, while contact with the nutrient feed and the aqueous sample has to be avoided.

For these reasons, the extremely simple device that we developed seems so far to be the best option and we feel is a crucial step forward to the development of a cheap in-field arsenic measurement device. The fabrication of microfluidics chips from a mold is relatively inexpensive with one mold serving for more than one hundred chips. Chips have replicate flow-lines, increasing the accuracy of the measurement. Also from the biological point of view the costs are extremely low because from a single batch of beads produced from 0.2 ml of cell culture one can fill more than 20 chips. The next immediate step is now the development of an optical detection system and an instrument housing to integrate the microfluidics cage. Once this step will be accomplished, it will also be relatively easy to accommodate bioreporter cell lines with other target specificities, of which many exist. The

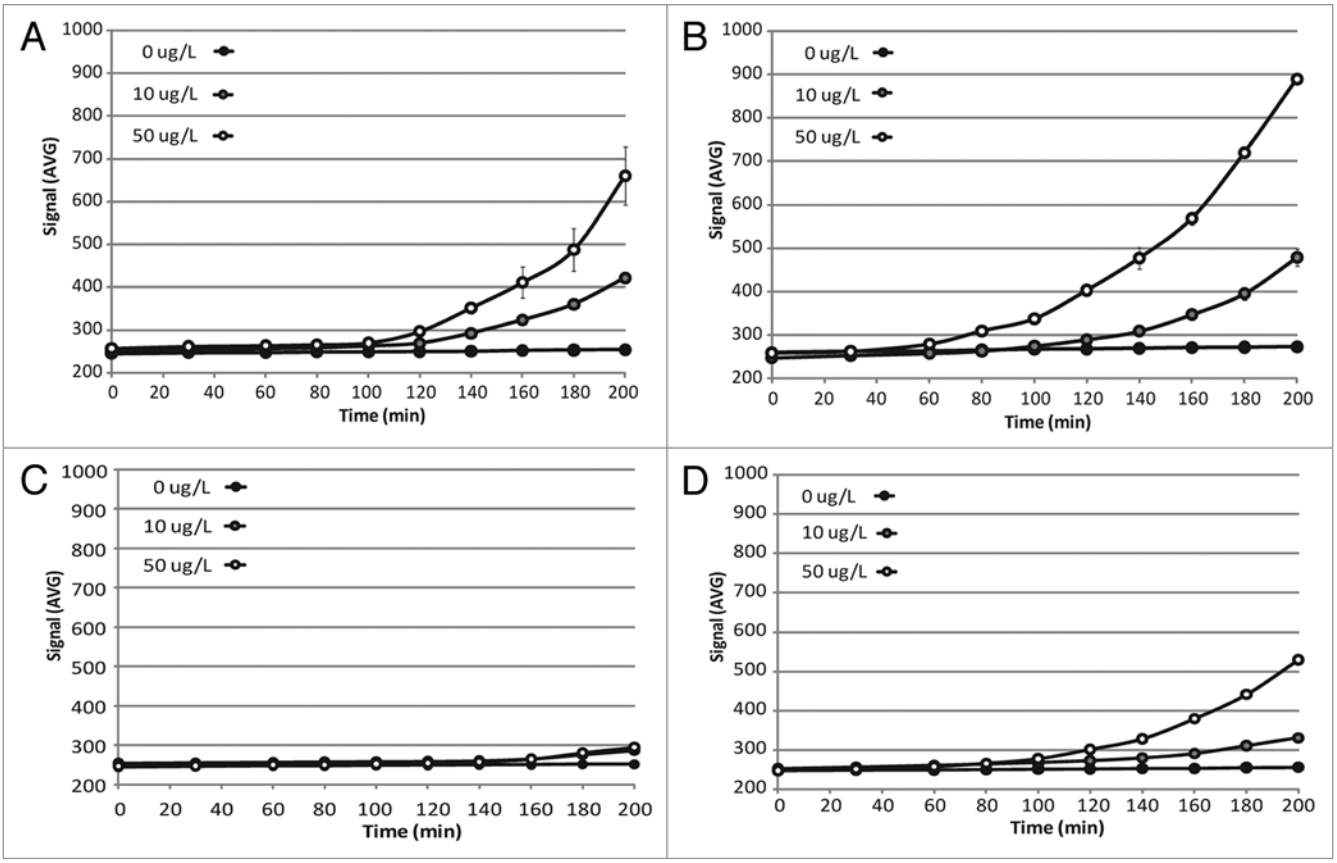

Figure 1. Effect of pre-incubation with LB medium on the reporter development of cells in beads in response to arsenic. (A and B) Cell-beads stored for 7 days at -20°C, measurement without or with LB pre-incubation step, respectively. (C) Cell-beads stored for 14 days at -20°C, measurement without pre-incubation step. (D) Cell-beads stored for 28 days at -20°C, measurement with LB pre-incubation step. All data are averages from triplicate assays simultaneously carried out in a four-lane PDMS chip. Error bars denote calculated deviations from the average. Error bars not shown are smaller than the symbol size.

device is thus relevant for environmental research, for related fields of biotechnology and industry applications that need easy measurement of chemical parameters, for agrotechnology, and possibly even for medical analytics.

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