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A miniature porous aluminum oxide-based flow-cell for online water quality monitoring using bacterial sensor cells

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ABSTRACT

The use of live bacterial reporters as sensing entities in whole-cell biosensors allows the investigation of the biological effects of a tested sample, as well as the bioavailability of its components. Here we present a proof of concept for a new design for online continuous water monitoring flow-cell biosensor, incorporating recombinant reporter bacteria, engineered to generate an optical signal (fluorescent or bioluminescent) in the presence of the target compound(s). At the heart of the flow-cell is a disposable chip made of porous aluminum oxide (PAO), which retains the sensor microorganisms on its rigid planar surface, while its high porosity allows an undisturbed access both to the sample and to essential nutrients. The ability of the bacterial reporters to detect model toxic chemicals was first demonstrated using a "naked" PAO chip placed on solid agar, and later in a chip encased in a specially designed flow-through configuration which enables continuous on-line monitoring. The applicability of the PAO chip to simultaneous online detection of diverse groups of chemicals was demonstrated by the incorporation of a 6-member sensor array into the flow-through chip. The selective response of the array was also confirmed in spiked municipal wastewater effluents. Sensing activity was retained by the bacteria after 12-weeks storage of freeze-dried biochips, demonstrating the biochip potential as a simple minimal maintenance "plug-in" cartridge. This low-cost and easy to handle PAO-based flow-cell biosensor may serve as a basis for a future platform for water quality monitoring.

1. Introduction

Traditional approaches for detecting the presence of undesired chemicals in water are mostly based on chemical or physical analyses, which allow highly accurate and sensitive determination of the exact composition of the tested sample. Such methodologies, however, fail to provide information regarding the bioavailability of pollutants, their effects on living systems, and their synergistic or antagonistic behavior in mixtures. They also demand a substantial investment of time, skilled personnel and sophisticated equipment, particularly when there is no preliminary information concerning the sample's composition. A complementary approach is based on the use of living systems in a variety of bioassays: test organisms, ranging in complexity from fish to microbes, are exposed to the sample, and changes in their behavior, morphology, or other biological responses that indicate the presence of toxic substances are monitored (Gerhardt et al., 1998; Shedd et al., 2001; Gerhardt et al., 2006). Unicellular microorganisms, in particular bacteria, are attractive for such purposes due to their large population sizes, rapid growth rates, low cost, easy maintenance and their amenability to genetic engineering (Belkin, 2003; van der Meer and Belkin, 2010).

Bacterial bioreporters have been molecularly engineered to detect specific chemicals, groups of chemicals, or global biological effects such as toxicity or genotoxicity (van der Meer and Belkin, 2010). In most cases, the engineered constructs harbor a sensing element that detects the presence of the target compound(s), fused to a reporter element, the expression of which yields a quantifiable output. The most common sensing elements used are DNA segments harboring gene promoter regions involved in the cellular response to the target chemical(s); of several available reporter elements, most commonly are the *lacZ, gfp* or *lux* genes, yielding colorimetric, fluorescent or bioluminescent signals, respectively. Numerous examples of bacterial sensors for diverse classes of chemicals have been reported, including the detection of genotoxic agents and oxidative stress (Lee et al., 2007; Biran et al.,

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2009), toluene and related organic compounds (Applegate et al., 1998; Willardson et al., 1998), antibiotic substances (Melamed et al., 2012), tri- and di-nitrotoluene and related compounds (Yagur-Kroll et al., 2013), heavy metals (Trang et al., 2005; Magrisso et al., 2009) and more.

To turn such bacterial strains into on-line biosensors, they need to be integrated into a hardware platform containing all the components essential for continuous flow, combined with a sensing device (Elad et al., 2008). Elad et al. (2011) described a flow-through biosensor with disposable modular poly(dimethylsiloxane) (PDMS) chips, incorporating agar-immobilized bioluminescent recombinant reporter bacteria, with a continuous water flow for up to 10 days. It was shown that a 4-reporters panel detected all simulated contamination events within 0.5–2.5 h, and its response was indicative of the nature of the contaminating chemicals. Buffi et al. (2011) described a microfluidics biosensor for fluorescent *Escherichia coli* bioreporter cells immobilized in agarose beads for arsenite detection in aqueous samples. The microfluidic cartridge was composed of a PDMS block, containing channels and cages bonded on a microscope glass slide. Charrier et al. (2011) reported an on-line multi-channel biosensor for the detection of heavy metals and general toxicity, in which bioluminescent bacteria were agarose-immobilized in a removable card. The simultaneous on-line detection of one or more heavy metals as well as the measurement of the overall toxicity of the sample were demonstrated. Other researchers have alginate- or agarose-immobilized reporter bacteria on optical fiber tips (Eltzov et al., 2009), or on a multi-well transparent polycarbonate platform (Horry et al., 2007).

Here we describe a proof of concept for a new design for an online continuous water monitoring flow-cell with integrated recombinant reporter bacteria. The heart of the device is a disposable chip made of porous aluminum oxide (PAO), a nanoporous matrix that is a highly suitable for microbial culture support (Ingham et al., 2007): it is exceptionally porous (40% by volume), inert, and retains microorganisms on the rigid planar surface while allowing them full access to nutrients diffusing through the chip. In this study, the PAO-based chip has been employed in two forms. For initial examination of its capacity to support bacterial bioreporters, it was first tested as a "naked" PAO chip with cavities etched on its surface loaded with bacterial reporters. A dose-dependent fluorescent response was observed when the chip was placed on solid agar containing different concentrations of a target analyte. For demonstrating the suitability of the chip for online water quality monitoring, the PAO layer was incorporated into a specially designed flow system, and a concentration-dependent fluorescent response was demonstrated by a hydroquinone sensor strain. The induction of a 6-member bacterial reporter array by different chemicals was demonstrated, both in laboratory media and in wastewater effluents, as well as the detection of different optical outputs (green or red fluorescence and bioluminescence). To simplify future practical deployment of any live-cell biosensor system, it is highly desirable that the sensor cells are stored as an integral component of the device; for this end we have also demonstrated the applicability of on-chip live cell preservation by freeze drying. This easy to handle PAO-based flow-cell may provide a basis for a future online monitoring system for environmental, industrial or security applications.

2. Materials and methods

2.1. Bacterial reporters used in this study and growth conditions

The bacterial reporter strains used in this study, all of them previously characterized, are listed in Table 1. Fluorescent strains were grown in TGA medium (10 g L^{-1} Bacto tryptone, 5 g L^{-1} NaCl, 2 g L^{-1} D-(+)glucose, 11.9 g L^{-1} HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, $C_8H_{18}N_2O_4S$), pH 7.0). The bioluminescent strains were grown in Lysogeny Broth (LB), pH 7.0. Antibiotics for plasmid maintenance were employed according to Table 1.

2.2. Bacteria reporter induction on PAO chips: stationary system

Microbial PAO culture chips (8×36 mm^2; MicroDish model MDCC160.40, Ingham et al., 2007) containing more than 4000 round microwells (40 μm deep, 160 μm in diameter), coated by a 10 nm layer of platinum over the upper surface to limit autofluorescence (Fig. 1A, top), were used. The chips were sterilized by plasma cleaning (10 s, low power setting, Harrick PDC-002 Plasma

Table 1
Bacterial reporter strains used in this study.

	E. coli host	Sensing element (gene promoter)	Reporting element	Chemicals detected	Model chemical, mg/l	Antibiotic resistance[1]	Reference
1	MG1655	*recA*	*gfpmut2'*	DNA damaging agents	Nalidixic acid, 10	Amp	Lab collection
2	MG1655	*recA*	*gfpmut2*	DNA damaging agents	Nalidixic acid, 10	Kan	(Zaslaver et al. 2006)
3	MG1655	*micF*	*gfpmut2*	Oxidative stress agents	Paraquat, 400	Kan	Yagur-Kroll, unpublished
4	MG1655	*zntA*	*gfpmut2*	Heavy metals	Cadmium chloride, 25	Kan	(Zaslaver et al. 2006)
5	MG1655	*yqjF*	*gfpmut2*	2.4-DNT and byproducts	Hydroquinone, 200	Kan	(Zaslaver et al. 2006)
6	DH5α	*yqjFB2A1*	*gfpmut2*	2.4-DNT and byproducts	Hydroquinone, 200	Kan	Yagur-Kroll, unpublished
7	RFM443	*recA*	*DsRed-Express*	DNA damage	Nalidixic acid, 10	Amp	(Hever and Belkin 2006)
8	DH5α	*yqjFB2A1*	*luxCDABE*	2.4-DNT and byproducts	Hydroqinone, 200	Amp	(Yagur-Kroll et al. 2013)
9	MG1655	*CP38*	*gfpmut2*	Constitutive expression (no induction)		Amp	Yagur-Kroll, unpublished

[1]Amp, ampicillin (100 μg/ml); Kan, kanamycin (30 μg/ml).