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Review

Microbial whole-cell arrays

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Summary

The coming of age of whole-cell biosensors, combined with the continuing advances in array technologies, has prepared the ground for the next step in the evolution of both disciplines – the whole-cell array. In the present review, we highlight the state-of-the-art in the different disciplines essential for a functional bacterial array. These include the genetic engineering of the biological components, their immobilization in different polymers, technologies for live cell deposition and patterning on different types of solid surfaces, and cellular viability maintenance. Also reviewed are the types of signals emitted by the reporter cell arrays, some of the transduction methodologies for reading these signals and the mathematical approaches proposed for their analysis. Finally, we review some of the potential applications for bacterial cell arrays, and list the future needs for their maturation: a richer arsenal of high-performance reporter strains, better methodologies for their incorporation into hardware platforms, design of appropriate detection circuits, the continuing development of dedicated algorithms for multiplex signal analysis and – most importantly – enhanced long-term maintenance of viability and activity on the fabricated biochips.

Introduction

The array concept: nucleotides, proteins and cells

Within a very short period, microarrays have revolutionized our ability to identify, characterize and quantify bio-

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logically relevant molecules. The principle in all arrays is almost identical: a large family of well-defined reactive molecules is fixed onto a mapped solid surface grid, and exposed to a multicomponent analyte mixture. Sites at which a recognition event has occurred (such as by a complementary nucleic acid sequence) are identified by one of several possible detection techniques (e.g. fluorescence). The characteristics of the sample – and hence the constituents and/or the response of the studied system – can then be discerned from the identity and nature of the bioreceptor molecules occupying these sites. Using this principle, an increasingly large number of applications are being developed in medicine, biology, toxicology, drug screening and more.

Most of the arrays in use today are based on nucleic acid oligonucleotides (Ehrenreich, 2006; Lee and Saeed, 2007; Petersen and Kawasaki, 2007); other arrays contain antibodies (Wingren and Borrebaeck, 2006), enzymes (Reymond and Babiak, 2007) or other proteins (Kricka *et al.*, 2006). In all of these configurations, the advantages mostly stem from the extremely high specificity inherent in each of the individual recognition events. A different analytical approach may be generated if the array components are not molecules but rather live cells. Although much of the specificity characterizing molecular recognition is lost, this is more than compensated for by the ability to directly assay biological effects on live systems. While the approach was initially aimed towards gene expression studies (Van Dyk *et al.*, 2001), it can be successfully utilized for numerous applications, efficiently combining effect-testing with analyte identification. Consequently, a variety of future applications are envisaged for such cell-based sensors in the medical, industrial, pharmaceutical and environmental realms, including drug and chemicals screening or environmental monitoring (Daunert *et al.*, 2000; Belkin, 2003; Gu *et al.*, 2004; Wang *et al.*, 2005; Sørensen *et al.*, 2006). In this article we review the state-of-the-art in this rapidly developing field, focusing on microbial whole-cell arrays.

Advantages of cell array technology

In contrast to biosensing technologies based on molecular recognition, the use of whole cells as sensing entities allows the investigation of the activity of the tested sample

rather than the identity of its components. Functional cellular responses that can be analysed in this manner include gene expression, metabolic activity, viability, bio-availability, toxicity and genotoxicity, measuring either specific or global biological effects of the target analyte(s). Most of these vital responses can only be assayed by the use of live systems; while chemical methods can often yield lower detection thresholds and provide a more sensitive analytical performance, no chemical assay can provide information on the effects of the tested compound as sensed by a live cell. Furthermore, the use of live cells allows for reagent-less, non-destructive real-time monitoring of the biological effects as they develop, with no need for preparatory and analytical steps such as staining or hybridization. In an array format, these advantages are supplemented by the combinatorial effects of multiplexed sensors, comparable to the principles inherent in electronic nose (Thaler and Hanson, 2005) or tongue (Vlasov *et al.*, 2002) devices. If the technical hurdles outlined in the following sections will be surmounted, a miniaturized array format should permit a high-throughput sample analysis, superior to current microtitre plate-based screening technologies. The envisaged miniaturization can eventually lead to the construction of portable instrumentation for both laboratory and field use, for applications such as toxicity assessment or mutagenicity testing. Finally, the rapidly developing field of molecular systems biology could make use of such tools for the unravelling of complex biological processes.

Cell array biochips

Eukaryotic array biochips. While the current review focuses on prokaryotic cell arrays, eukaryotic systems serve as good examples for the growing interest in the field of whole-cell arrays for high-throughput screening. It has been suggested that arrays of eukaryotic cells can be used in a variety of applications including gene function analysis, micro-physiometry, biosensing, single-cell analysis and therapeutic agent identification (McConnell *et al.*, 1992; Aravanis *et al.*, 2001; Ziauddin and Sabatini, 2001; Palkov *et al.*, 2004; Waterworth *et al.*, 2005; Lee *et al.*, 2006; Palmer *et al.*, 2006). The techniques used for the generation of such arrays and for the positioning of cells vary, ranging from photolithography to inkjet technology (Beske and Goldbard, 2002; Park and Shuler, 2003; Alper, 2004). Specific examples include patterning of cell-adhesive self-assembled monolayers using microcontact printing (Mrksich and Whitesides, 1995), photo- and electropatterning of hydrogel-encapsulated living fibroblasts arrays (Albrecht *et al.*, 2005) and inkjet printing of viable mammalian cells (Xu *et al.*, 2005). A multiphenotype array of mammalian hepatocytes in a polyethylene glycol hydrogel was challenged by Pishko and co-workers with chemi-

cal and biological toxins, and the viability and sensing capability of the cells were examined (Koh *et al.*, 2003; Iltle and Pishko, 2005).

Prokaryotic array biochips. Whereas eukaryotic cell arrays, particularly those based on mammalian systems, possess the unique advantage of more closely simulating human cellular responses, prokaryotic cell systems have numerous compensatory benefits. The cells are easy to grow and maintain, large and homogenous populations are readily obtainable, and suitable cell immobilization and preservation methodologies are available. Prokaryotic cells are also more robust and less sensitive to their physical and chemical environment, and less susceptible to biological contamination. Furthermore, microbial cells are also much more amenable to the physical and/or chemical manipulations required to pattern them in the array format. Possibly the most important bacterial characteristic in this context is the facility by which they can be genetically tailored to emit the desired signal in the presence of the target compound(s) or specific environmental conditions. In most cases, this is achieved by the fusion of a sensing element – a selected promoter – to a suitable molecular reporter system (Belkin *et al.*, 1996; Belkin *et al.*, 1997; Vollmer *et al.*, 1997; Lee and Gu, 2003; Min and Gu, 2003). The expression of two independent reporter systems in a single organism has also been reported (Mitchell and Gu, 2004a,b; Hever and Belkin, 2006). Furthermore, such sensor cells can detect analytes in different media, such as water, gas and soil (Gu *et al.*, 1999; Gil *et al.*, 2000; Gu and Chang, 2001).

To progress from a panel of genetically engineered sensor cells to an actual array on a solid platform, several biological and physical issues need to be addressed (Fig. 1). These are covered in the following sections.

Bacterial cell array technology

Genetic engineering of microbial reporter cells and the panel concept

One of the main advantages inherent in the use of microorganisms as building blocks for whole-cell arrays is the facility by which they can be genetically engineered to respond by a dose-dependent signal to environmental stimuli. Two parallel research approaches have been employed for this purpose, focusing on either constitutive or inducible reporter gene expression, often referred to as 'lights off' and 'lights on' assays (Belkin, 2003).

The 'lights off' concept is based on measurement of the decrease in signal intensity, such as the one produced by a naturally luminescent bacterium (*Vibrio fischeri*; Quershi *et al.*, 1998) or a genetically modified one (Belkin, 2003). More relevant to the present review is the 'lights on'

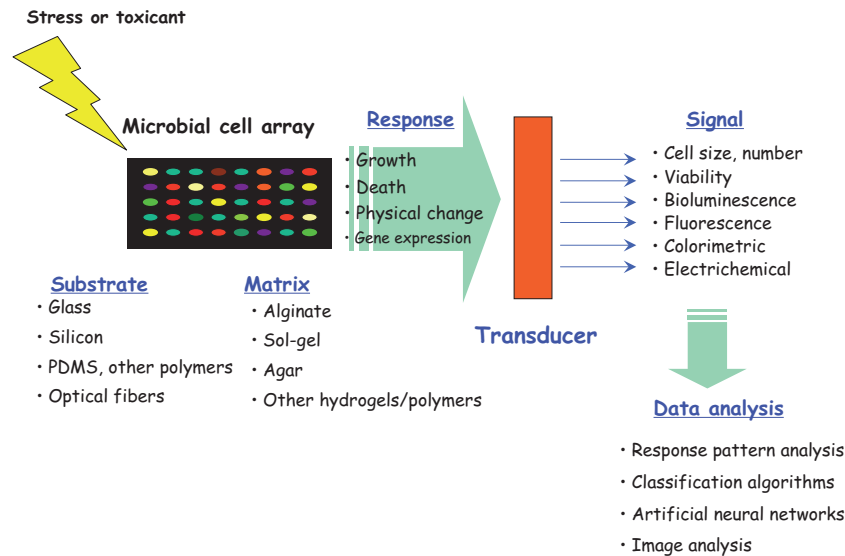


Fig. 1. Generalized concept of cell array biochip technology.

approach, based on the molecular fusion of a reporting gene system to gene promoters from selected stress response regulons. It has been demonstrated that with the use of the appropriate stress-responsive promoters it is possible to construct bacterial reporter strains which generate a dose-dependant signal in response, for example, to the presence of heat shock-inducing agents (Van Dyk *et al.*, 1994), oxidants (Belkin *et al.*, 1996; Lee and Gu, 2003) or membrane-damaging substances (Choi and Gu, 2001). As no single reporter strain is expected to cover all potential cellular stress factors, it has been proposed that a panel of such stress-specific strains be used (Belkin *et al.*, 1997). Similar panels have been shown to sensitively respond to environmental pollutants such as dioxins (Min *et al.*, 2003) and endocrine disruptors (Gu *et al.*, 2002). For the development of genotoxicity assays, the promoters serving as the sensing elements were selected from among DNA-repair operons such as the SOS system; the reporters used were either bacterial *lux* or β -galactosidase (Nunoshiba and Nishioka, 1991; Vollmer *et al.*, 1997). Other reports proposed the use of a green fluorescent protein (GFP) gene from the jellyfish *Aequorea Victoria*, or its variants, as an alternative reporter system for the same purpose (Kostrzynska *et al.*, 2002; Sagi *et al.*, 2003). Using GFP as a reporter, Norman and colleagues (2005) have demonstrated that the ColD plasmid-borne *cda* gene promoter was preferable to other SOS gene promoters *recA*, *sulA* and *umuDC*. A yeast-based (*Saccharomyces cerevisiae*) GFP system for genotoxicity assessment is being continuously improved upon by Walmsley and co-workers (Knight, 2004; Knight *et al.*, 2004; Walmsley, 2005).

Another class of inducible systems includes those that can sensitively detect a specific chemical or a group of

chemicals. They are usually based on promoters of genes involved in the metabolism pathway of, or the resistance mechanism to the compound to be detected. Since the pioneering work of Saylor and co-workers in the construction of a *lux* fusion for the specific detection of naphthalene and salicylate (Burlage *et al.*, 1990; Heitzer *et al.*, 1992), there has been a steady stream of similar constructs responsive to different organic or inorganic pollutants or classes of pollutants. Bioluminescence has served as the reporter in many of these cases, with a few examples of β -galactosidase activity and GFP accumulation (Köhler *et al.*, 2000; Belkin, 2003; Gu *et al.*, 2004; Vollmer and Van Dyk, 2004; Ron, 2007).

Reporters and signals

As indicated in the preceding section, the signals emitted by the array members can be generated either by the presence of a protein (e.g. GFP or other fluorescent proteins; Kuang *et al.*, 2004; Fesenko *et al.*, 2005a), a carotenoid (*crtA*; Fujimoto *et al.*, 2006; Maeda *et al.*, 2006), or by the activity of an enzyme. The latter category includes bacterial luciferase (*lux*; Lee *et al.*, 2005) and β -galactosidase (*lacZ*; Biran *et al.*, 2003; Popovtzer *et al.*, 2005), as well as others: insect luciferase (*luc*), alkaline phosphatase (*phoA*), β -glucuronidase (*uidA*) and β -lactamase (*bla*) (Yoon *et al.*, 1991; Beck and Burtscher, 1994; Bronstein *et al.*, 1994; Willardson *et al.*, 1998; Shao *et al.*, 2002; Barsalobres-Cavallari *et al.*, 2006). Depending on the reporter gene used, the emitted signals can be detected optically, colorimetrically or electrochemically (Köhler *et al.*, 2000). Additional assays, also applicable to array formats, may be based on cell viability, for example, by the Live/Dead system (Heo *et al.*, 2003) or by surface

plasmon resonance analysis (Choi *et al.*, 2005), cell length (Umehara *et al.*, 2003) or cellular well-being and growth. The latter has been demonstrated microscopically, using single YFP-tagged cells in narrow microfluidic channels (Balaban *et al.*, 2004).

Data analysis: interpreting the array response pattern

Data emanating from the individual responses of an array of specific stress-responsive bacteria should not only allow the detection of a wide range of toxic chemicals but also indicate the type of biological activity involved. With a sufficient number of array members, each chemical or group of chemicals will be characterized by its own specific signature, which can then be used both to identify the chemicals in the sample and to indicate their biological activity.

Indeed, Lee and colleagues (2005) demonstrated the different response patterns of a cell array chip constructed of 20 luminescent reporter strains following a separate exposure to three chemicals of different biological activities. The same group has also reported the fabrication of an oxidative stress-specific bacterial cell array chip (Lee *et al.*, 2007). Their results demonstrate that such an array can be used to elucidate the nature of the adverse effect of toxic chemicals, and suggest that response pattern-based classification should be made according to the chemicals' mode of action rather than their structure.

An attempt to identify toxicants on the basis of their biologic fingerprint by a pattern classification algorithm based on discriminant analysis was made by Ben-Israel and colleagues (1998), using *Escherichia coli* strains carrying *lux* genes fused to several stress-responsive gene promoters (including *micF*, *lon*, *fabA*, *katG* and *uspA*). Of the 25 tested compounds, 23 were identified by this strategy in a 3 h procedure. The signature of a binary mixture was predicted by use of the learning data characterizing each toxicant separately and a good correlation ($R^2 \geq 0.85$) was found between the observed and the predicted response patterns.

To evaluate different options for array data analysis we have generated a very large data set using five bioluminescent reporter strains exposed to five model toxicants and to a buffer control. Forty randomly arrayed repeats were carried out in 384-well microtitre plates, and the emitted light was quantified every 5 min for 2 h (Benovici, 2003). The data were then analysed using different mathematical and statistical approaches. Five of the six treatments were identified by an artificial neuron network 30 and 60 min after exposure, while all six were identified by the same method after 120 min. Bayes decision theory and the non-parametric nearest-neighbour technique (Duda and Hart, 1973) were also applied to the collected data (Elad, Magrisso, Belkin, in preparation). Similarly,

classifiers were designed based on the data collected 30, 60 and 120 min after exposure, with the Bayesian classifiers showing the lowest error rate estimates (no more than 2.1% in a leaving-one-out procedure) and zero false negatives. The use of Bayes decision theory in employing the responses of two *E. coli* gene promoters (*nhoA* and *grpE*) to discriminate between two toxicants (nitrogen mustard and potassium cyanide) is exemplified in Fig. 2.

Array platforms

Substrate. Similar to oligonucleotide or protein arrays, cell arrays need to be spotted on a compatible substrate. Numerous materials are available for this purpose, including silicon, glass and various polymers. Silicon, extensively used within the semiconductor field, is an attractive option, as integrated circuit technologies can easily be employed for cell array fabrication (Bolton *et al.*, 2002; Bhattacharya *et al.*, 2007). Popovtzer and colleagues (2005) developed a silicon biochip with 100 nl electrochemical chambers, harbouring genetically engineered *E. coli* cells. Cell arrays can also be generated directly on electrical components, such as photo-diodes, light-emitting diodes or field effect transistors. Micromachining technologies make it possible to tailor the silicon chip to the required topographical specifications, by patterning microfluidic channels, micro-chambers, valves and additional structures (Thorsen *et al.*, 2002; Yoo *et al.*, 2007).

Although well-established, the machining of silicon platforms is relatively complex, time-consuming and expensive. Glass, being highly biocompatible and transparent, is an attractive and cost-effective alternative. Similarly to silicon, it is amenable to the etching of microfluidic channels and chambers (Inoue *et al.*, 2001), as well as to various chemical or physical treatments that modify surface characteristics and cell attachment (Ruckenstein and Li, 2005; Fukuda *et al.*, 2006; Thirumalapura *et al.*, 2006).

Various polymers are widely used to assemble microfluidic channels and other surface structures. Poly(dimethylsiloxane) (PDMS) is often preferred as an array material because of its ease in handling, optical transparency and biocompatibility. Tani and colleagues (2004) reported a three-dimensional microfluidic network system for constructing on-chip bacterial cell bioassays. Microchannels fabricated on the two separate PDMS layers were connected via perforated microwells on the silicon chip to form a three-dimensional microfluidic network. A PDMS maze was used to observe bacterial motion under nutrient depletion to study quorum formation (Park *et al.*, 2003). PDMS channels have been similarly used to study the persistence of bacteria under stress (Balaban *et al.*, 2004), where the proliferation of single cells could be monitored.

Other substrates reported for the support of cell arrays included optical fibre bundles (Biran and Walt, 2002) and

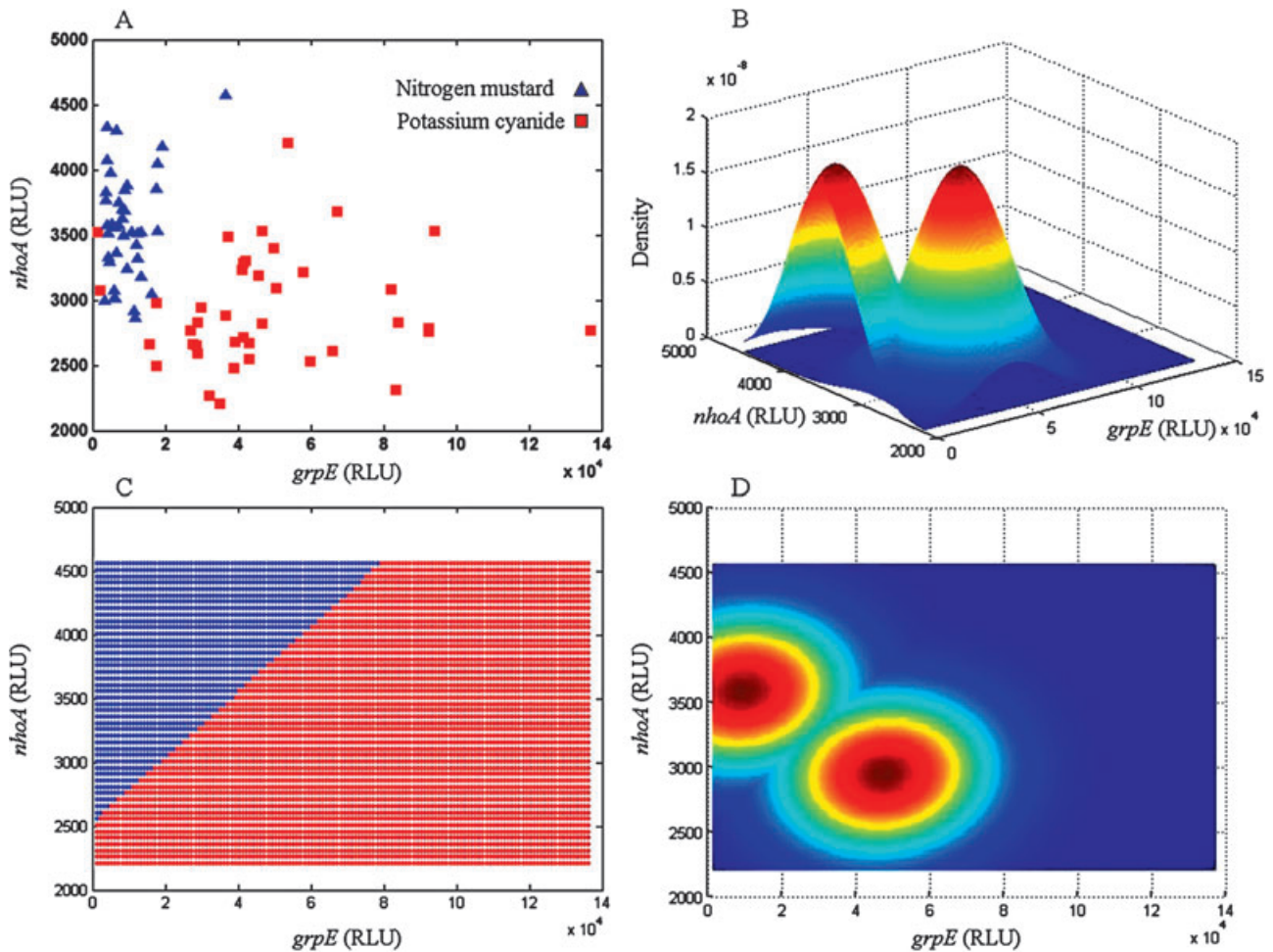


Fig. 2. Bayesian decision boundary for potassium cyanide (red, ■) and nitrogen mustard (blue, ▲) based on the luminescent response of two reporter strains: one harbouring a *grpE'*::*lux* fusion (*x*-axis) and the other a *nhoA'*::*lux* fusion (*y*-axis).

A. Raw data (40 repeats).

B. Estimated state-conditional probability density functions assuming normal distribution and identical covariance matrices.

C. Decision boundary assuming equal *a priori* probabilities.

D. Estimated densities, a look from above.

RLU, bioluminescence intensity, expressed in arbitrary Relative Light Units.

gold surfaces (Choi *et al.*, 2005). In the former case, the responses from individual cells could be measured independently (Kuang *et al.*, 2004). In the latter, cells were immobilized on gold plates using self-assembled cysteine-terminated synthetic oligopeptides. An interesting tack towards a broad use of cell array biochips was pursued by Ingham and colleagues (2007). The researchers have fabricated a miniaturized, disposable microbial culture chip, a 'micro-Petri dish', by micro-engineering growth compartments on top of porous aluminum oxide (PAO). The chip, placed on nutrient agar, acts as the surface on which an exceptional number of microbial samples (up to one million wells per 8×36 mm chip) can be grown, assayed and recovered.

Techniques for cell array deposition. Several approaches have been proposed and demonstrated for arraying the cells in the required pattern. Flickinger and colleagues (2007) formulated reactive microbial inks; piezo tips were used to generate ink-jet deposited *pmerR*::*lux E. coli* dot arrays using a latex ink formulation. Fesenko and colleagues (2005a) suggested the fabrication of an acrylamide-based hydrogel bacterial microchip with an array of hemispherical gel elements, 0.3–60 nl in volume, attached to a hydrophobic glass surface and containing immobilized microbial cells. Both Fesenko and colleagues (2005a) and Thirumalapura and colleagues (2006) have used arrayers originally destined for DNA array fabrication.

Boland and co-workers developed a method for fabricating bacterial colony arrays on soy agar using commercially available ink-jet printers (Xu *et al.*, 2004). Barron and colleagues (2004) used a laser-based printing method to transfer genetically modified bioreporters bacteria onto agar-coated slides. Both authors describe how the developed technique enables to fabricate an array consisting of different bacterial strains.

Another recently proposed methodology for the construction of bacterial arrays is microcontact printing. The utilization of PDMS stamps for printing an *E. coli* array on agarose was demonstrated by Xu and colleagues (2007). Taking a somewhat different approach, Weibel and colleagues (2005) described the use of microcontact agarose stamps prepared by molding against PDMS masters to print bacterial colony arrays on agar plates. Alternatively, microcontact printing of an adhesive organic monolayer was used in a four-step soft lithography process to fabricate 12 µm square bacteria 'corrals' on silicon wafer substrate (Rowan *et al.*, 2002).

Cell immobilization, maintenance of viability and long-term storage

The live cell 'spots' on the array surface need to be deposited in such a manner that will not only place the cells in the appropriate pattern in relation to each other and to the sensing device's signal transducer, but will also allow long-term cell preservation. Various approaches for viability and activity maintenance of live reporter cells over prolonged periods of time for environmental monitoring and toxicity assessment have been reviewed by Bjerketorp and colleagues (2006). Reported solutions include freeze/vacuum drying (Ulitzur *et al.*, 2002; Stocker *et al.*, 2003; Pedahzur *et al.*, 2004) as well as cell encapsulation and entrapment in a large variety of polymers. Methods used for the fabrication of bacterial cell arrays (mostly of genetically engineered bioluminescent reporters) included agar (Lee *et al.*, 2005; 2007), agarose (Tani *et al.*, 2004), alginate (Fesenko *et al.*, 2005b), latex (Flickinger *et al.*, 2007), polyacrylamide (Fesenko *et al.*, 2005a) and carrageenan (Held *et al.*, 2002). Noteworthy is the work of Akselrod and colleagues (2006), who, following the pioneering work of Ashkin and co-workers in optical force-based particle manipulation (Ashkin and Dziedzic, 1987; Ashkin *et al.*, 1987), have assembled microarrays of living bacterial cells in a polyethylene glycol diacrylate (PEGDA) hydrogel with optical traps. Using PEGDA as a scaffold to support the optically organized arrays and fix the position of the cells, a 5 × 5 two-dimensional array of *E. coli* was formed in the hydrogel and cell viability after 43 h was confirmed by *gfp* induction. An entirely different approach to on-chip long-term cell viability maintenance can be inferred from Balagaddé and colleagues (2005). A

chip-based bioreactor that uses microfluidic plumbing networks to actively prevent biofilm formation was created. The device allows steady-state growth in six independent 16 nl reactors which serve as 'microchemostats' and enable long-term culture.

Only a few of the reports referred to above have investigated long-term cell viability maintenance as well as the ability to store the fabricated array for long periods. Recombinant bioluminescent *E. coli* responsive to nalidixic acid and laser-printed on agar-coated slides maintained their activity after shipment at ambient temperature followed by storage for up to 2 weeks at 4°C (Barron *et al.*, 2004). Furthermore, it was indicated that the orifice-free aspect of this laser procedure may be useful in the design of an off-the-shelf bacterial biosensor that can be stored without loss of activity, as it allows for transfer of lyophilized bacteria. The active sensor lifetime and the shelf lifetime of an optical imaging fibre-based live bacterial cell array biosensor were investigated as well (Kuang *et al.*, 2004). The sensors retained their sensing ability for at least 6 h when stored in an ambient environment and demonstrated a shelf life of 2 weeks at 4°C.

Regardless of the inherent analytical qualities of any live cell array and of its performance when freshly deposited, future implementations of such arrays will depend on successful long-term storage and viability maintenance. With this objective in mind, the results of the studies summarized in this section, while providing an improvement over earlier reports, are nevertheless unsatisfactory. Future studies will need to address this essential aspect of any future whole-cell sensor array; possibly one of the more promising avenues of research will focus on the synthesis and formulations of new immobilization matrices (Bjerketorp *et al.*, 2006). Another promising approach may be in the testing of new osmo- and cryo-protectants, including novel compounds isolated from highly desiccation- or freeze-resistant organisms.

Enhancement of array sensitivity

One of the drawbacks inherent in the array format is that the signal emanating from the small amount of the cells that can be concentrated in microlitre- or even nanolitre-size spots may be very low. An increase in signal intensity would allow more sensitive analyte detection, as well as the use of simpler (and thus cheaper) detectors.

Enhancement of signal intensity, or modifications in response sensitivity and the timing of its onset, can be achieved by genetic manipulations of either the promoter region, the reporter gene(s) or the host cell. Insights as to possible avenues for modifying reporter specificities were provided by Galvao and de Lorenzo (2006); considerations for performance optimization were elegantly outlined by van der Meer and colleagues (2004) as well as by

Table 1. Cell array biochips and technologies used for toxicity assessment.

Name	Signal	Substrate	Immobilization matrix	Type	Reference
Optical imaging fibre-based cell array	Light (F)	Fibre optic	Suspension	Array	Kuang <i>et al.</i> (2004)
On-chip whole-cell bioassay	Light (B)	Silicon	Agarose	Microfluidic	Tani <i>et al.</i> (2004)
Cell array biosensor	Light (B)	Plastic	Agar	Array	Lee <i>et al.</i> (2005)
Electrochemical nano-biochip	Electrical current	Silicon	Suspension	Array	Popovtzer <i>et al.</i> (2005)
LuxArray	Light (B)	Nylon membrane	Agar	Array	Van Dyk <i>et al.</i> (2001)
Hydrogel bacterial microchip (HBMChip)	Light (F)	Glass	Acrylamide	Array	Fesenko <i>et al.</i> (2005a)

F, fluorescence; B, bioluminescence.

Marqués and colleagues (2006). Pathways for achieving enhanced sensitivity in optical signal acquisition, down to single-molecule detection, were demonstrated by Wells and colleagues (2005, 2006). Mitchell and colleagues (2005) demonstrated an enhancement of biosensor capability by modifying the origin of reporter genes. For example, according to their work, *E. coli* strains carrying fusions of selected oxidative stress-responsive promoters to *Photobacterium luminescens lux* showed higher bioluminescent levels than strains carrying the same promoters fused to the *luxCDABE* genes from *V. fischeri*, while the sensitivities of the strains were similar, regardless of the luciferase used. A substrate-specific approach to electrochemical signal amplification was reported by Neufeld and colleagues (2006); *p*-aminophenol, the end-product of the activity of the β -galactosidase reporter enzyme, acted as an activator of the sensor element used in that construct, *fabA*.

Applications of bacterial cell arrays

While the envisaged applications of live whole-cell arrays are numerous, many of the current reports either address environmental applications or test the effect of environmentally relevant chemicals. Most prominent among these are the attempts to use the arrays as tools in toxicity testing. Standard approaches to toxicity bioassays centre around the quantification of the negative effects of the tested sample on a test organism population. Originally based on the use of live organisms, recent years have seen a shift to cellular and subcellular alternatives; particularly attractive in this respect is the possibility of genetically tailoring microorganisms to respond to specific sets of toxic chemicals (Belkin, 2003). Microbial cell arrays are a logical step forward: a panel of genetically engineered microorganisms, each modified to respond to a different class of chemicals, and together covering a broad range of potential toxic effects. In each of the panel members, different gene cascades are elicited in response to different stress factors and different biochemical responses are expressed (Phadtare *et al.*, 1999; Storz and Imlay, 1999; Yura and Nakahigashi, 1999; Workman *et al.*, 2006). The use of such a strain panel for multiplexing toxicity analysis has been proposed by several authors (Belkin *et al.*, 1997;

Ahn *et al.*, 2004; Galluzzi and Karp, 2006; Ron, 2007). Published reports on cell arrays designed for toxicity assessment purposes are listed in Table 1.

Biran and Walt (2002) introduced a high-density ordered array of single cells, individually addressed, occupying thousands of microwells etched on the distal end of an optical imaging fibre. In two subsequent studies, Walt and co-workers have detected mercury (Biran *et al.*, 2003) and genotoxins (Kuang *et al.*, 2004) using such cell arrays. In the first study, a genetically modified *E. coli* strain, containing the *lacZ* reporter gene fused to the heavy metal-responsive gene promoter *zntA*, was used to fabricate a mercury biosensor. Single-cell *lacZ* expression was measured when the array was exposed to mercury and a response to 20 ng ml⁻¹ Hg²⁺ could be detected after 1 h. In the second study, mitomycin C at similar concentrations was detected by *E. coli* cells carrying a *recA*::*gfp* fusion after 90 min. The researchers have further demonstrated an optical decoding system for monitoring the location of each randomly dispersed individual cell (Biran and Walt, 2002).

Tani *et al.* (2004) presented an on-chip format for high-throughput whole-cell bioassays. Using two multichannel layers and one microwell array chip, the interactions between various types of samples and strains could be monitored in one assembly in a combinatorial fashion. The operation of the array was exemplified by the detection of mitomycin C at concentrations down to 0.02 mg l⁻¹ with *E. coli* harbouring a *umuD*::*lac* fusion.

Lee and colleagues (2005) used a standard 384-well plate and a 96-well acryl chip as platforms for the development of two biosensor arrays. Twenty recombinant bioluminescent bacteria, harbouring different promoters fused to bacterial *lux* genes, were deposited in the wells of either the chip or the 384-well plate after agar immobilization and the responses from the cell arrays were characterized using three chemicals that cause either superoxide damage (paraquat), DNA damage (mitomycin C) or protein/membrane damage (salicylic acid); only 2 h was needed for analysis. On the same acryl chip platform, Lee and colleagues (2007) have fabricated an oxidative stress-specific cell array. The chip consisted of 12 agar-immobilized bioluminescent strains, each responsive to a different type of oxidative stress. Array performance,

tested with nine chemicals, displayed the desired selectivity: not only did the array respond to paraquat and four of its radical-producing structural analogues, but also exhibited different response patterns to each of the five substances.

The use of an electrode platform for toxicity detection was demonstrated by Popovtzer and colleagues (2005), who constructed an electrochemical biochip for water toxicity detection. A clear electrical signal was produced following the exposure of recombinant *E. coli* reporter strains to ethanol (6%) or phenol (1.6 mg l⁻¹). Similarly, Held and colleagues (2002) described a microbial biosensor array electrode platform, integrated in a flow-injection system, for mono- and disaccharide determination. Transport mutants of *E. coli* were immobilized in carrageenan in front of an O₂-sensing gold electrode; selectivity was endowed by the specific sensitivity of each mutant to a different sugar.

Several recent reports have presented the construction of single-strain arrays that can also be adapted to the assembly of multigenotype patterns. Flickinger and colleagues (2007), using ink-jet technology, applied latex ink formulation to deposit *pmerR::lux E. coli* dot arrays for high-throughput microbial toxicity screening. Similarly to a previous report (Biran *et al.*, 2003), luminescence was induced with 20 ng ml⁻¹ Hg²⁺, and the array responded within 1 h. Mercury was also detected with similar immobilized or non-immobilized constructs at lower concentrations, down to the ng/L scale, albeit not in an array format (Lyngberg *et al.*, 1999; Hakkila *et al.*, 2002; Ivask *et al.*, 2007). Also among the environmental applications is the report by Fesenko and colleagues (2005a), who developed an acrylamide-based hydrogel bacterial microchip and investigated two possible application modes: monitoring cell populations and biosensing, the latter illustrated by the detection of sodium meta-arsenite.

For gene expression analysis, Van Dyk *et al.* (2001) – among the first to advance the concept of using live cells as array components – described the LuxArray: a high-density nylon membrane print of a set of bioluminescent *E. coli* reporter strains, harbouring functional promoter fusions to *P. luminescens luxCDABE*. For a similar purpose, the same substrate was used to construct a bacterial colony array out of recombinant *E. coli* clones containing plasmid-encoded copies of 4608 individual expressed sequence tags (ESTs) (Barsalobres-Cavallari *et al.*, 2006). A bacterial cell array biochip was also applied to antibody detection (Thirumalapura *et al.*, 2006): Gram-negative and Gram-positive strains were deposited on nitrocellulose-coated glass substrates by a microarray printer. Antibody recognition events were identified using a microarray scanner, at a sensitivity of 0.1 µg ml⁻¹. In another proposed application, an array biochip was devel-

oped in which recombinant bacteria expressing specific surface capture proteins were spatially arrayed in microfluidic channels by dielectrophoresis, to detect protein molecules that are difficult to purify and immobilize (Oh *et al.*, 2006).

Concluding remarks and future outlook

Recent advances in array technologies on the one hand, and the coming of age of whole-cell biosensors on the other hand, have prepared the ground for the next step in the evolution of both disciplines – the whole-cell array. As indicated in the previous section, envisaged applications for such arrays are numerous; nevertheless, it should be clearly stated that to date these applications have yet to be implemented outside the walls of the research laboratory. The present review, which highlights the state-of-the-art in different disciplines essential for a functional bacterial array, also serves to bring forward the numerous hurdles which need to be passed before the technology matures. Possibly the most urgent need is to dramatically improve maintenance of cell activity and viability over prolonged periods after array fabrication; this challenge has hardly been addressed to date. Also essential are an improved arsenal of reporter strains, better methodologies for incorporation of such cells into the hardware platforms, development of appropriate detection circuits and the availability of dedicated algorithms for multiplex signal analysis. As the paths to all of these objectives are relatively straightforward, it is tempting to envisage how, within a relatively short period, microbial cell arrays may turn into efficient tools for basic microbiological studies as well as for industrial high-throughput chemical/pharmaceutical screening applications, environmental monitoring and food safety.

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