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Living Bacterial Cell Array for Genotoxin Monitoring

Yina Kuang, Israel Biran, and David R. Walt*

Department of Chemistry, Tufts University, 62 Talbot Avenue, Medford, Massachusetts 02155

A biosensor composed of a high-density living bacterial cell array was fabricated by inserting bacteria into a microwell array formed on one end of an imaging fiber bundle. The size of each microwell allows only one cell to occupy each well. In this biosensor, *E. coli* cells carrying a *recA::gfp* fusion were used as sensing components for genotoxin detection. Each fiber in the array has its own light pathway, enabling thousands of individual cell responses to be monitored simultaneously with both spatial and temporal resolution. The biosensor was capable of performing cell-based functional sensing of a genotoxin with high sensitivity and short incubation times (1 ng/mL mitomycin C after 90 min). Dose−response curves for several genotoxins were obtained. The biosensors demonstrated an active sensing lifetime of more than 6 h and a shelf lifetime of two weeks.

To assess environmental threats, living organism-based assays and biosensors have advantages over conventional chemical or biochemical technologies because they can perform functional sensing. While most chemical sensors respond only to molecular binding, living organism-based biosensors can provide functional measurements such as bioavailability, genotoxicity, or general toxicity.[1,2] Several living animal tests have been used for environmental monitoring, such as the canary test for suffocation,[1] the rabbit eye test for irritants,[3] and the fish test for general toxicity.[4] With advances in understanding cellular and subcellular systems, a significant amount of research has been conducted in attempts to replace these animal-based methods with living cell-based biosensors for monitoring the quality of various samples such as groundwaters or surface waters or the activity of new drug candidates. Living cell biosensors are devices in which live cells, acting as the sensing component, are coupled to a transducer that converts the cell responses into readable signals. A variety of cell types, such as neurons, cardiomyocytes, immune cells, hepatocytes, yeasts, bacteria, and viruses, have been used previously to fabricate living cell-based biosensors.[2,5−7]

Among these living cell systems, bacteria are especially attractive due to their rapid growth rates, low cost, and easy handling. In addition, bacteria are versatile in that there are well-developed techniques for genetically manipulating them to expand their responses. For example, bacterial cells can be genetically engineered to contain a sensing−reporting construct such as a promoter−reporter fusion. In such a construct, a reporter gene, referred to as the reporter element, is fused downstream to an adjacent, inducible gene promoter, called the sensing element. The promoter−reporter fusion is then inserted into the host bacteria. When these transformed cells are exposed to a solution containing the inducer molecule, transcription of the adjacent reporter gene is activated. The reporter protein can be detected by a variety of methods including fluorescence,[8−11] luminescence,[12−22] colorimetry,[23,24] or electrochemistry.[25]

There are several technical challenges with cell-based biosensors. For example, living cell-based biosensors exhibit a significant cell-to-cell variation. In addition, cell activities are affected by environmental factors such as temperature, pH, and toxicity.

* Corresponding author: (e-mail) david.walt@tufts.edu; (phone) 617-627- 3470; (fax) 617- 627-3443.

(1) McFadden, P. *Science* **2002**, *297*, 2075−2076.
(2) Belkin, S. *Curr. Opin. Microbiol.* **2003**, *6*, 206−212.
(3) York, M.; Steiling, W. *J. Appl. Toxicol.* **1998**, *18*, 233−240.
(4) Kolarova, I. *Fresenius Environ. Bull.* **2003**, *12*, 848−851.
(5) Karlsson, A. M.; Bjuhr, K.; Testorf, M.; Oberg, P. A.; Lerner, E.; Lundstrom, I.; Svensson, S. P. S. *Biosens. Bioelectron.* **2002**, *17*, 331−335.
(6) Stenger, D. A.; Gross, G. W.; Keefer, E. W.; Shaffer, K. M.; Andreadis, J. D.; Ma, W.; Pancrazio, J. J. *Trends Biotechnol.* **2001**, *19*, 304−309.
(7) Baeumner, A. J. *Anal. Bioanal. Chem.* **2003**, *377*, 434−445.
(8) Kostrzynska, M.; Leung, K. T.; Lee, H.; Trevors, J. T. *J. Microbiol. Methods* **2002**, *48*, 43−51.
(9) Arai, R.; Makita, Y.; Oda, Y.; Nagamune, T. *J. Biosci. Bioeng.* **2001**, *92*, 301−304.
(10) Stiner, L.; Halverson, L. J. *Appl. Environ. Microbiol.* **2002**, *68*, 1962−1971.
(11) Roberto, F. F.; Barnes, J. M.; Bruhn, D. F. *Talanta* **2002**, *58*, 181−188.
(12) Ptitsyn, L. R.; Horneck, G.; Komova, O.; Kozubek, S.; Krasavin, E. A.; Bonev, M.; Rettberg, P. *Appl. Environ. Microbiol.* **1997**, *63*, 4377−4384.
(13) Davidov, Y.; Rozen, R.; Smulski, D. R.; Van Dyk, T. K.; Vollmer, A. C.; Elsemore, D. A.; LaRossa, R. A.; Belkin, S. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2000**, *466*, 97−107.
(14) Polyak, B.; Bassis, E.; Novodvorets, A.; Belkin, S.; Marks, R. S. *Sens. Actuators, B* **2001**, *74*, 18−26.
(15) Choi, S. H.; Gu, M. B. *Biosens. Bioelectron.* **2002**, *17*, 433−440.
(16) Min, J.; Pham, C. H.; Gu, M. B. *Environ. Toxicol. Chem.* **2003**, *22*, 233−238.
(17) Belkin, S.; Smulski, D. R.; Vollmer, A. C.; VanDyk, T. K.; LaRossa, R. A. *Appl. Environ. Microbiol.* **1996**, *62*, 2252−2256.
(18) Rupani, S. P.; Gu, M. R.; Konstantinov, K. B.; Dhurjati, P. S.; VanDyk, T. K.; LaRossa, R. A. *Biotechnol. Prog.* **1996**, *12*, 387−392.
(19) Vollmer, A. C.; Belkin, S.; Smulski, D. R.; VanDyk, T. K.; LaRossa, R. A. *Appl. Environ. Microbiol.* **1997**, *63*, 2566−2571.
(20) Bechor, O.; Smulski, D. R.; Van Dyk, T. K.; LaRossa, R. A.; Belkin, S. *J. Biotechnol.* **2002**, *94*, 125−132.
(21) Turner, N. L.; Horsburgh, A.; Paton, G. I.; Killham, K.; Meharg, A.; Primrose, S.; Strachan, N. J. C. *Environ. Toxicol. Chem.* **2001**, *20*, 2456−2461.
(22) Weitz, H. J.; Ritchie, J. M.; Bailey, D. A.; Horsburgh, A. M.; Killham, K.; Glover, L. A. *FEMS Microbiol. Lett.* **2001**, *197*, 159−165.
(23) Guan, X. Y.; Daunert, S.; D'Angelo, E. M. *Abstr. Pap. Am. Chem. Soc.* **2002**, *223*, 152-ANYL.
(24) Serebriiskii, I. G.; Toby, G. G.; Golemis, E. A. *Biotechniques* **2000**, *29*, 278.
(25) Biran, I.; Babai, R.; Levcov, K.; Rishpon, J.; Ron, E. Z. *Environ. Microbiol.* **2000**, *2*, 285−290.

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Another challenge is maintaining cell viability; once cells are immobilized to the transducer (e.g., electrode or optical fiber), they must be kept alive and functional over a reasonable period.

Our laboratory has recently developed a new technology in which we fabricate a high-density (2×10^7 sensors/cm^2) living bacterial cell array.[26,27] The basic platform is a 1-mm-diameter imaging fiber bundle containing ~50 000 individual optical fibers. Each optical fiber in the bundle has its own independent light pathway. One end of the fiber bundle is chemically etched to form a high-density microwell array.[28] The width of the microwells is determined by the diameter of the optical fiber core material (3.1 μm), and the depth is determined by the etching time. Living bacteria are randomly inserted into the microwells to form a high-density cell array. Fluorescent signals from individual cells at the distal end of the fiber can be transmitted[29] through the imaging fiber and captured by a CCD camera located at the proximal end of the fiber bundle. Each microwell is sized such that it holds only one cell, and since each fiber has its own light pathway, thousands of individual cells can be monitored simultaneously with both spatial and temporal resolution.

In this study, we employed this high-density living bacterial cell array for genotoxin detection. *Escherichia coli* cells carrying a *recA::gfp* fusion plasmid were used. This sensing mechanism is based on the bacterial SOS response system. In *E. coli* cells, *recA* gene transcription is induced upon DNA damage including the formation of pyrimidine dimers, cross-linked strands, and quinolone antibiotic-induced breaks in DNA.[30] The resultant RecA protein mediates the self-cleavage of LexA protein, leading to the induction of the SOS regulon involved in DNA damage tolerance and error-prone replication. GFPmut2 protein was used as the reporter protein.[31] This reporter protein is a variant of GFP isolated and cloned from the jellyfish *Aequorea victoria*,[32] and it has been optimized for expression in *E. coli* to provide maximal sensitivity. Compared with the LacZ reporter protein used in our previous study,[27] the GFP protein has higher stability, has higher quantum yield, and does not require the addition of a substrate or cofactors since it is intrinsically fluorescent.[33,34] As a result, the biosensor fabrication and measurement procedures are simple, making this living cell array biosensor suitable for potential monitoring applications.

EXPERIMENTAL SECTION

Unless stated otherwise, all chemicals were obtained from Sigma (St. Louis, MO).

Strain and Media. *E. coli* strain MG1655+pUA2699 (host strain MG1655 transformed with a low copy number *recA::gfpmut2* fusion plasmid pUA2699),[35] received from Prof. Uri Alon, Department of Molecular Cell Biology and Department of Physics of Complex Systems, Weizmann Institute of Science, Israel, was incubated overnight in M9 minimal medium (Becton Dickinson, Le Pont de Claix, France) supplemented with 50 μg/mL kanamycin sulfate (Fisher Scientific, Fair Lawn, NJ) at 37 °C in an incubator shaker (New Brunswick Scientific, Edison, NJ). Fresh culture was prepared by diluting the overnight culture 1:50 and incubating at 37 °C until the OD_{600} reached 0.1 (Beckman DU 530 Life Science UV/VIS spectrophotometer, Beckman Coulter, Inc., Fullerton, CA).

Microtiter Plate Assay. Aliquots of fresh cell culture were distributed into wells of a microtiter plate. Different concentrations of the following genotoxins were added in triplicate to different wells: mitomycin C (MMC), *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), hydrogen peroxide, nalidixic acid (NA), formaldehyde (FA) (Fisher Scientific, Pittsburgh, PA) in M9, or M9 (control). The final volume in each well was adjusted to 100 μL. Fluorescence intensity (excitation at 480 nm and emission at 520 nm) was measured by a Gemini microplate spectrophotometer (Molecular Devices, Sunnyvale, CA) every 5 min for 90 min. Fluorescence intensity was recorded with arbitrary units and expressed as fluorescence intensity increase $(I_t - I_0)/I_0$ (I_t denotes the fluorescence intensity at time t; I_0 denotes the fluorescence intensity before applying the genotoxins) to compensate for the variations in the starting cell number in each well and for environmental or instrumental variations.

Imaging Fiber-Based Living Cell Array Fabrication. The etched end of an imaging fiber bundle containing 3.1-μm microwells (Illumina, San Diego, CA) was covered with a thin layer of polyethylenimine by applying 1% polyethylenimine solution and allowing the solution to dry. A 10-mm-length polyurethane tube with a 1-mm inner diameter (Small Parts Inc., Miami Lakes, FL) was attached to the etched end of the fiber to form a microvial in which the etched end of the fiber serves as the bottom. Aliquots (10 μL) of 1:100 cell dilutions from fresh cultures were loaded into the microvial, and the fiber was horizontally held onto a centrifuge (IEC Micromax centrifuge, IEC International Equipment Co., Needham Heights, MA). Centrifugation was performed at 4000 rpm for 2 min, causing the cells to deposit into the individual microwells. The supernatant was then removed with a pipet. M9 medium with or without genotoxin, MMC or MNNG, was immediately added to the microvial.

Measurements and Data Analysis. The imaging fiber-based living bacterial cell array was mounted on an epifluorescence microscope (model BX61, Olympus America Inc., Melville, NY) and focused on the proximal end of the fiber. A charge-coupled device (CCD) camera (Hamamatsu Orca-ER) acquired the signals through an objective lens located at the proximal end of the optical fiber (Figure 2). Fluorescent signals from individual cells at the distal end of the fiber were transmitted through the imaging fiber to the proximal end. The fluorescence intensity was analyzed by IPLab software (Scanalytics, Fairfax, VA) and displayed as arbitrary units.

Fluorescent signals (excitation at 480 nm and emission at 520 nm) from individual cells were measured with a 500-ms acquisition

(26) Biran, I.; Walt, D. R. *Anal. Chem.* **2002**, *74*, 3046−3054.
(27) Biran, I.; Rissin, D. M.; Ron, E. Z.; Walt, D. R. *Anal. Biochem.* **2003**, *315*, 106−113.
(28) Pantano, P.; Walt, D. R. *Chem. Mater.* **1996**, *8*, 2832−2835.
(29) Epstein, J. R.; Walt, D. R. *Chem. Soc. Rev.* **2003**, *32*, 203-214.
(30) Garrett, R. H.; Grisham, C. M. *Biochemistry*, 2nd ed.; Saunders College Pub.: Fort Worth, TX, 1999.
(31) Cormack, B. P.; Valdivia, R. H.; Falkow, S. *Gene* **1996**, *173*, 33−38.
(32) Kain, S. R.; Adams, M.; Kondepudi, A.; Yang, T. T.; Ward, W. W.; Kitts, P. *Biotechniques* **1995**, *19*, 650−655.
(33) Yang, T. T.; Cheng, L. Z.; Kain, S. R. *Nucleic Acids Res.* **1996**, *24*, 4592−4593.
(34) Kohler, S.; Belkin, S.; Schmid, R. D. *Fresenius J. Anal. Chem.* **2000**, *366*, 769−779.
(35) Ronen, M.; Rosenberg, R.; Shraiman, B. I.; Alon, U. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10555−10560.

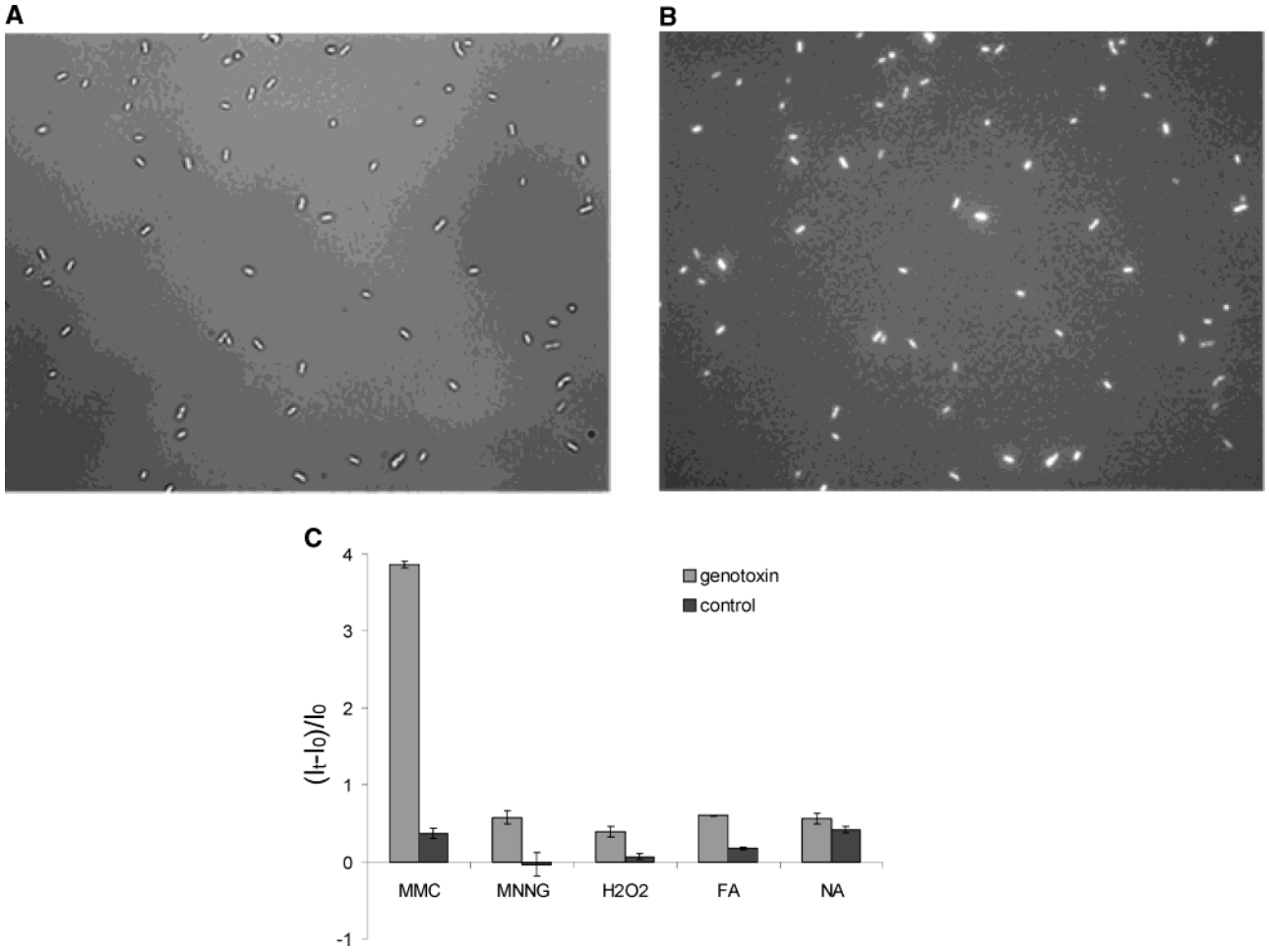

Figure 1. Visualizing GFP expression from *E. coli* MG1655+pUA2699 cells. Cells were treated with 0.1 mg/mL FA for 90 min and placed on a microscope slide. (A) White light image. (B) Fluorescent image of the same cells at 480 nm/520 nm excitation/emission wavelengths. (C) Microtiter assays of *E. coli* MG1655+pUA2699 cell responses to genotoxins. Cells were incubated with M9 medium containing 10 μg/mL MMC, MNNG, H_2O_2, FA, and NA for 90 min. Fluorescent signal was expressed as fluorescence intensity increase $(I_t - I_0)/I_0$.

time immediately after exposing the cell array to M9 medium containing MMC, MNNG, or only M9 medium as the control. Fluorescent signals were measured every 5 min for 90 min. Cell locations in the array were determined by the GFP fluorescence intensities that were higher than the background fluorescence level from empty wells. Some cells had very low background fluorescence so their locations were determined after the cells were induced instead of using background fluorescence. Cell responses were expressed as a fluorescence intensity increase $(I_t - I_0)/I_0$. One hundred cells with the highest intensity increases were analyzed.

RESULTS AND DISCUSSION

***E. coli* MG1655+pUA2699 Strain Genotoxin Detection Capabilities.** Before fabricating the imaging fiber-based living bacterial cell array for genotoxin detection, *E. coli* cells containing the *recA::gfp* fusion plasmid were tested using standard microscopy methods and microtiter plate assays. In our initial experiments, freshly cultured cells had more uniform responses than overnight-cultured cells (data not shown). As a result, freshly cultured cells in the early exponential stage (with OD_{600} ~0.1) were used in all experiments.

To visualize the expression of GFP protein in *E. coli* cells, FA was used initially as the genotoxin to induce gene expression. FA is a ubiquitous environmental contaminant and its genotoxic effect has been demonstrated in bacteria since 1973.[36] The fluorescent signals from exposed cells were observed by placing the cells on a microscope slide. Figure 1 shows both the white light image (Figure 1A) and the fluorescence image (Figure 1B) of the same cells after a 90-min incubation with 0.1 mg/mL FA. Cells incubated with FA had much higher fluorescence intensities compared to those in control experiments in which cells were incubated with only M9 medium (data not shown). All cells exposed to FA showed a relatively high and stable fluorescence, with no dead or nonresponsive cells. This experiment indicates that *E. coli* strain MG1655+pUA2699 can effectively respond to the presence of the genotoxin FA.

To check whether this *E. coli* strain could respond to other known genotoxins, a microtiter assay was performed. Figure 1C shows the results from such an assay, in which aliquots of cells were exposed to 10 μg/mL MMC, MNNG, H_2O_2, FA, and NA for 90 min. *E. coli* cells showed different fluorescence intensity increases with these different genotoxins. In all cases, the fluorescence intensity increase was higher than the control experiments. The degree of the fluorescence intensity increase probably corresponds to the genotoxic potential of each com-

(36) Nishioka, H. *Mutat. Res.* **1973**, *17*, 261−265.

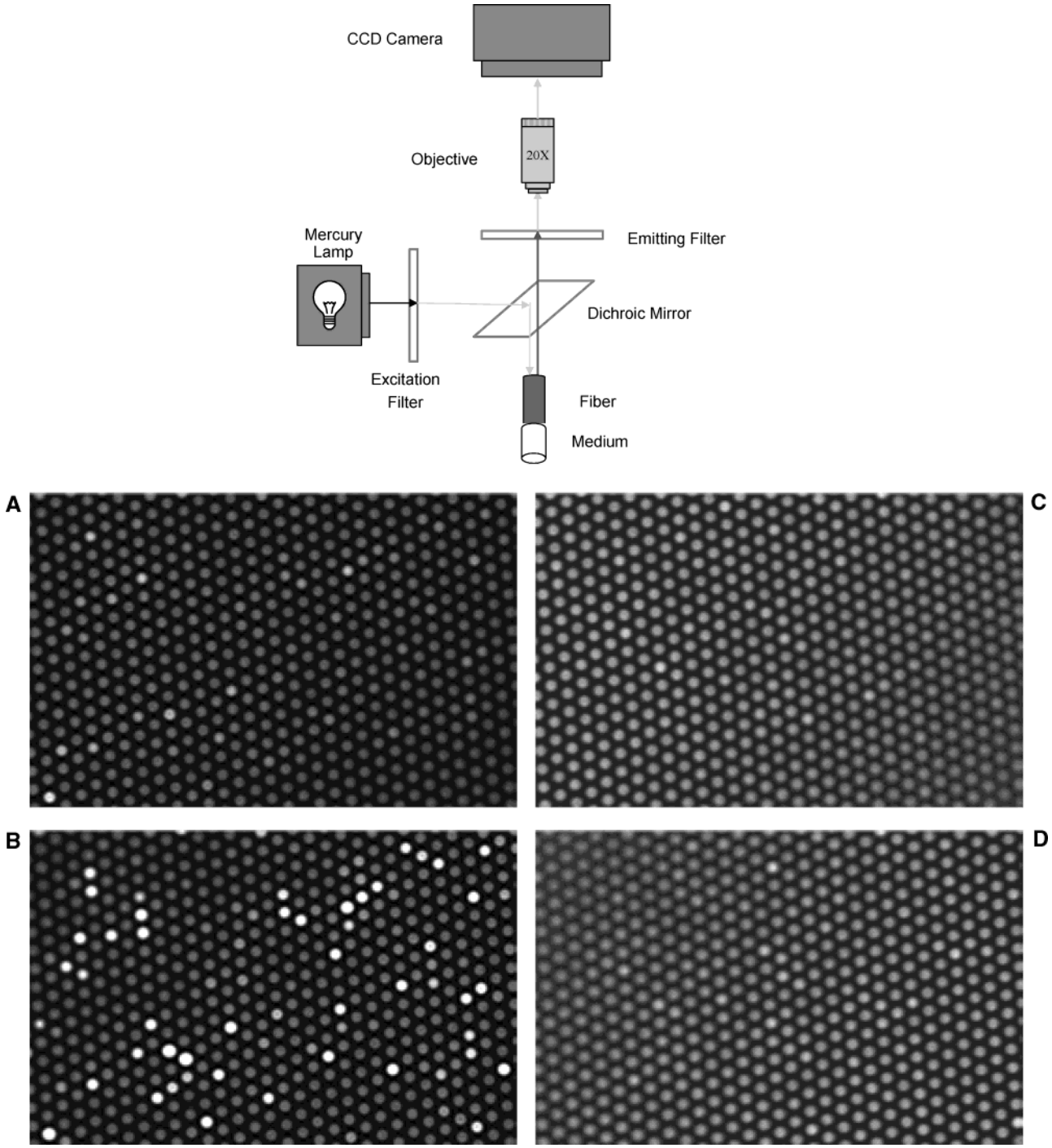

Figure 2. Single-cell array biosensor platform setup and fluorescent images from a small portion of two imaging fiber-based arrays. The left panel shows an array where cells were incubated with medium containing 5 μg/mL MMC. Fluorescent signals were acquired at (A) $t = 0$ and (B) 90 min. The right panel shows the control array where cells were incubated with only medium. Fluorescent signals were taken at (C) $t = 0$ and (D) 90 min. The bright spots indicate an increase in fluorescence. The fluorescence increases from cells exposed to MMC were much higher than the control.

pound.[12] This experiment demonstrated the generic sensing capabilities of *E. coli* strain MG1655+pUA2699 for genotoxin detection.

Optical Imaging Fiber-Based *E. coli* Cell Array Biosensor. Principle of Operation. *E. coli* cells were prepared and loaded onto the etched end of an imaging optical fiber as described in the Experimental Section. Based on microtiter assay results (Figure 1C), MMC was chosen as the model genotoxin to induce expression of GFP due to its superior gene induction effect on this cell strain compared to the other genotoxins. Figure 2 shows fluorescence images of a small portion of the proximal end of an imaging fiber transmitting signals from cells immobilized in the microwells on the distal end of the fiber. The fluorescence intensities were recorded in arbitrary units and converted into a gray scale image, with the brightest spots corresponding to the highest GFP expression levels. As shown in Figure 2, when a cell array was exposed to medium containing 5 μg/mL MMC (Figure 2B), after 90 min the cells exhibited fluorescence intensities that

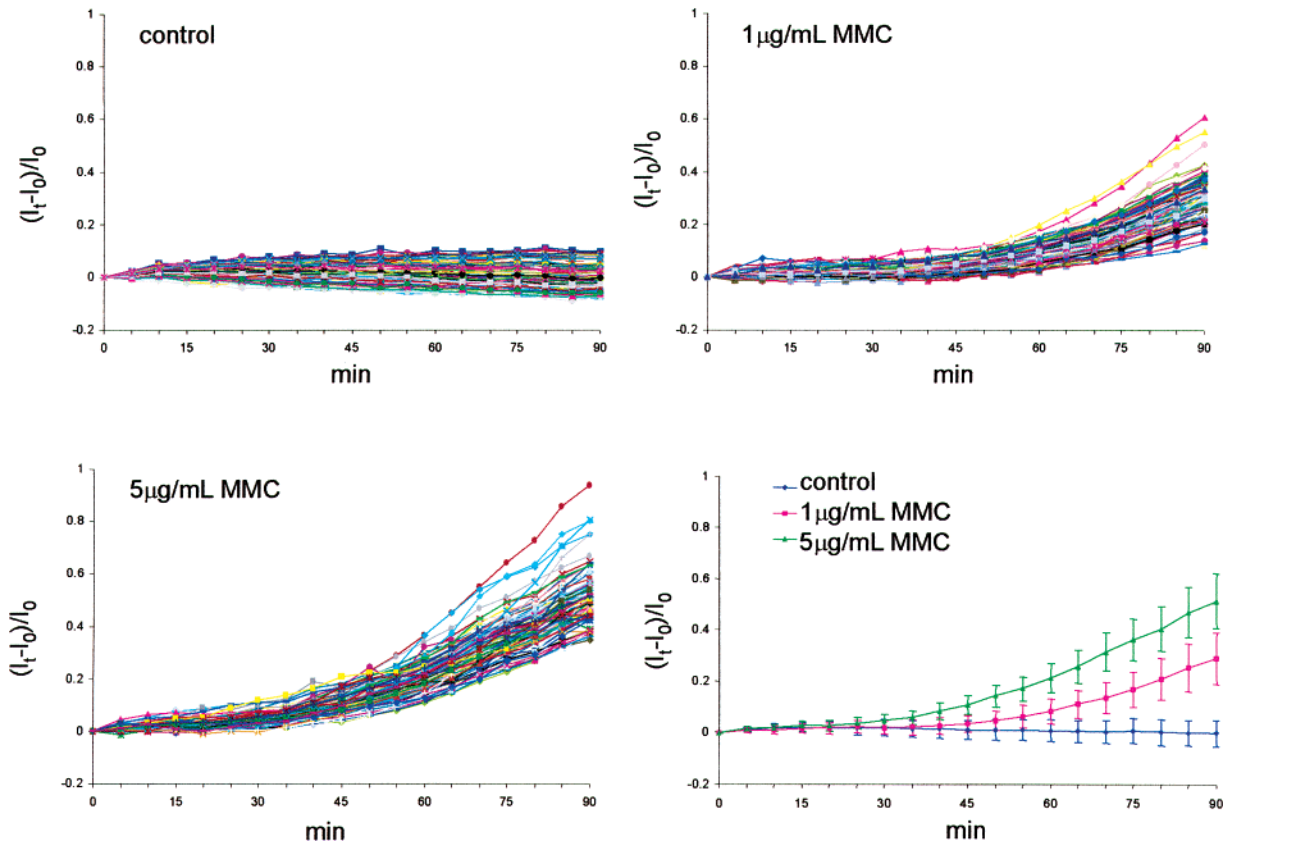

Figure 3. Kinetics of GFP expression induction in single cells. Cells were incubated with M9 medium containing (A) 0, (B) 1, and (C) 5 μg/mL MMC. Fluorescent signals were expressed as a fluorescence intensity increase $(I_t - I_0)/I_0$. The average fluorescence intensity increases from ~100 cells in each experiment are shown in (D).

were much higher than at time 0 (Figure 2A). In a control cell array, where the cells were not exposed to MMC, the fluorescence intensities were similar at time 0 (Figure 2C) and after 90 min (Figure 2D).

Measuring Individual Cell Induction Kinetics. To determine whether the cell arrays could quantify genotoxin concentrations, the arrays were exposed to M9 medium supplemented with different concentrations of MMC. The induction kinetics, defined as fluorescence intensity increase $(I_t - I_0)/I_0$ over time, from thousands of individual cells were simultaneously recorded every 5 min over a 90-min period. Figure 3 shows the induction kinetics of ~100 cells with the highest fluorescence intensity increases at three MMC concentrations (0, 1, and 5 μg/mL MMC). All three experiments were conducted with cells from the same cell culture assembled onto different arrays. These kinetics graphs indicate that the cell responses to the genotoxin MMC were both time and dose dependent. After an initial lag phase, the GFP expression level increased with the incubation time. The length of this initial lag phase was inversely proportional to the genotoxin concentration; with higher MMC concentrations, the time required to obtain a significant response was shorter. As shown in Figure 3, 30 min was required at 5 μg/mL MMC and 45 min at 1 μg/mL MMC. Because cells clearly showed detectable fluorescence signals at 1 μg/mL MMC after a 90-min incubation period, a 90-min response time was used in all the following experiments.

Figure 3A−C shows that all the cells in an array did not uniformly respond to MMC although they were all derived from the same single colony and same culture and were experimentally monitored under the same conditions. Some cells had faster responses or higher GFP expression levels than others. This stochastic gene expression in isogenic cell populations is mainly due to inherent cell-to-cell variations. Such variations have been observed and reported previously[37−39] for both prokaryotic and eukaryotic cells.

To obtain maximum sensitivity, the genotoxin detection was based on the average responses of many individual cells as described in our previous research.[40] Because each single cell works as an individual sensor, by averaging the cell responses from multiple cells, the signal/noise ratio is increased by a coefficient of n,[1/2] where n is the number of cells in the array. In Figure 3D, the average fluorescence intensity increases from Figure 3A−C were used to determine MMC concentrations. As expected, with the same incubation time, the higher the inducer concentration, the higher the expression levels of GFP, which led to higher fluorescence intensities. It is important to note that the GFP expression level in Figure 3D is different from the microtiter plate assay (Figure 1) when MMC was used as the inducer. In the microtiter plate assay, the GFP expression level was obtained by collecting the overall signal from an ensemble of millions of

(37) Attfield, P. V.; Choi, H. Y.; Veal, D. A.; Bell, P. J. L. *Mol. Microbiol.* **2001**, *40*, 1000−1008.
(38) McAdams, H. H.; Arkin, A. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 814−819.
(39) Elowitz, M. B.; Levine, A. J.; Siggia, E. D.; Swain, P. S. *Science* **2002**, *297*, 1183−1186.
(40) Michael, K. L.; Taylor, L. C.; Schultz, S. L.; Walt, D. R. *Anal. Chem.* **1998**, *70*, 1242−1248.

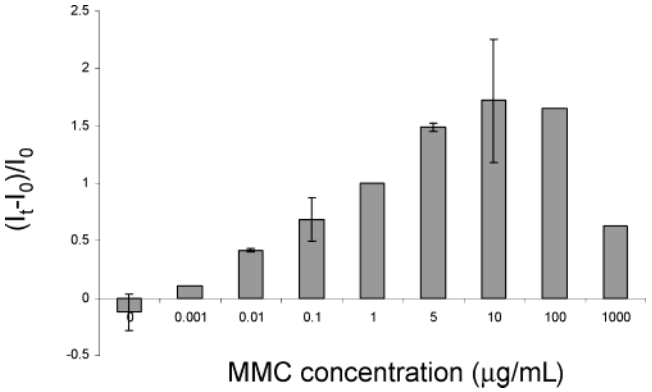

Figure 4. Biosensor dose-dependent response. Average fluorescence intensity increases from cells exposed to M9 medium containing different MMC concentrations were normalized to the average fluorescence intensity increase induced by 1 μg/mL MMC from the same-day assay. Any point with an error bar represents the averages of at least three replicate experiments from different days.

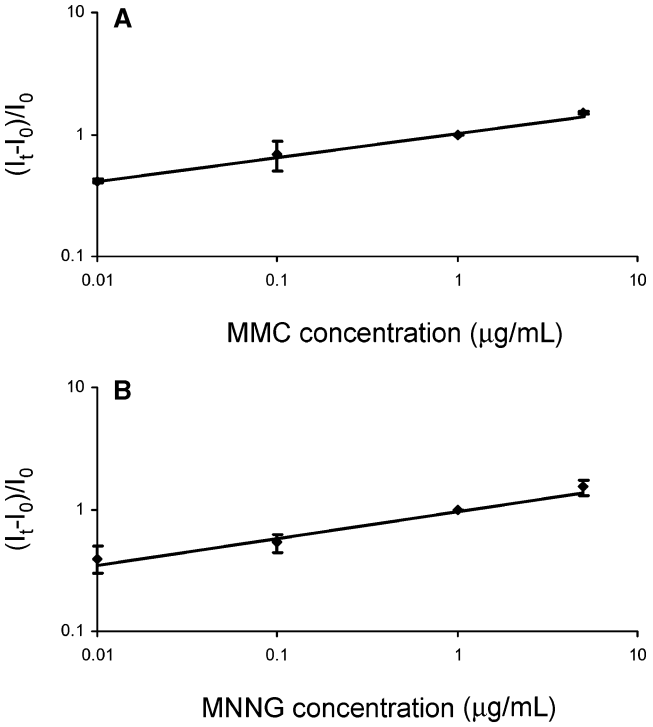

Figure 5. Biosensor linear detection range for MMC and MNNG. Linear dose-dependency relationships were obtained when cell arrays were exposed to (A) MMC or (B) MNNG in the concentration range of 0.01−5 μg/mL.

cells in the microtiter well whereas the results in Figure 3D were obtained by calculating an average signal from the individual signals of many single cells.

Biosensor Calibration. When experiments similar to the one shown in Figure 3 were conducted with cell cultures derived from different cell colonies, the same trend in response pattern was obtained, in which higher concentrations of genotoxin resulted in higher fluorescence signals; however, the actual fluorescence intensities varied (data not shown). This high variability of gene expression can be influenced by parameters such as temperature, pH, or sample matrix composition.[41] These factors may have a direct impact on cell metabolic rates or cell viability and usually fluctuate from day to day.

To examine colony-to-colony or day-to-day variability, we compared GFP expression levels from different experiments conducted on seven different days. Although all the assays were performed using the same MMC concentration (1 μg/mL), the average fluorescence intensity increase $(I_t - I_0)/I_0$ varied from 0.12 to 0.34. It was therefore necessary to develop a methodology to compare experiments conducted on different days using different cell colonies. The average fluorescence intensity increases at different MMC concentrations were normalized to the average fluorescence intensity obtained with 1 μg/mL MMC. More specifically, for arrays derived from the same cell culture and conducted on the same day, the signals obtained from arrays exposed to different MMC concentrations were divided by the average signal from the array exposed to 1 μg/mL MMC. We used this approach to compare results obtained from experiments conducted on different days using different cell cultures (Figure 4). The results showed a clear dose-dependent fluorescence intensity increase up to 10 μg/mL. When the MMC concentration reached 10 μg/mL, however, the fluorescence intensity increase rate was lower. In addition, normalized average responses from different days at this concentration showed a large standard deviation. The 10 μg/mL is probably close to the threshold concentration at which MMC causes direct cytotoxic effects. When the MMC concentration is higher than 10 μg/mL, a direct cytotoxic effect was observed (Figure 4). This cytotoxic effect was also observed when high concentrations of MNNG were used as inducer (data not shown), demonstrating the array's ability to monitor cytotoxic effects in addition to genotoxic effects. Cells can also be transformed to express a second reporter protein[39] or stained with a fluorescent dye that can directly indicate cell viability.[42] This approach is much more convenient and faster than the existing method in which cytotoxic effects are measured by comparing the percent colony forming units of cells before and after being treated with toxicants.[14]

Under lower MMC concentrations (from 0.01 to 5 μg/mL), a linear dose-dependent relationship was obtained (Figure 5A). To further validate this calibration method, another genotoxin, MNNG, was chosen as a *recA* inducer and dose-dependent measurements were performed. With the same data normalization procedure as with MMC, a linear dose-dependent relationship was obtained (Figure 5B). These experiments demonstrate the broad detection capabilities of this optical imaging fiber-based living cell biosensor. To identify specific genotoxins, the same platform potentially could be used with multiple recombinant cell types, with each cell type carrying a unique promoter that senses a specific genotoxin fused to a different fluorescent protein. In this case, the identity of a specific analyte could be obtained by correlating the different strains' response patterns.

The optical imaging fiber-based biosensor system was previously shown to be a highly sensitive platform as was further demonstrated in this study, in which low copy number gene expression was observed at the single-cell level. The detection

(41) Mirasoli, M.; Feliciano, J.; Michelini, E.; Daunert, S.; Roda, A. *Anal. Chem.* **2002**, *74*, 5948−5953.

(42) Boulous, L.; Prevost, M.; Barbeau, B.; Coallier, J.; Desjardins, R. *J. Microbiol. Methods* **1997**, *37*, 77−86.

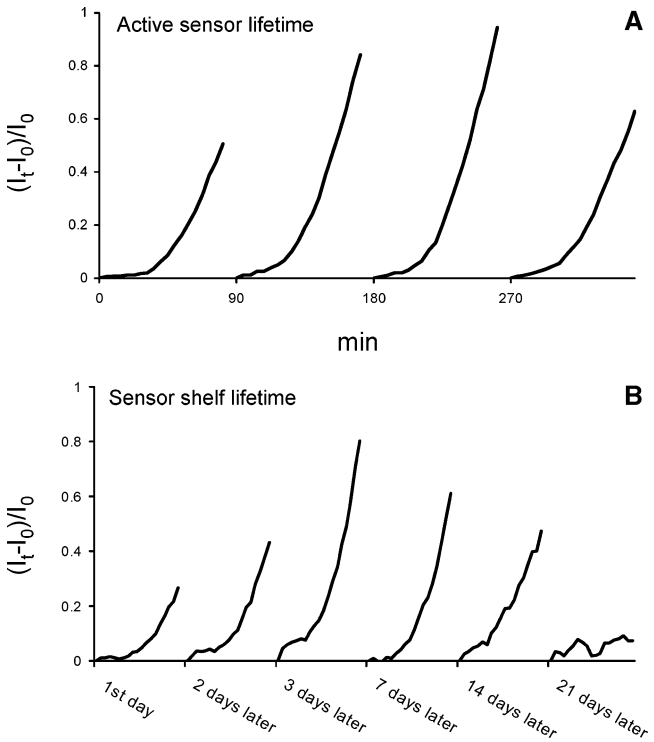

Figure 6. (A) Active sensor lifetime tests. Four sensors were prepared from the same culture and exposed to 10 μg/mL MMC for 90 min at $t = 0$, and after 1.5, 3, and 4.5 h. Sensors retained activity in the ambient environment for at least 6 h without any compromise in sensing ability due to cell aging or other factors. (B) Biosensor shelf lifetime tests. A batch of biosensors was prepared and tested over a consecutive three-week period by exposing them to M9 medium containing 10 μg/mL MMC for 90 min. Compared to control experiments, the biosensors still produced significant signals when exposed to MMC after storage for two weeks without any special treatment.

limit was in the same range as with a microtiter plate assay, in which the measurements from individual wells in the plate are from the responses of many cells. Compared to other optical-based biosensors, the present system exhibits several advantages. First, the cell immobilization step is simple and does not have any matrix effect, which usually decreases the measurement's sensitivity.[14] Second, only a 90-min incubation period is required to detect MMC concentrations as low as 1 ng/mL (Figure 4). It is expected that, with longer incubation times, it should be possible to detect even lower MMC concentrations.

Active Lifetime and Shelf Lifetime. To examine the potential applicability of such biosensors for environmental monitoring, two additional parameters, the active sensor lifetime and the shelf lifetime, were investigated. Active sensor lifetime is defined as the length of time in which the sensor can remain in the ambient environment and retain its sensing ability after monitoring is initiated. This attribute is important for many monitoring or screening applications. To assess the active sensor lifetime, four sensors were prepared from the same cell culture and kept at room temperature in M9 medium. The four sensors were exposed individually to M9 medium containing 10 μg/mL MMC after 0, 1.5, 3, and 4.5 h. The kinetics of GFP expression in a 90-min incubation period is displayed in Figure 6A. The figure shows that these sensors remained active in medium for at least 6 h without any compromising their sensing ability.

Long shelf lifetime allows sensors to be prepared in a batch and stored without losing their sensing abilities. A long shelf lifetime is critical in developing cost-effective and time-efficient biosensor devices. To test the shelf lifetime of these sensors, stock sensors were made as described in the Experimental Section. The ends of the polyurethane tubes were wrapped in Parafilm to keep the cell medium from evaporating. The sensors were then immediately stored at 4 °C. Upon testing, sensors were reactivated by incubating at 37 °C for 90 min. Over a consecutive three-week period, sensors stored in this manner were reactivated and treated with M9 medium containing 10 μg/mL MMC. Figure 6B shows the kinetics of GFP expression levels of these sensors during a 90-min incubation over three consecutive weeks. After 15 days, the fluorescence intensity obtained from biosensors exposed to medium containing 10 μg/mL MMC was still three times as high as signals from the control experiment (for figure clarity, the control graphs are not shown). These kinetics graphs demonstrate that sensors remained functional for at least two weeks without any special treatment. The results shown in Figure 6 demonstrate the potential of this technology for screening or monitoring applications. Most microtiter plate assays cannot be conducted outside of a laboratory because they require a large volume of fresh cell culture.

CONCLUSIONS

Because of their ability to perform functional sensing, living cell-based biosensors are drawing increased attention. The work reported in this paper demonstrates the ability to fabricate an optical imaging fiber-based living bacterial cell array for genotoxin detection. The bacterial biosensor demonstrated sensitive genotoxin dose-dependent responses, reasonable active sensor lifetime, and relatively long "shelf" storage lifetime.

This high-density living cell array provides several benefits for genotoxin detection. First, the array can provide improved sensitivity. One source of this high sensitivity is the efficient signal transmission and collection process. Since the cells are located in the wells very close to the fiber surface (i.e., the bottom of the wells), the isotropic fluorescence signals are gathered by the individual fibers and transmitted to the CCD camera with minimal light attenuation. Because the cell responses were measured individually, the results can be presented as a summed signal, an averaged signal, or individual cell responses. Second, the array format has the potential to provide a simple method for multianalyte sensing by combining multiple cell types in the array, with each cell containing a unique fluorescent reporter with a different emission wavelength. This multianalyte sensing capability has previously been described in a proof of concept reported from our laboratory.[26]

The size of the microwells is determined by the diameter of the individual fibers in the fiber bundles; therefore, this optical imaging fiber-based microarray can be easily tailored to virtually any cell size. It provides a potentially universal platform for other living cells such as yeast or mammalian cells. Due to the small feature sizes, thousands of individual cells can be monitored simultaneously and repetitively with both spatial and temporal resolution. The ability to observe the responses of individual cells and to measure response distributions is a capability of the array

that is being further investigated. In addition to the biosensing applications described here, this cell array could be employed for high-throughput drug screening, where only small quantities of drug candidates are available. In addition, the arrays can be used to monitor single-cell gene expression and cellular responses, where information from the culture average can mask the heterogeneity of the individual cell responses. Novel applications of this biosensor platform for investigating gene expression in arrays of single living cells are the focus of our ongoing research.

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