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Cell-based biosensor for rapid screening of pathogens and toxins

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ABSTRACT

Development and validation of a mammalian cell-based biosensor for application in food defense and food safety was investigated. Three prototypes of the biosensor capable of handling different sample types were developed and tested with food and beverages. The sensing element is a B lymphocyte Ped-2E9 cell-line, encapsulated in collagen matrix in 3D scaffold. The uniqueness of this biosensor is that it detects analyte interaction with mammalian cells and is able to distinguish pathogenic from non-pathogenic and active from inactive toxins, rendering accurate estimation of the risk associated with the agents. This sensor gave positive signal for a broad range of bacterial pathogens; *Listeria monocytogenes*, enterotoxigenic *Bacillus*, *Vibrio*, *Micrococcus* and *Serratia*, and toxins; α-hemolysin from *Staphylococcus aureus*, phospholipase C from *Clostridium perfringens*, cytolysin from sea anemone *Stoichactis helianthus*, listeriolysin O from *L. monocytogenes*, and enterotoxin from *Bacillus*. Detection limit for toxins was 10–40 ng in 2 h while for a model bacterial pathogen, *L. monocytogenes*, 10^3–10^4 CFU/ml in 4–6 h, even in the presence of a mixture of higher concentrations of non-pathogenic species of the same genera or common background microflora. With inoculated food and beverage, the sensor detected *L. monocytogenes* and *Bacillus cereus* at a low initial concentration of 10^2–10^4 CFU/g from ready-to-eat meat and rice, and only active toxins at nanogram quantities from rice, milk and water samples. Though all the three prototypes performed well with beverages, Devices II & III are most suitable for testing particulate foods. These data present promising evidence for possible application of this biosensor for rapid detection of multiple pathogens or toxins for food defense and food safety application.

1. Introduction

In the field of sensing technologies, the need of a system capable of providing information about the biological activity of an analyte is of great interest. Especially in food safety and biosecurity area, rapid identification and differentiation of pathogenic from non-pathogenic species, viable from non-viable cells or active from inactive toxins is important as the presence of non-viable, inactive or non-pathogens do not pose any safety concerns. Furthermore, the emergence of new or modified pathogens augments the threat of bioterrorism and food safety (Rasco and Bledsoe, 2005). Cell-based detection systems are emerging as a dependable and promising approach to sense the functionality or biological activity of an analyte by monitoring perturbations in 'normal' physiological activities of mammalian cells following exposure (Banerjee and Bhunia, 2009; Stenger et al., 2001; Ziegler, 2000). A cell-based biosensor (CBB) is capable of detecting the presence of pathogens in clinical, environmental or food samples (Banerjee and Bhunia, 2009; Liu et al., 2009; Rider et al., 2003; Tencza and Sipe, 2004); however, further improvement in shelf-life and the stability of CBB is needed for onsite application (Banerjee and Bhunia, 2009).

A majority of CBBs measure optical properties of cellular metabolites or intracellular enzymes (Banerjee and Bhunia, 2009; Banerjee et al., 2007; Curtis et al., 2008; Gray et al., 2005; Rider et al., 2003). Mammalian cells have been used to detect and differentiate virulent *Listeria* (Roche et al., 2001) and enterotoxigenic *Bacillus* (Fletcher and Logan, 1999; Pedersen et al., 2002) from avirulent ones. *Listeria monocytogenes* can infect and produce cytotoxicity to murine hybridoma B cells (Ped-2E9) by releasing alkaline phosphatase (ALP) that can be detected in 5–6 h and is directly related to its virulence potential (Bhunia et al., 1994; Bhunia and Westbrook, 1998; Bhunia et al., 1995). The assay time was further shortened to 2 h when the reducing agent dithiothreitol was added to the Ped-2E9 during incubation (Westbrook and Bhunia, 2000). Ped-2E9 was also employed to detect enterotoxin from *Bacillus cereus* (Banerjee et al., 2008; Gray et al., 2005).

The presence of trace amount of toxins in foods, beverages or drinking water supply pose huge food safety and biosecurity risks (Casadevall and Pirofski, 2004; Rasco and Bledsoe, 2005) and the need for rapid screening tools is of great demand to establish comprehensive biosecurity preparedness (Bicout, 2004; Kurth, 2004; World, 2004). CBBs have several advantages: CBBs detect and

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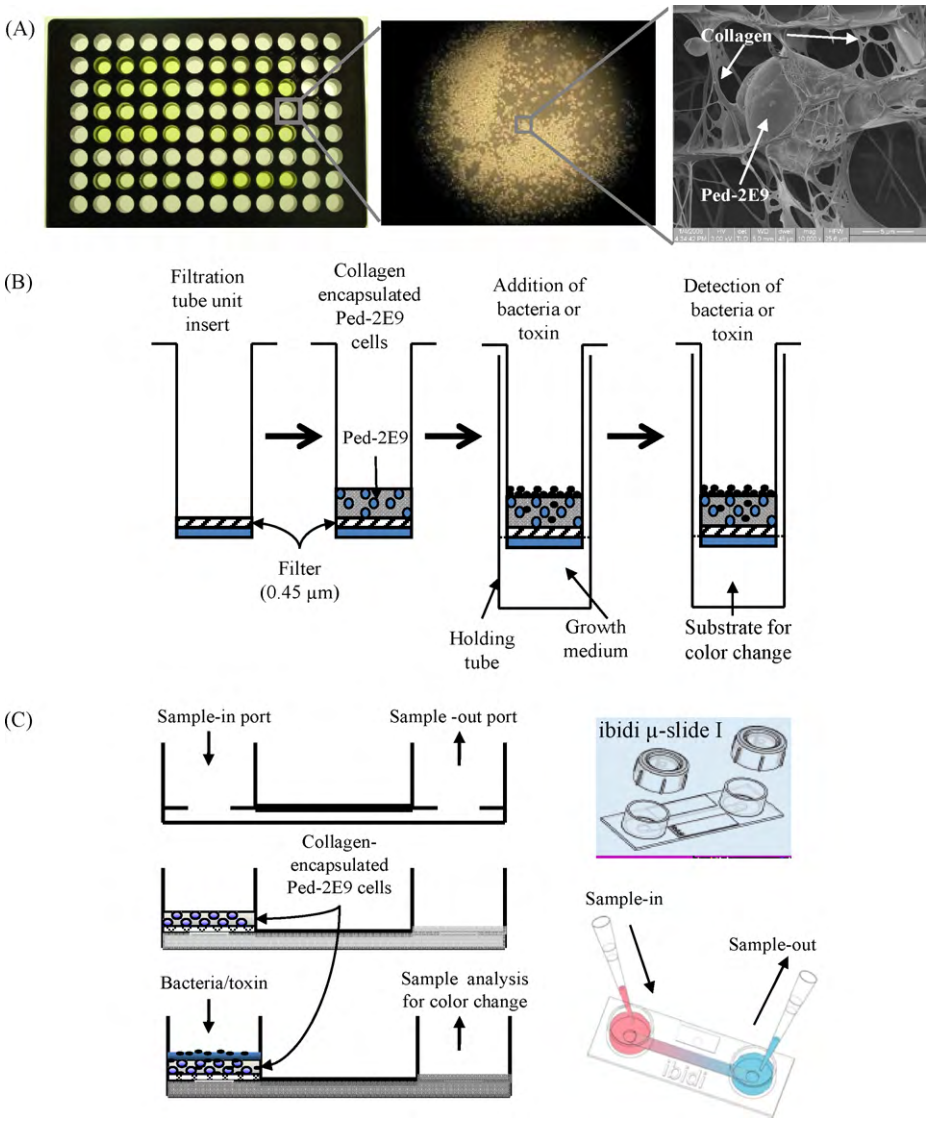

Fig. 1. Design of different cell-based sensor devices: (A) Device I (96-well plate), (B) Device II (Filtration tube), and (C) Device III (μ-Slide). (A) Wells containing collagen embedded Ped-2E9 cells (Banerjee et al., 2008): (right panel) scanning electron micrograph of Ped-2E9 embedded in collagen. (B) The filtration tube insert was first seeded with Ped-2E9 in collagen (200 μl/well) and was put back into the tube holders before testing. After analyte exposure, the device was centrifuged to allow liquid permeation from the tube insert to the holding tube. The liquid was transferred to cuvette containing ready-to-go ALP liquid substrate for color reaction. (C) Device III is made of high optical quality slide (μ-Slide). A 0.22 μm filter was placed in one of the two wells designated sample delivery-port. Ped-2E9-collagen (250 μl) was dispensed into sample delivery-port and allowed to solidify. After exposure to analytes, liquid from sample-out port was collected and reacted with ready-to-go ALP substrate for color reaction.

respond to only live or active threat agents, and more importantly, the response to threat agents or toxins is equivalent to "human or animal" physiological responses, providing an excellent robust first line of screening tool (Banerjee and Bhunia, 2009). The motivation of the present work is to develop a portable cell-based sensor that can fulfill the above criteria enabling detection of pathogens and toxins from food and beverages.

CBBs fabricated with three-dimensional (3D) cell culturing systems are gaining significant interests because the cell system mimics in vivo cellular properties (Pampaloni et al., 2007). Hence analyte-induced response has more physiological relevance (Banerjee and Bhunia, 2009). We have recently developed a collagen encapsulated Ped-2E9 cell-based biosensing system to screen pathogenic bacteria and toxins (Banerjee et al., 2008). Here, we report the design and development of handheld prototype biosensors to integrate the gel encapsulated Ped-2E9, and testing and validation of the biosensor in rapid detection of pathogens and toxins of food safety and food biosecurity significance.

2. Materials and methods

2.1. Mammalian and bacterial cells

Lymphocyte origin Ped-2E9 cells (Bhunia et al., 1994) were prepared as before (Banerjee et al., 2008) (Supplementary Materials). Bacterial cultures used are listed in Table S1. To validate the sensor performance to detect model *L. monocytogenes* in the presence of other microbes, we prepared three different blends designated Group A (*Listeria innocua*, *Listeria grayi*, *Listeria seeligeri*, *Listeria welshimeri*, *Listeria ivanovii*), Group B (*Enterobacter aerogenes*, *Citrobacter freundii*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Carnobacterium gallinarum*), and Group C

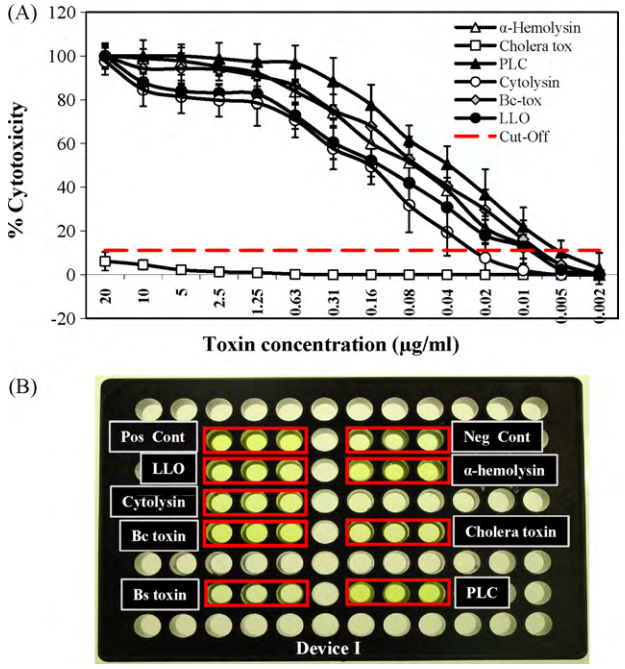

Fig. 2. (A) Analysis of LOD for toxins: α-hemolysin, cholera toxin, phospholipase C (PLC), cytolysin, *Bacillus cereus* toxin (Bc-tox), listeriolysin O (LLO), and *Bacillus subtilis* (Bs toxin) on Device I after 6 h of exposure. (B) Device I was tested with toxins at 10 μg/well for 6 h, and the wells showing yellow color are positive. Positive control (Pos cont) wells are treated with Triton X, negative control (Neg Cont) media only.

(*Bacillus subtilis*, *E. coli* O157:H7, *Enterococcus faecalis*, *Salmonella typhimurium*, *Serratia marcescens*).

2.2. Toxins

Pure toxins including α-hemolysin from *S. aureus*, cholera toxin from *Vibrio cholerae*, phospholipase C (PLC) from *Clostridium perfringens* and cytolysin from sea anemone *Stoichactis helianthus* were purchased from Sigma (St. Louis, MO). Crude toxins were prepared from *Bacillus* (Gray et al., 2005) and *Listeria* (Banerjee et al., 2008) culture supernatants (Supplementary Materials).

2.3. Preparation of sensor prototypes

Three different sensor device configurations were used: Device I, Costar 96-well plates (Fig. 1A) (Banerjee et al., 2008); Device II, Filtration tube units (Cat#UFC40HV0S, Millipore, Billerica, MA, Fig. 1B); Device III, μ-Slide I (Cat#80106, ibidi GmbH, Munich, Germany, Fig. 1C). This sensor responds to membrane active cytolytic toxin analytes and the principles of reaction mechanism have been described previously (Banerjee and Bhunia, 2009; Banerjee et al., 2008). Here, we made some minor modifications of encapsulating the Ped-2E9 in collagen gel. Briefly, Ped-2E9 cells were collected, counted, washed, and about 2.5×10^7 viable cells/ml were resuspended in complete serum-free medium. The cell suspension was mixed with neutralized type I collagen (final pH was adjusted to 7 by adding 1N NaOH in PBS) at a final concentration of 1.5 mg/ml and seeded into three devices at different volumes: 30 μl/well (Device I), 200 μl/well (Device II), and 250 μl/well (Device III). All devices were held at 37 °C for 15–45 min or until the gel was solidified. The wells of Device I (200 μl/well) and Devices II and III (each with 1 ml/well) were filled with DMEM–phenol red free (PRF) with 2.5% FBS and maintained at 37 °C in a humidified CO_2 incubator for 24–48 h and the medium was changed once at 24 h. All three devices were initially optimized using a strain of pathogenic and non-pathogenic *Listeria* and *Bacillus* (Supplementary Materials).

2.4. Testing of sensors with bacteria or toxins

Before testing, spent medium from each device was aspirated and replenished with serum-free DMEM–PRF. The cytotoxicity effects of 53 bacterial cultures (Table S1) were measured by ALP release assay (Banerjee et al., 2008; Bhunia and Westbrook, 1998). Briefly, 100–200 μl of bacteria cell suspension (1×10^8 CFU/ml) or toxins (100 μl) was added and held at 37 °C in a humidified incubator with 7% CO_2 for 3–6 h. In Device I, Ready-to-go ALP liquid substrate (Sigma) was added (100 μl/well) and color change was read ($A_{405/595\,nm}$) after 5–15 min in a plate reader (Bio-Rad). Device II was centrifuged ($300 \times g$, 10 min) and the permeate (200 μl) was collected in the cuvette. The sample (200 μl) from Device III (μ-Slide) was collected from the sample-out port. After addition of 200 μl of ready-to-go ALP substrate, color changes were read ($A_{405/595\,nm}$) at intervals of 3–5 min using a handheld USB2000 mini-spectrophotometer (Ocean Optics).

2.5. Limit of detection and specificity

To determine the limit of detection (LOD) of the sensors for bacteria or toxin, fresh culture of *L. monocytogenes* Scott A was washed and serially diluted (2×10^8 to 2×10^1 CFU/ml) in sterile PBS (pH 7.4). A 100–200 μl of bacteria cell suspension was delivered to the devices and incubated at 37 °C with 7% CO_2 for 2, 4 and 6 h.

For toxins, 100 μl of each serially diluted (40.0 μg/ml to 2.0 ng/ml) toxin preparations were added to each well of Device I and incubated for 2–4 h. ALP activity was determined in Ped-2E9 supernatants. Ped-2E9 with DMEM only, served as a negative control while Triton X-100 (0.1%)-treatment served as a positive control. A cytotoxicity value, 2× higher than that produced by the negative controls, non-pathogenic bacteria or toxin preparations from the same was considered positive.

Specificity of the biosensor to discriminate *L. monocytogenes* from closely related *Listeria* (Group A mix) and from a mixture of other microorganisms (Groups B and C mix) was evaluated in Devices II and III. In one set of experiments, inoculation for all bacteria (Groups A–C) was 20 μl (each) from an overnight culture in BHI into 1 ml of DMEM without serum (where each bacterial culture was adjusted to uniform concentration of $\sim 1.0 \times 10^9$ CFU/ml). In another set, three low concentrations of the *L. monocytogenes* (2×10^5, 2×10^4, 2×10^3 CFU/ml) with the Group B were tested. Each culture in the Group B was adjusted to 1×10^7 CFU/ml. In all cases, 0.5 ml of the bacterial mix was added to the wells of the devices and incubated as above for 1–6 h.

2.6. Detection of pathogens and toxins from spiked foods or beverages

L. monocytogenes was inoculated into 25-g of each hotdog and salami with initial concentrations of 10^1–10^6 CFU/g and enriched in 225 ml of Buffered Listeria Enrichment Broth (BLEB, Oxoid) for 18 h (Supplementary Materials). A 100–200 μl of broth was directly added to the devices and incubated for 3–6 h. At the same time, bacterial cells were also recovered from BLEB by immunomagnetic separation (Gray and Bhunia, 2005) using Dynabeads®anti-Listeria (Invitrogen) and added to the devices.

B. cereus and *B. subtilis* were inoculated into fried rice or pasteurized milk (10^4 CFU/g or ml) and enriched in BHI for 18 h (Supplementary Materials). Samples were centrifuged and supernatants (source of toxins) were filter-sterilized and tested with sensors immediately or stored at 4 °C for not more than 48 h. Also, milk samples were directly inoculated with α-hemolysin and PLC,

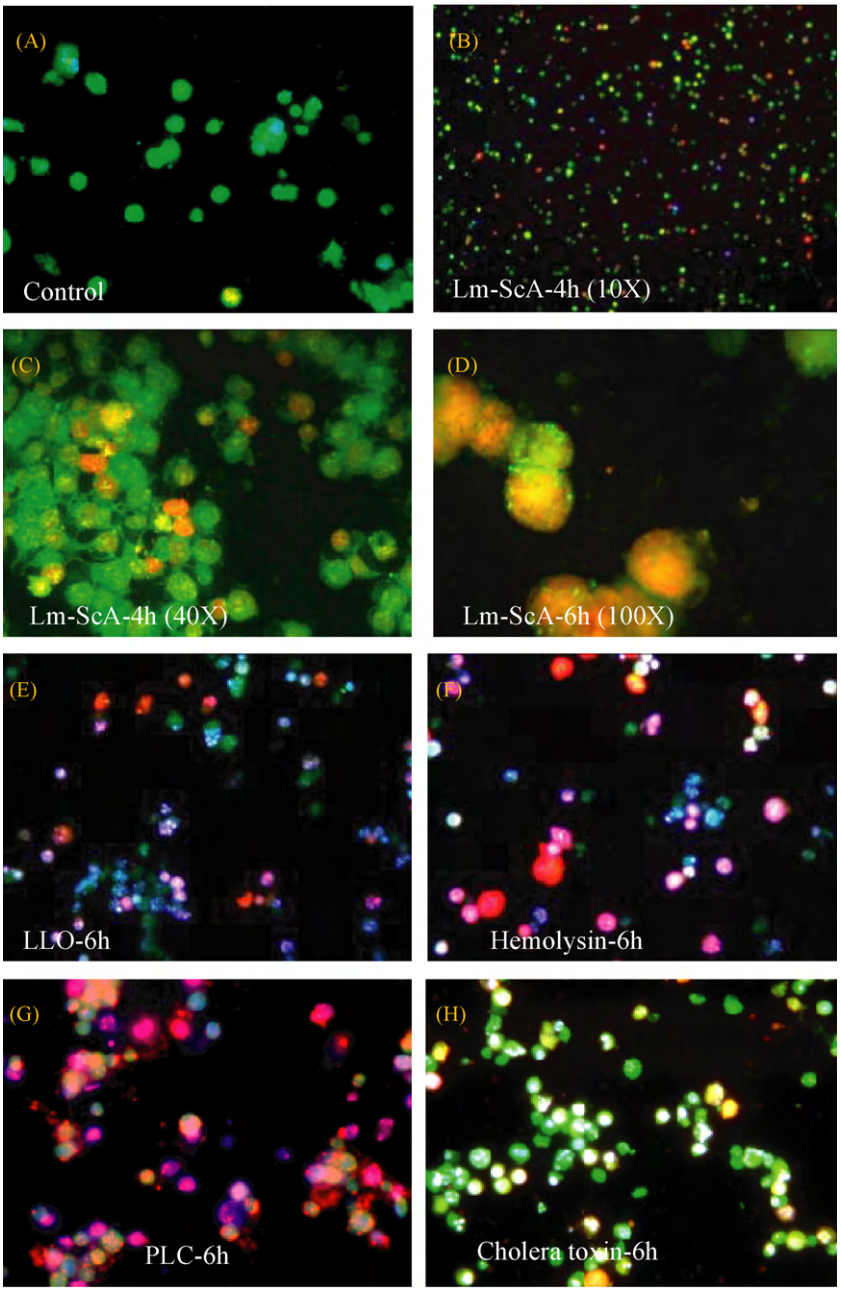

Fig. 3. Fluorescence microscopic analysis of Ped-2E9 death after 4–6 h exposure to analytes. Ped-2E9 cells were stained with solution containing acridine orange (green = living), propidium iodide (red = necrotic) and DAPI (blue = apoptotic). Apoptotic cells exhibit characteristic blebs, and lysed cells are necrotic. Treatments include: (A) Control; (B–D) *L. monocytogenes* Scott A (Lm-ScA) for 4 h or 6 h, imaged at 10×, 40× or 100×; (E) LLO (20×) for 6 h; (F) α-hemolysin (20×), 6 h; (G) PLC (20×), 6 h; and (H) cholera toxin (20×), 6 h.

and toxins from *B. cereus* and *B. subtilis* at10 μg/ml and held at 25 °C for 30 min, at 4 °C for 24 h or at 70 °C for 15 min, and processed as above. Bottled water (5 ml) was inoculated with cholera toxin or cytolysin at 10 μg/ml, held at 25 °C for 30 min, filter-sterilized, and tested (Fig. S1).

3. Results and discussion

3.1. Gel encapsulated Ped-2E9 cells as biosensors: evaluation of pathogen and toxin-mediated cytotoxicity

Collagen encapsulated Ped-2E9 in each of the three devices was exposed to *Listeria* for 6 h and assayed (Table S1). Pathogenic *Listeria* (*L. monocytogenes* Scott A, V7, F4244, 101M, 103M, CHLR2) produced cytotoxicity ranging from 44.6% to 78%. *L. monocytogenes* lacking key virulence determinants (*hly* and *prfA*) showed much lower cytotoxicity (1.2–2.5%). Among the other species, two out of three *L. ivanovii* produced higher cytotoxicity (47–58.7%) than *L. grayi* (0.09–0.6%), *L. innocua* (1.4–2.4%), *L. seeligeri* (0–5.7%) or *L. welshimeri* (0%). *L. ivanovii*, an animal pathogen, has similar sets of virulence genes as *L. monocytogenes* (Hain et al., 2006), hence similar levels of cytotoxicity.

Among the Gram-positive bacteria tested ($n = 16$), four, including *B. cereus* 4AC and A926 (22.6–44%), *Bacillus thuringiensis* (42.5–46%) and *Micrococcus luteus* (37.2% to 42.7%) were cytotoxic (Table S1). Of 20 Gram-negative bacteria (Table S1), only four, *S. marcescens*, *Vibrio vulnificus*, *Vibrio fluvialis*, and *Vibrio parahaemolyticus* showed cytotoxicity value of 15% or higher.

Cytotoxicity of *Bacillus* can be attributed to the presence of cytotoxin genes; *cytK*, *nhe*, and *hblA* (Fagerlund et al., 2008; Gray et al., 2005). *M. luteus* and *S. marcescens* were also cytotoxic similar to a previous report (Shroyer and Bhunia, 2003). *S. marcescens* is known to produce cytolysin, a 56-kDa metalloprotease and ShlA hemolysin (Carbonell et al., 1996; Marty et al., 2002). All produce hemolysins but *V. vulnificus* and *V. fluvialis* were more cytotoxic than *V. parahaemolyticus* (Chakraborty et al., 2005; Lee et al., 2004).

3.2. Assessment of toxin-mediated cytotoxicity

α-Hemolysin, PLC and cytolysin at 0.5–10.0 μg/ml evoked very high cytotoxicity (62.5–100%) and also confirmed to be cytolytic, showing strong haemolysis of sheep blood (Table S2). On the contrary, cholera toxin induced only 4.5% cytotoxicity and is also non-hemolytic on blood agar. Crude toxin from *L. monocytogenes* (LLO) and *B. cereus* (Bc-tox) showed 88% and 94.4% cytotoxicity, respectively, in 6 h; while toxin preparations from non-pathogenic *L. innocua* and *B. subtilis* showed only 1.2% and 5.6% cytotoxicity, respectively (Table S2).

The LOD for α-hemolysin, PLC, LLO and Bc-tox was 10 ng/ml (Fig. 2A) and the respective cytotoxicity values were 13.7%, 22.0%, 12.8% and 16.2% while for cytolysin the LOD was 40 ng/ml (19.5% cytotoxicity). The cut-off value for positive cytotoxicity was 11.2%, which is two-fold greater than the cytotoxicity elicited by the non-pathogenic *B. subtilis* (5.6% cytotoxicity, at 10.0 μg/ml; Table S2). Fig. 2B shows the wells in Device I with visible yellow color (positive cytotoxicity) in response to different toxins (10.0 μg/ml, 6 h).

All devices responded to toxins in a dose-dependent manner from 20 ng/ml to 40 μg/ml. All the cytolytic toxins yielded higher cytotoxicity than a non-hemolytic toxin (cholera toxin) indicating that membrane damage by cytotoxin allowed ALP release and cytotoxicity (Banerjee et al., 2008; Gray et al., 2005; Shroyer and Bhunia, 2003). The emetic toxin producing *B. cereus* F4810 (Haggblom et al., 2002) showed lower cytotoxicity than the diarrheagenic strains (*B. cereus* A926, MS1-9, ATCC14579) (Beattie and Williams, 1999; Schoeni and Wong, 2005). This indicates that the hemolysin BL (HBL) or *nhe* from diarrheagenic *B. cereus* (Fagerlund et al., 2008) has profound membrane damaging effect on Ped-2E9 than the emetic toxin producing strains (Gray et al., 2005).

3.3. Assessment of cell death by apoptosis or necrosis

The nature of cell death in collagen encapsulated Ped-2E9 induced by pathogens or toxins was determined by staining (Supplementary Materials). *L. monocytogenes* induced apoptosis in 56.5% of cells and necrosis in 32% at 6 h (Fig. 3). When LLO (10.0 μg/ml) was tested, the extent of necrotic cell death significantly increased (65.7%). Other toxins also exhibited increased necrotic death: *B. cereus* MS1-9 toxin (81.7%), α-hemolysin (87.4%) and PLC (90%) (Fig. 3). Likewise, all the cytolytic toxins exhibited higher necrotic than apoptotic cell death possibly due to rapid toxin-induced membrane damage (Banerjee et al., 2008). *L. monocytogenes* cells or LLO is known to induce apoptosis on Ped-2E9 (Banerjee et al., 2008; Banerjee et al., 2007; Menon et al., 2003) and similar results were also obtained here.

3.4. LOD and specificity

The estimated LOD for *L. monocytogenes* was 10^3 CFU at 4 and 6 h (Fig. 4 A–C) and 10^6 CFU at 2 h (Fig. 4A) indicating the sensor to be very sensitive compared to other methods (Bhunia, 2008; Kim et al., 2009). The cut-off value was set at 10%, which is about two-fold greater than *L. innocua*.

When the sensor was screened for *L. monocytogenes* in the presence of other *Listeria* (Group A), competitive (Group B) or pathogenic (Group C) microorganisms, *L. monocytogenes* produced 41% (3 h) and 83% (6 h) cytotoxicity (Fig. 5A). These values are slightly higher ($P > 0.05$) than the values when *L. monocytogenes* was tested alone (Fig. 5A), indicating that the sensor can detect *L. monocytogenes* in the presence of other *Listeria*. In the presence of naturally occurring (Group B) microorganisms (*E. aerogenes*, *C. freundii*, *L. acidophilus*, *S. aureus*, *P. aeruginosa*, *C. gallinarum*), the biosensor successfully detected *L. monocytogenes* at both 3 h (37% cytotoxicity) and 6 h (72% cytotoxicity) (Fig. 5B). When *L. monocytogenes* at 2×10^3, 2×10^4, and 2×10^5 CFU/ml were grown for 2–6 h with the Group B (each adjusted to 1×10^7 CFU/ml), the sensor detected 2×10^5 CFU/ml (31% cytotoxicity) at 4 h but showed lower cytotoxicity at the two other lower concentrations (25.6% and 17.2%) (Fig. 5D). While at 6 h, the sensor detected 2×10^4 CFU/ml (33% cytotoxicity), and at the lower concentration (2×10^3 CFU/ml) the values were less when compared to 10^4 CFU/ml but significantly higher ($P < 0.05$) than the cytotoxicity caused by *L. innocua* (Fig. 5D). These results indicate that the presence of competitive microflora can reduce the LOD of the sensor by an order of 1–2 logs probably due to reduced *Listeria* growth resulting from nutrient

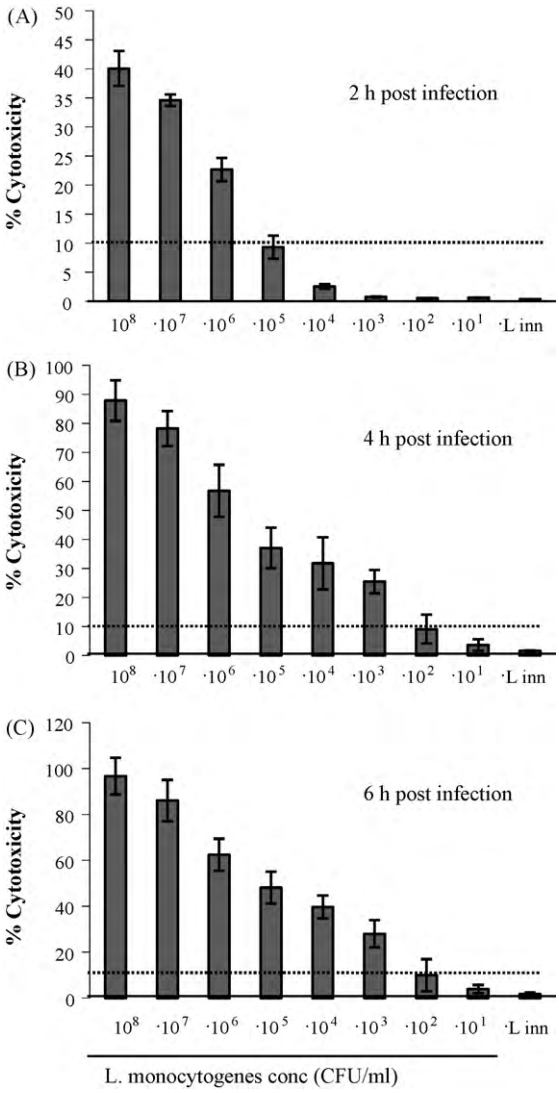

Fig. 4. LOD analysis of *L. monocytogenes* Scott A (10^1–10^8 CFU/ml) and the control *L. innocua* (Linn, 10^9 CFU/ml) at three different exposure time points; 2 h (A), 4 h (B) and 6 h (C). Values are average of two experiments performed in duplicate for all the three different devices; values are presented as Mean ± SEM. The cut-off value is set to 10% which is about two-fold higher than the *L. innocua* values.

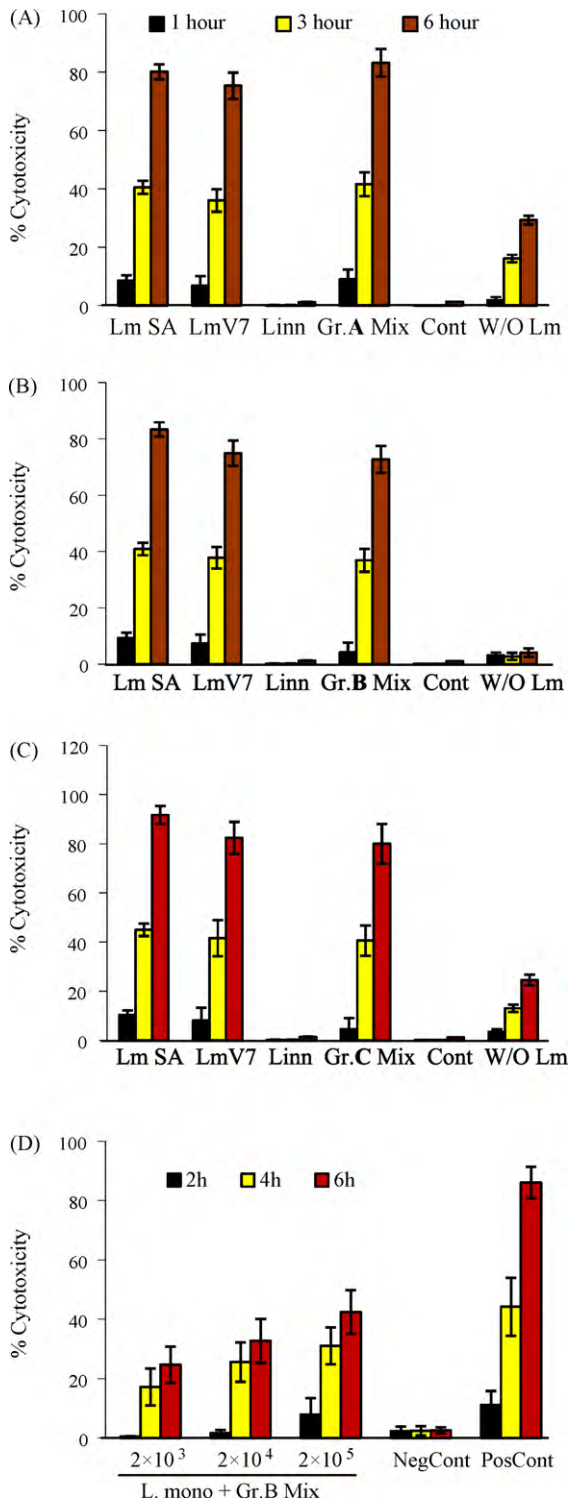

Fig. 5. Evaluation of the discriminatory power of the cell-based sensor for *L. monocytogenes* in the presence of different bacteria at 1, 3 and 6 h: Cytotoxicity of *L. monocytogenes* Scott A (LmSA) in the presence of (A) Group (Gr.) A Mix (*L. innocua*, *L. grayi*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*), (B) Gr. B Mix (*Enterobacter aerogenes*, *Citrobacter freundii*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Carnobacterium gallinarum*), and (C) Gr. C Mix (*Bacillus subtilis*, *E. coli* O157:H7, *Enterococcus faecalis*, *Salmonella typhimurium*, *Serratia marcescens*), and (D) at different concentrations (2×10^3, 2×10^4, 2×10^5) in the presence of Group B mix (10^7 CFU each). Negative control (Neg Cont) contained no bacteria while positive control (Pos Cont) contained LmSA only. Other treatments were, *L. monocytogenes* V7 (LmV7), *L. innocua* F4247 (Linn), mixtures without *L. monocytogenes* (W/O Lm), and control (no bacteria). Values are average of three experiments performed in duplicate in Device II and Device III ± SEM. The cut-off value for A, B, and C is established to be about 15% which is about two-fold higher than the values given by the mixture without Lm at 1 h.

deficiency from overgrowth of other microbes (Kim and Bhunia, 2008; Oravcova et al., 2008).

In the presence of Group C (*B. subtilis*, *E. coli* O157:H7, *E. faecalis*, *S. typhimurium*, *S. marcescens*), the sensor detected *L. monocytogenes*, which produced 40.5% (3 h) and 80% (6 h) cytotoxicity (Fig. 5C). Likewise, these cytotoxicity values were slightly less than the values obtained at the same time points when *L. monocytogenes* alone was used. *E. coli* O157:H7, and *Salmonella* also did not show any cytotoxicity and this may be due to lack of tropism of toxins produced by these pathogens to Ped-2E9 (Shroyer and Bhunia, 2003).

3.5. Detection of pathogens from inoculated food samples

In Devices II and III, *L. monocytogenes* inoculated hotdogs at 10^2, 10^3 and 10^4 CFU/g followed by 18 h enrichment and immunomagnetic separation produced 24.5%, 43.8% and 62.2% cytotoxicity, respectively, which were significantly ($P < 0.05$) higher than the value (3.4%) obtained from the non-pathogenic *L. innocua* (10^6 CFU/g initial inoculation). Performance of both devices was comparable for all pathogens tested (Tables S1 and S2). The cytotoxicity values from inoculated salami were comparatively lower than the values obtained from hotdogs at a lower initial inoculation (10^2, 10^3 and 10^4 CFU/g). This is possibly due to the presence of preservatives and inhibitors in salami that affected the bacterial growth (Kim and Bhunia, 2008) and ALP activity. Nevertheless, food-testing data indicated that conventional enrichment (18 h) followed by on-device enrichment (6 h) is suitable for detection of low levels (10^3 CFU/g) of pathogens, which is considered to be one of the strengths for this sensor.

3.6. Detection of cytolytic toxins from food or beverage samples

To test the sensor for the presence of preformed or nascent toxins in food or beverage, contaminated fried rice, pasteurized milk and bottled water were tested. *B. cereus* strains A926 and MS1-9 (10^4 CFU/g) in rice, produced 71.2% and 60.6% cytotoxicity while in milk, 45.5% and 43.3% cytotoxicity, respectively (Table 1). Water containing cholera toxin produced 3.87% and cytolysin produced 83% cytotoxicity (Table 1), and these results were comparable with data presented in Table S2.

Milk inoculated with α-hemolysin and PLC at 10 μg/ml each produced 66.5% and 71% (no treatment), 65% and 72.5% (4 °C for 24 h) and 5.65% and 6.15% (70 °C for 15 min) cytotoxicity, respectively, indicating toxins to be heat-labile. Similar results were obtained for *B. cereus* toxin in milk (Table 1). However, overall cytotoxicity values of toxin-spiked milk samples were consistently lower than the toxin-spiked cell culture medium, PBS or distilled water, indicating the presence of toxin inhibitor or ALP inhibitor in milk (Table 1 and Table S2). Presence of ALP inhibitor in milk is not unprecedented (Jasinska et al., 1985).

For each pure analyte, all three devices performed well and there were no statistically significant differences in the cytotoxicity values (Table S1). However for sample handling and preparation, Device I is superior since multiple samples can be tested at a time without additional manipulation. Device II requires a centrifugation step which is not required by the other two. Device III requires minimum manipulation. For food testing, all three devices performed optimally with beverages (milk and water) with minimal interference (Table S3); however, particulate foods (such as hotdog, salami, and rice) severely interfered with the assay in Device I causing physical obstruction of the light path during the endpoint reading in the well which was not a concern in Device II or III (Fig. 1).

Table 1
Performance evaluation of cell-based biosensor with inoculated food and beverage samples.

Food/beverage	Bacterial cultures or toxin (concentration)	Treatment	% Cytotoxicity
Hotdog	*L. monocytogenes* Scott A (10^6 CFU/g)	Enrichment in BLEB at 37 °C for	81.46 ± 4.2
	Lm Scott A (10^5 CFU/g)	18 h followed by 6 h on the device	70.35 ± 6.5
	Lm Scott A (10^4 CFU/g)		62.24 ± 5.1
	Lm Scott A (10^3 CFU/g)		43.83 ± 4.4
	Lm Scott A (10^2 CFU/g)		24.54 ± 4.7
	Lm Scott A (10^1 CFU/g)		11.72 ± 3.6
	L. innocua F4247 (10^6 CFU/g)		3.41 ± 2.1
Salami	*L. monocytogenes* Scott A (10^6 CFU/g)	Same as above	70.10 ± 5.5
	Lm Scott A (10^5 CFU/g)		56.43 ± 3.1
	Lm Scott A (10^4 CFU/g)		43.82 ± 6.1
	Lm Scott A (10^3 CFU/g)		25.80 ± 2.1
	Lm Scott A (10^2 CFU/g)		10.81 ± 2.6
	Lm Scott A (10^1 CFU/g)		9.75 ± 2.5
	L. innocua F4247 (10^6 CFU/g)		3.53 ± 0.7
Fried rice	*Bacillus cereus* A926 (10^4 CFU/g)	Enrichment in BHI at 37 °C for 18 h	71.23 ± 10.45
	Bacillus cereus MS1-9 (10^4 CFU/g)	followed by 6 h on the device	60.66 ± 7.80
	Bacillus subtilis (10^4 CFU/g)		2.15 ± 0.65
Milk	*Bacillus cereus* A926 (10^4 CFU/ml)	Same as above	45.58 ± 7.94
	Bacillus cereus MS1-9 (10^4 CFU/ml)		43.27 ± 9.75
	Bacillus subtilis (10^4 CFU/ml)		1.73 ± 0.68
	α-Hemolysin (*S. aureus*) (10 μg/ml)	Direct mix	66.54 ± 8.98
		24 h, 4 °C	64.96 ± 9.25
		70 °C, 15 min	5.65 ± 4.21
	Phospholipase C (*C. perfringens*) (10 μg/ml)	Direct mix	71.04 ± 8.74
		24 h, 4 °C	72.55 ± 6.19
		70 °C, 15 min	6.15 ± 0.65
	Crude toxin (*B. cereus* A926) (10 μg/ml)	Direct mix	59.44 ± 5.68
		24 h, 4 °C	55.13 ± 8.01
		70 °C, 15 min	2.75 ± 0.97
	Crude toxin (*B. cereus* MS1-9) (10 μg/ml)	Direct mix	54.52 ± 7.71
		24 h, 4 °C	48.99 ± 8.95
		70 °C, 15 min	1.84 ± 0.93
	Crude toxin (*B. subtilis*) (10 μg/ml)	Direct mix	1.98 ± 0.07
		24 h, 4 °C	2.34 ± 0.45
		70 °C, 15 min	0.00 ± 0.00
Water	Cholera toxin (*V. cholerae*) (10 μg/ml)	Direct mix	3.87 ± 2.36
	Cytolysin (*Stoichactis helianthus*) (10 μg/ml)	Direct mix	82.96 ± 7.47

Cytotoxicity data are average values obtained from Device II (Filtration tube) and Device III (μ-Slides) and each experiment was performed three times in duplicate. BLEB, buffered enrichment broth; BHI, brain–heart infusion

4. Conclusion

With the growing concerns of food borne disease outbreaks, food product recalls and threats related to food biosecurity there is an ever-increasing need for better detection and screening strategies for food and beverages. Modern methods such as nucleic acid and antibody-based (Banada and Bhunia, 2008; Byrne et al., 2009; Suo et al., 2010), and biosensor-based (Bhunia, 2008; Gooding, 2006; Maraldo et al., 2007) are sensitive and specific, but lack robustness, portability, or unable to provide biological activity of the analyte. In contrast, the cell-based biosensors can determine the lethality of an analyte very quickly (Banerjee and Bhunia, 2009; Stenger et al., 2001; Ziegler, 2000). Three prototype devices with collagen encapsulated Ped-2E9 in 3D format successfully detected a broad range of pathogens and toxins in food and beverage even in the presence of background microflora. Device I with 96-wells is attractive not only for its ability to screen large number of beverage samples but also for the ease of use. Besides beverages, Devices II and III also worked well with particulate foods but require additional handling and manipulation. The LOD for pathogens was ~10^3 cells while for toxins, 10–40 ng. With its inherent ability to distinguish viable pathogens or active toxins from their inactive counterparts and with the ease of use, this cell-based sensor shows promise in future application in the field of rapid food diagnostics, biosecurity and environmental monitoring.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.05.020.

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