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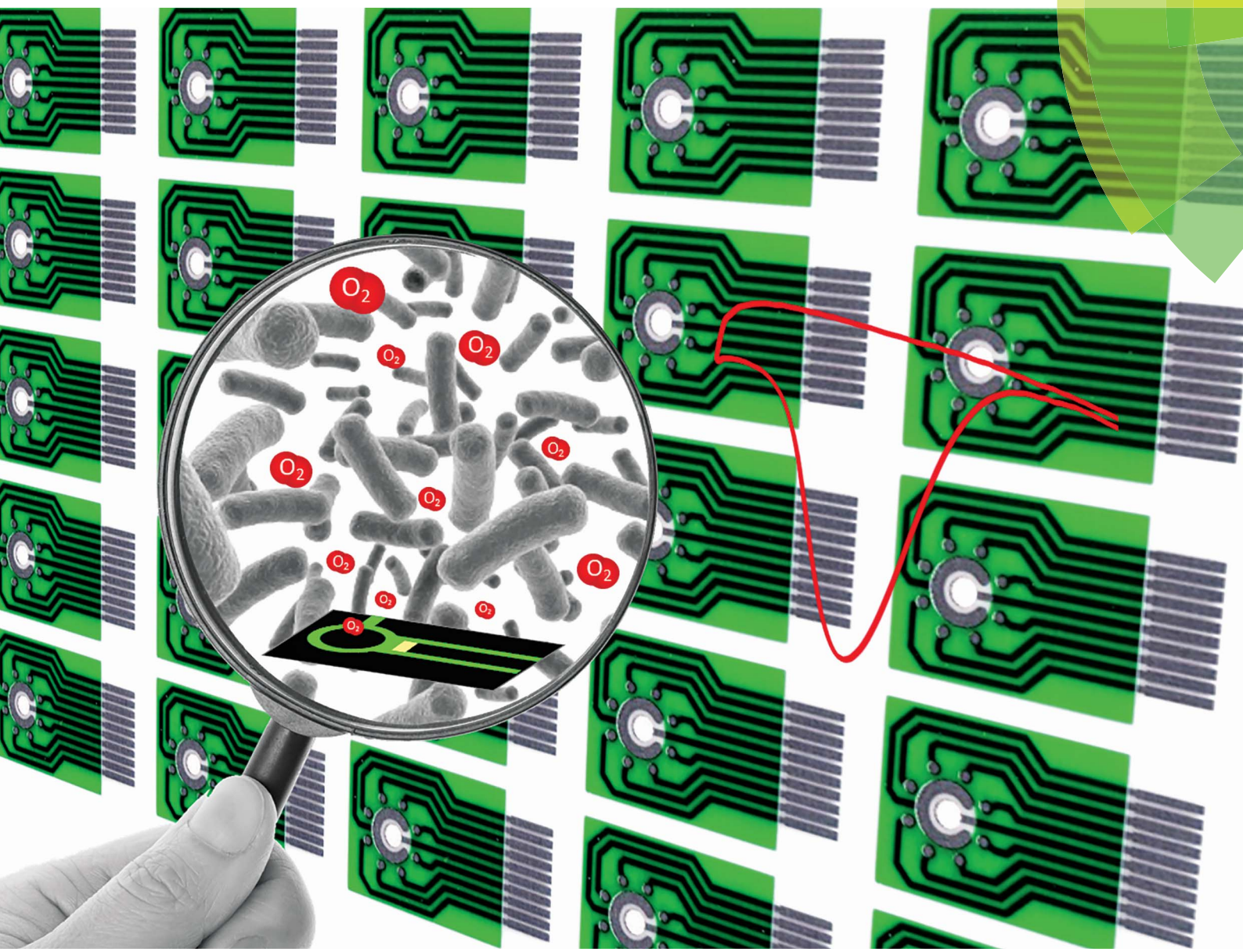

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Instant enumeration of total viable bacterial counts for food quality assurance using 'DEP-On-Go' sensor†

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Food quality has to be constantly monitored from farm-to-table to control foodborne illness. Total viable count (TVC), which estimates the population of live microbial load, can be an extremely useful tool for food quality assurance. This work presents the utility of an electrochemical 'DEP-On-Go' sensor to determine TVCs instantly and quantitatively in milk and other food samples, including vegetables, and meat, using the oxygen consumption activity of viable microbes. We present the analytical performance of our device for the sensitive detection of the TVC in packed milk with a detection limit of ≤300 CFU mL^{-1}, which fulfills the acceptable limit (<30 000 CFU mL^{-1}) recommended by Prevention of Food Adulteration (PFA) standards. We detected the TVC in raw or packed milk immediately without any pretreatment or incubation. The calibration curve of the TVC obtained by the standard plate count method and peak current resulting from the oxygen consumption activity of bacteria in milk samples showed linearity with a correlation coefficient of 0.9907 over the range of 1.8–8.7 log CFU mL^{-1}. The 'DEP-On-Go' sensor was further utilized to selectively determine the coliform bacteria count in milk samples with a linear coefficient of 0.9455 over the range of 0.6–7.0 log CFU mL^{-1} within 4 hours of incubation time using selective broth media. Our results support the easy-to-detect, low cost (1–5 USD), rapid and reliable monitoring of microbial contamination using the 'DEP-On-Go' device, which can become a powerful mobile platform in modern food industries.

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1. Introduction

Foodborne illness caused by microbes that contaminate food, such as *Salmonella*, *Listeria* or *Escherichia coli* O157, is a serious public health issue.[1] Unhygienic handling and uneven distribution of the food supply from the production site to the consumer site can be responsible for this contamination. For example, a major proportion of milk produced in developing countries is distributed through highly fragmented, unorganized sectors, including local milk vendors, wholesalers, retailers, and the producers themselves. As milk is an ideal growth medium that supports the growth of omnipresent germs or microbes originating from raw milk, an uneven distribution of cold chain facilities for milk supply from farm-to-table can lead to the growth of coliforms and aerobic bacteria in milk and thus lead to milk-borne illness.[2–4] It has been reported that approximately 90% cases of foodborne diseases related to milk spoilage are of bacterial origin.[5] Thus, determining the population of viable cells (total viable bacterial counts, TVCs), which estimates the microbial population load that can grow at ambient temperature and in the presence of oxygen, can be an extremely useful tool in the evaluation of quality of food and beverage samples for human consumption. In recent studies, a higher number of TVCs, coliform counts and other specific bacteria, including *Escherichia coli*, and *Staphylococcus aureus*, were detected in milk samples collected from a milk vendor as well as in pasteurized packed milk from supermarkets in developing countries.[6–10] Therefore, a rapid surveillance system for quality checks of milk and other food products as a control measure strategy, such as on-site 'test and store', can minimize the occurrence of foodborne illnesses.

The traditional culture-based plate count technique is available for quantitative enumeration of TVCs; however, it requires excessively long times in the range of minimum 24 hours to a few days of incubation time, sophisticated laboratory facilities and is subjective due to visual assessment, which prevents its prevalence as a primary surveillance system in resource-limited regions. In order to minimize the incubation

time of plate count techniques, a design for a sensitive impedimetric array of microelectrodes to monitor the growth of bacterial colonies has recently been described.[11] Interestingly, several optical sensing systems including RABIT (Don Whitley Scientific Ltd), BacTrac (Sy-Lab), MicroFoss (FOSS Analytical), have been commercially established as a high-throughput, automated and reliable methods for indirect growth detection of bacteria *via* monitoring of consumption and/or release of their key metabolites, such as CO_2 or O_2.[12–14] In this stream, an interesting approach is exemplified for dairy products called GreenLight (Luxcel Biosciences) which is based on respirometric screening technology (RST) that employs the fluorescence-based oxygen probes.[15–17] On the other side, non-culture based methods which are relatively rapid have also been established, such as immunological methods and nucleic acid-based methods,[18] but none of these has proven ideal as they are a multistep process requiring specialized reagents, equipment, and highly skilled technicians; thus, these methods are less accessible for decentralized and rapid on-site analysis by non-specialists. In addition, differentiation of viable and nonviable cells by non-culture methods is often a problematic, resulting in false positive results. Another non-culture and very rapid method that use a fluorescent labelling technique to detect viable cells directly in liquids by flow cytometry has also been used.[19] Conclusively, limited flexibility and ultra-high setup and assay costs associated with all these established methodologies make it practically impossible for field testing. Therefore, a highly accessible and reliable on-site approach is requires that can be utilized to detect TVCs instantly and quantitatively beyond the minimum acceptable limit, *i.e.*, 1000 CFU per gram.[20]

Electrochemistry offers a simple alternative to traditional analytical methods and provides a high degree of sensitivity and flexibility for field-scale applications. Published research work from our group and others have described the development of an electrochemical oxygen sensor that detects cellular oxygen consumption due to bacterial respiratory activity based on the principle that measurement of available dissolved oxygen can estimate the number of viable cells in a sample.[21–28] In a very recent report, the change in electrochemically active metabolite production from microorganisms was used to calculate bacterial counts.[29] Although these results have been encouraging, electrochemical sensors have yet to become mainstream in the evaluation of food safety in the modern food industry. To address this issue, our intention in this study was to develop a ready-to-use electrochemical sensing platform and to demonstrate the application transition of an electrochemical oxygen sensor to resource-limited settings for instant enumeration and real-time monitoring of TVCs and bacterial strains for general food quality checks. Recently, we introduced a prototype of a simple and portable electrochemical sensor termed the 'Disposable electrode printed (DEP)-On-Go' system for the simultaneous sensing of multiple heavy metals.[30] We now apply this system and extend its application to demonstrate improved electrochemical sensing of foodborne pathogens in food samples. In addition, we introduce a portable multichannel sensor equipped with automated bacterial count software that facilitates on-site monitoring of multiple samples simultaneously. First, TVCs from fresh food samples were determined by the 'DEP-On-Go' sensor and then evaluated using the standard dry sheet plate count method.[31] This system was also applied to detect specific bacterial pathogens, such as coliforms, using selective media. We present a detailed investigation of the utility of an electrochemical oxygen sensor to test a variety of commonly used food products in markets and households, including milk, vegetables, and meat, and describe our progress to develop a working bacteria-sensing system for food in partnership with our commercial electrochemical manufacturing technologies (BioDevice Tech., Ishikawa, Japan). We show improved performance and assay sensitivity over both previously published systems and standard plate count methods. We believe that the 'DEP-On-Go' system, which is simple and determines TVCs instantly and quantitatively, and, in principle, is extendable to selectively determining microbes such as *Salmonella*, *Shigella*, *Staphylococcus*, and *Listeria* using an appropriate selective broth media, could significantly enhance the food quality control process in the modern food industry.

2. Experimental

2.1. Materials and apparatus

Samples of pasteurized packed milk, vegetables and meats were purchased by random sampling from local supermarkets in Japan (Nomi city, Ishikawa) and India (Jaipur city, Rajasthan). Thirty fresh vegetables (mainly fresh leafy and salad vegetables), and thirty-six fresh meats (mainly beef, pork and chicken), were cut into small pieces, and bacterial contamination was permitted at room temperature for up to 24 h. Pasteurized milk was opened at room temperature, and bacterial contamination was permitted for up to 36 h. The coliforms were extracted from cabbage after incubation overnight at 35 °C. *E. coli*-selective broth was obtained from Kanto Chemical Co., Inc., Japan. Ready-to-use dry culture sheets (Sanita-kun coliform count and Sanita-kun aerobic counts) were purchased from JNC Corp., Tokyo, Japan.

The disposable electrode-printed chip (DEP chip) was provided by BioDevice Technology Co. Ltd. (Ishikawa, Japan). The DEP chip consisted of a miniaturized three-electrode system, including a carbon working electrode, a carbon counter electrode and an Ag/AgCl reference electrode. The potential of reference electrode in saturated KCl is +0.199 V. Two types of DEP chip series, DEP-SP-N and TG-1, were used in this work. The total length of DEP-SP-N was 12 mm, and the area of the working electrode was 3.042 mm^2. TG-1 had the same shape as DEP-SP-N but its total length was 2.5 times larger and its working electrode area was 3 times bigger than DEP-SP-N. Cyclic voltammetry (CV) measurements were taken with a Potentiostat BDT miniSTAT 100 (Bio Device Technology Co. Ltd.) connected to a Windows tablet that was loaded with electrochemical system software (KME UsbStat version 2 and an automatic multichannel KME Nmk Sensor for automatic data analysis of vegetable, and meat samples (Bio Device Technology Co. Ltd., Ishikawa, Japan)). All measurements were performed

at room temperature (25 °C). For the CV measurements, each DEP chip was used once and discarded after each measurement to avoid contamination during analysis.

2.2. Sample preparation for electrochemical measurement

For the milk samples, 8 drops (approximately 350 μL) of milk sample contaminated with different numbers of TVCs or coliforms was directly added in a measuring cell, and the DEP-SP-N-chip was inserted in the cell for electrochemical analysis of dissolved oxygen (DO). To contaminate milk samples selectively, the coliforms were isolated from cabbage (100 μL), then inoculated into 37 g L^{-1} of EC broth solution (10 mL) and cultured overnight at 37 °C. The identification of coliforms and their concentration ($\sim 3.8 \times 10^8$ CFU mL^{-1}) in the culture broth media was confirmed by Sanita-kun coliform plates. Coliform colonies were counted after 24 h of incubation at 35 °C.

For other food sample preparations, the samples of vegetables, and meat were collected exactly as they were displayed for sale in the supermarket, put in sterilized polyethylene bags, and taken to the laboratory for analysis. Approximately 50 g of chopped vegetables and meats were mixed with 100 mL of sterilized PBS buffer (pH 7.45) solution in a filter bag, shaken for 1 min and drained. The drained solution was filtered by 0.45 μm filtered membrane to concentrate the bacteria on the membrane surface. Approximately 100 μL of yeast extract (YE) medium containing 5.0 g L^{-1} YE (Wako Pure Chemical Industries Ltd., Japan) at pH 7.0 was poured on the membrane (for details, see ESI, Fig. S1 and S2†). The TG-1 DEP chip was placed onto the membrane surface and connected to the USB-powered hand-held potentiostat.

2.3. TVCs and coliform count analysis using standard plate count method

The milk samples were serially diluted in sterilized water (as required), inoculated onto the Sanita-kun aerobic count-type and coliform-type dry sheets and incubated at 35 °C. All sheets were analyzed to calculate the number of TVCs and coliform colonies after 48 h and 24 h of incubation, respectively. The extracted solutions of vegetables and meats in sterilized PBS buffer (pH 7.45) were sent to Shokuhin Biseibutsu Center, Co. Ltd., Japan, for TVC analysis using the standard agar medium method.

2.4. TVC analysis using DEP-On-Go sensor

The electrochemical detection of oxygen consumed by bacteria was carried out using the protocol previously described by our group.[28] In principle, the DEP chip connected to a USB-powered hand-held potentiostat was used to measure the current flow by CV mode. The oxygen peak current was linearly correlated with the concentration of DO. The calibration curve was developed against the oxygen peak current and bacteria counts in vegetables, meats and milk. To measure the oxygen peak current in the samples of vegetables and meats, the TG1-type DEP chip was connected to a potentiostat and placed onto the membrane with an accumulated concentration of bacteria from the sample extract. The membrane with the DEP chip was covered with polythene bag to eliminate oxygen diffusion from the environment during oxygen consumption by bacteria. The DO signal was measured after 10 min for vegetable samples and 30 min for meat samples. The milk sample was directly deposited on the DEP chip without any pretreatment or incubation time, and the DO peak current was measured by CV. The CV parameters were as follows: beginning potential, 150 mV; first vertex potential, −1500 mV; second vortex potential, 150 mV; and scan rate, 100 mV s^{-1}.

2.5. Total coliform counts analysis using DEP-On-Go sensor

To determine the coliforms in pasteurized milk, the assay was developed using the CV method. The milk samples were diluted by adding 100 μL of EC broth and 100 μL of cultured coliform medium to 800 μL of pasteurized milk to prepare a series of culture mediums containing 10^7, 10^6, 10^5, 10^4, 10^3, 10^2, 10^1 and 1 CFU mL^{-1}. These diluted samples (600 μL) were incubated in capped Eppendorf tubes at 35 °C for 10, 60, 180 and 240 min. The calibration curve of the coliform count *versus* DO peak current was plotted at the detection time of 240 min. The CV parameters used for coliforms were the same as those for the TVCs.

3. Results and discussion

3.1. Characteristics of the DEP-On-Go sensor for instant TVC measurement

Fig. 1 illustrates the two simple operation steps for the instant detection of TVCs in food samples by the DEP-On-Go sensor: drop the sample into the measuring cell and run the proprietary software that is loaded on a tablet PC for cyclic voltammetry (CV) analysis. The primary components of this system were originally developed and are as follows: a compact PC software-controlled and USB-powered palm-sized potentiostat, a disposable screen-printed electrode chip (DEP chip), and a DEP chip holder for simple and easy sample processing. As shown in the photograph in Fig. 1, these components and other measurement tools are compactly packed into a hand-held box for mobile analysis. TVCs can be detected by electrochemical measurement based on the principle that oxygen serves as an electron acceptor in aerobic respiration and oxygen-based electrocatalytic reactions (as shown in the equations below), generating a small amount of electric current in the 3-electrode electrochemical system, which can be measured by cyclic voltammetry (CV).

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \text{ (working electrode)}$$

$$4OH^- \rightarrow O_2 + 2H_2O + 4e^- \text{ (counter electrode)}$$

By measuring a small change in the amount of current in CV mode, the change in the amount of bulk-dissolved oxygen (DO) can be determined. Therefore, the measurement of bulk oxygen uptake (consumption) rate due to aerobic cell respiration and growth of the contaminating TVC can be proportionally linked to the total number of live bacterial cells. Therefore, in this way, proliferation of bacteria can be easily detected by a 3-electrode

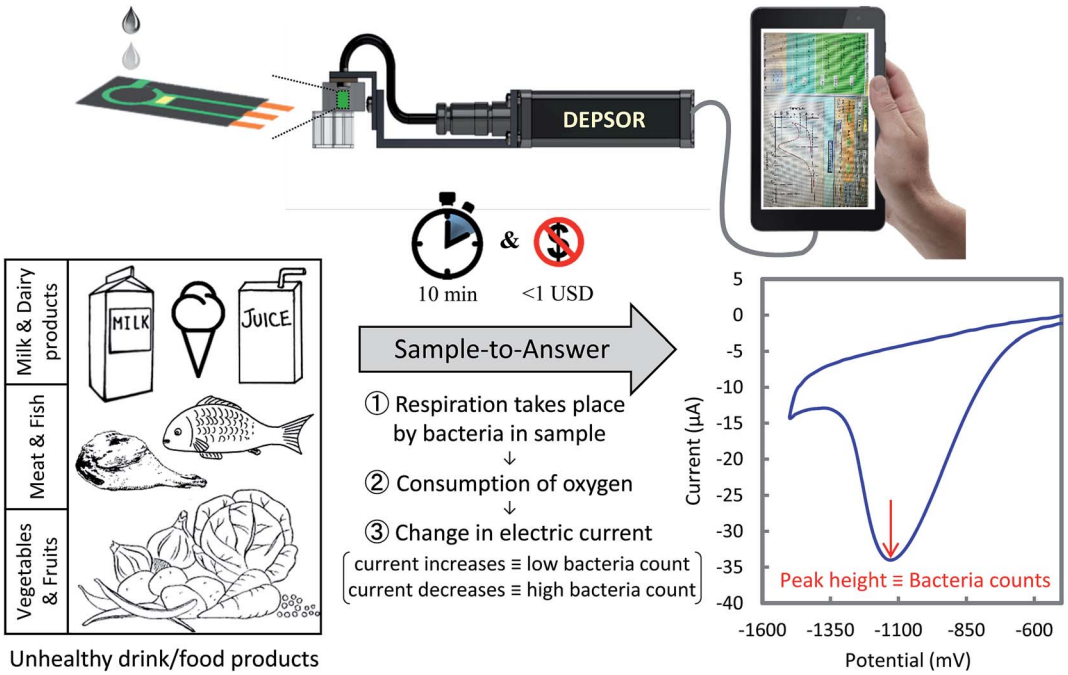

Fig. 1 A schematic drawing of the instant detection of total viable count in food samples using the DEP-On-Go sensor. A typical cyclic voltammogram recorded from a fresh milk sample is also shown.

electrochemical system. The intensity of the current peak represents the TVC of the sample. Most importantly, the TVC results can be accomplished instantly within a minute. A corresponding video demonstrating the instant enumeration of TVCs in a milk sample can be seen in the ESI (S1 Movie†).

3.2. Electrochemical detection of TVCs in milk samples

First, we evaluated the analytical performance of the 'DEP-On-Go' sensor by calibrating it with different TVC values calculated by the electrochemical method and standard plate count method. The TVC values (ranging from 1.8 to 8.7 log CFU mL^{-1}) were obtained by contaminating fresh pasteurized milk in an open environment for an increasing order of time intervals up to 36 h at room temperature. For electrochemical analysis, the DO in the milk samples was instantly measured by detecting the oxygen peak current in cyclic voltammetry (CV) mode using the DEP-On-Go sensor (Fig. 2a). For plate count analysis, aerobic TVCs in milk samples were determined using ready-to-use dry medium culture plates (Sanita-kun aerobic count). The cyclic voltammograms of DO showed that the oxygen peak current decreased as the number of bacteria increased in the milk samples (Fig. 2a). At 48 h of incubation at 35 °C, the TVCs in all milk samples grew and formed red colored colonies on the Sanita-kun plates. The calculated number of TVCs in all the Sanita-kun plates were consistent with the estimated TVCs added in the samples (see ESI, Fig. S3†). Consequently, a standard calibration curve relating oxygen peak current to TVCs was established with high linearity (the square of the regression coefficient (R^2) after the linear regression was 0.9907), which indicates that the effect of an electrochemical reaction resulting from the oxygen consumption activity of bacteria can be correlated to the microbial density of TVCs. Majorly, the calibration curve can be divided in three phases: lag-phase (<log 3 CFU mL^{-1}), exponential-phase (log 3–8 CFU mL^{-1}) and stationary-phase (>log 8 CFU mL^{-1}), which might be resulted because of the detection limit of this system. In the lag-phase, since the number of bacteria are less so the less consumption of oxygen and so the amount of electric current did not change sharply. However, the higher number of bacteria in exponential-phase rapidly consume the dissolved oxygen and so the amount of electric current sharply drops in proportion to the amount of oxygen consumed during bacterial respiration. The low drops of current in stationary-phase should be because of no net growth of bacterial population mainly because of the less available oxygen. The obtained results confirm that detection of TVCs ranging from 1.8 to 8.7 log CFU mL^{-1} in milk samples can be instantly performed using the DEP-On-Go sensor without any incubation time.

With the aim of verifying the practicability of the proposed DEP-On-Go system for TVC detection in unknown samples, we measured TVCs in packed or raw milk samples that were collected from local markets in Japan and India. Table 1 shows the estimated TVCs in total 29 milk samples. To obtain the correlated TVC concentration, the detected oxygen peak current data from all milk samples were entered into the calibration standard equation: $y = 1.6313x - 19.209$ (Fig. 2b). The results showed that TVCs in all six milk samples collected from supermarkets in Japan were within the acceptable range (log 4.47, *i.e.*, 30 000 CFU mL^{-1}). However, TVCs in packed milk samples collected from supermarkets in India was high (the highest mean TVC was log 7.1 CFU mL^{-1}). Only 16% of packed milk was graded as satisfactory based on TVC standards. Earlier

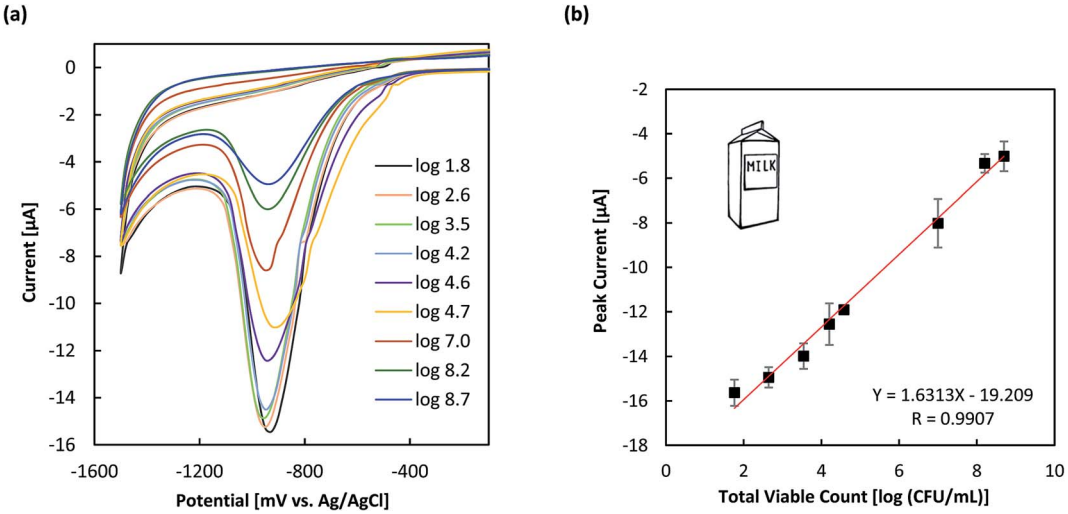

Fig. 2 Electrochemical measurement of TVC in milk samples. (A) Cyclic voltammograms and (B) corresponding calibration curves to determine the relationships between the electrocatalytic oxygen peak current obtained from cyclic voltammograms and the TVCs obtained from the standard plate count method (Sanita-kun aerobic count). The plot of current as a function of concentration of TVC with a linear trend line was calculated with regression analysis with an R-squared value of 0.9907 and a correlation coefficient of $r = 0.98$). The data are the average of three to seven independent measurements.

studies have also reported higher TVCs (log 5.77 CFU mL^{-1}) in packed milk samples collected from markets in India.[6] Among the analyzed samples, full cream milk samples were found to have very high TVCs, which is also consistent with the previously reported study.[8] Surprisingly, a similar brand sample collected at different days/times from different locations had different TVC values. This result clearly indicated the poor bacteriological quality of packed milk samples and that there is an urgent need to implement good hygiene practices in the milk supply chain in India. Fortunately, home pasteurization by boiling milk in the kitchen is a common practice in India, which might ensure the prevention of possible milk-borne illnesses. Based on these results and the simplicity, affordability, portability, and performance of the DEP-On-Go device, this technology is valuable for establishing an affordable and accessible electrochemical detection system for monitoring the bacterial contamination of milk samples.

3.3. Electrochemical detection of TVCs in general food samples

To validate the wide application of the DEP-On-Go sensor, TVCs were calculated in other food samples, namely, vegetables and meat sold in supermarkets in Nomi city, Japan. A total of 36, and 30 different types of meat, and vegetables, respectively, were collected and analyzed. To extract the microbial load from the solid food samples, a kit was designed and fabricated and a simple protocol was prepared for microbial load accumulation on the filter membrane (details are given in ESI, Fig. S1 and S2†). First, all the samples were analyzed by the DEP-On-Go sensor, and peak current for the DO in yeast extract media with the accumulated microbial load extracted from the food samples was measured. Next, a cross validation test using the standard plate count method for determining TVCs was performed using outsourcing services. Consequently, calibration plots between obtained values of peak current and TVCs were obtained. The results indicated that all samples in this study exhibited microbial contamination; however, the TVCs varied with the type of sample. Fig. 3 shows the TVCs for fresh vegetables, which ranged from 1.1×10^4 to 1.85×10^9; and fresh meat samples, which ranged from 1.23×10^4 to 2.05×10^9. As shown in Fig. 3a, we obtained a linear fitting calibration curve for all the tested vegetables with a reasonable detection limit of below 10^5 CFU g^{-1}, which fulfills the acceptable limit ($<10^6$ CFU g^{-1}) for the TVC or aerobic colony count in ready-to-eat vegetables recommended by Prevention of Food Adulteration (PFA) standards.[20] The calibration curves obtained by the standard plate count method and DEP-On-Go method show a low to high correlation coefficient for all the meat samples (Fig. 3b). Based on these plots, we developed automatic data analysis software (KME Nmk sensor software) and analyzed TVCs in the unknown food samples, which were vegetables (carrot, potato and cabbage (fresh and spoiled)) and chicken meat samples, using a multichannel hand-held potentiostat (see ESI, Fig. S4†). Table 2 shows the TVC results obtained using the DEP-On-Go sensor equipped with automatic software, demonstrating the simple and instant enumeration of TVCs in the food samples.

3.4. Electrochemical detection of coliforms in milk samples

Typical coliform bacteria include the organisms *Escherichia coli* and *Enterobacter aerogenes*, both of which are normal inhabitants of the large intestine. The presence of these organisms in milk therefore indicates fecal contamination and highly unhygienic handling; thus, the total coliform counts should be a useful indicator of assessing the quality of hygiene in milk and dairy products. We evaluated the performance of the DEP-On-

Table 1 Total viable counts in packed or raw milk samples detected by the DEP-On-Go sensor

No.	Pasteurized packed milk sample	Peak current (mean ± SD, μA)	Total viable counts (log 10 ± SD, CFU mL^{-1})
Packed milk from Nomi city, Ishikawa, Japan			
1	Nomi-A-0621	−14.7 ± 0.8	2.8 ± 0.2
2	Nomi-B-0621	−15.5 ± 0.9	2.0 ± 0.1
3	Nomi-C-0621	−13.9 ± 0.9	3.5 ± 0.2
4	Nomi-D-0621	−15.7 ± 0.8	1.7 ± 0.1
5	Nomi-E-0621	−13.4 ± 0.3	3.8 ± 0.1
6	Nomi-F-0621	−14.9 ± 0.3	2.7 ± 0.1
Packed milk from Jaipur city, India			
7	Jai-A1-0620	−11.1 ± 0.3	5.1 ± 0.1
8	Jai-A2-0630	−10.0 ± 0.2	5.5 ± 0.1
9	Jai-A3-0710	−12.3 ± 0.8	4.5 ± 0.3
10	Jai-B1-0710	−11.8 ± 0.5	4.7 ± 0.2
11	Jai-C1-0620	−11.1 ± 0.3	5.0 ± 0.1
12	Jai-C2-0630[a]	−7.3 ± 0.5	7.1 ± 0.5
13	Jai-C3-0710	−14.6 ± 0.1	2.9 ± 0.02
14	Jai-C4-0802	−11.7 ± 0.5	4.8 ± 0.2
15	Jai-D1-0620	−11.0 ± 0.6	5.1 ± 0.3
16	Jai-D2-0802	−9.7 ± 0.7	5.8 ± 0.4
17	Jai-D3-0812	−8.6 ± 0.2	6.3 ± 0.1
18	Jai-E1-0620	−11.4 ± 1.1	4.9 ± 0.5
19	Jai-E2-0630	−9.8 ± 0.8	5.7 ± 0.5
20	Jai-F1-0620	−10.4 ± 0.7	5.5 ± 0.4
21	Jai-G1-0620	−11.0 ± 0.3	5.2 ± 0.1
22	Jai-G2-0630	−9.4 ± 0.5	5.9 ± 0.3
23	Jai-G3-0812	−12.6 ± 0.7	4.3 ± 0.2
24	Jai-J1-0630	−11.7 ± 0.3	4.8 ± 0.1
Raw milk (after boiling) from Jaipur city, India			
25	Jai-H1-0802	−10.3 ± 0.6	5.5 ± 0.3
26	Jai-I1-0620	−8.9 ± 0.2	6.2 ± 0.1
27	Jai-I2-0630	−9.1 ± 0.5	6.1 ± 0.3
28	Jai-I3-0710	−11.3 ± 1.1	5.0 ± 0.5
29	Jai-I4-0812	−11.8 ± 0.6	4.7 ± 0.2

[a] Sample close to the expiration date.

Table 2 Total viable counts in various food samples detected by the DEP-On-Go sensor

No.	Food sample	Peak current (μA)	TVC (CFU mL^{-1})
Supermarket, Nomi city, Ishikawa, Japan			
1	Fresh cabbage	−14.8	2.7×10^4
2	Rotten cabbage	−8.6	1.0×10^7
3	Carrot	−13.7	6.1×10^4
4	Meat (chicken)	−16.2	1.0×10^4
5	Potato	−13.9	5.3×10^4

Go system for total coliform counts by measuring their oxygen consumption activity similar to the measurement of their TVCs. To count the coliforms selectively, the samples were incubated in selective broth media to stimulate the growth of coliforms only. The incubation time was optimized as the acceptable range of coliform count is negligible (nil in packed milk and <2000 CFU mL^{-1} in raw milk) and the limit of detection of current DEP-On-Go is above 300 CFU mL^{-1}. For this evaluation, the DO concentration in the milk samples (contaminated with different numbers of coliforms ranging from 4 to 10^7 CFU mL^{-1}) was detected by recording the oxygen peak current values at a peak potential of approximately 0.95 V using the CV method at 10, 30, 60, 180 and 240 min incubation times at 35 °C. The average oxygen peak current was not significant enough to detect a low number of coliforms for shorter incubation time, but it improved as the incubation time increased from 10 to 180 min (see ESI, Fig. S5†). We were able to detect coliforms as low as ≤16 CFU mL^{-1} in milk samples after 240 min of incubation.

For evaluation, coliforms bacteria in milk samples were also determined using ready-to-use dry medium culture plates (Sanita-kun aerobic count). At 48 h of incubation at 35 °C, the coliforms bacteria in all milk samples grew and formed blue colored colonies on the Sanita-kun plates. The calculated number of coliforms bacteria in all the Sanita-kun plates were consistent with the estimated TVCs added in the samples (see ESI, Fig. S6†). Fig. 4 shows the CV and calibration plots relating average peak current obtained from the DEP-On-Go sensor to total coliform counts obtained from the standard plate count method with high linearity (the square of the regression coefficient (R^2) after the linear regression was 0.9455). The average peak currents decreased depending on the number of

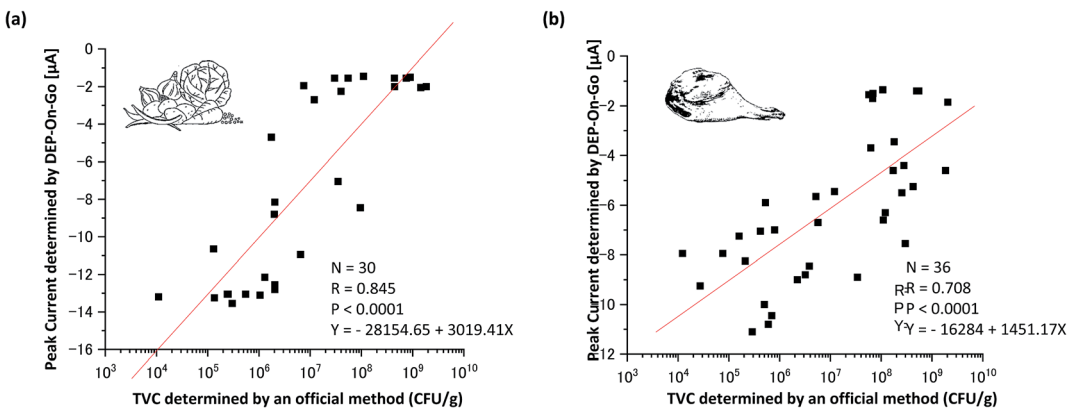

Fig. 3 Scatter plots of the electrocatalytic oxygen peak current obtained from cyclic voltammograms and the TVCs obtained from the standard plate count method in various fresh vegetables (a) and meats (b) food samples.

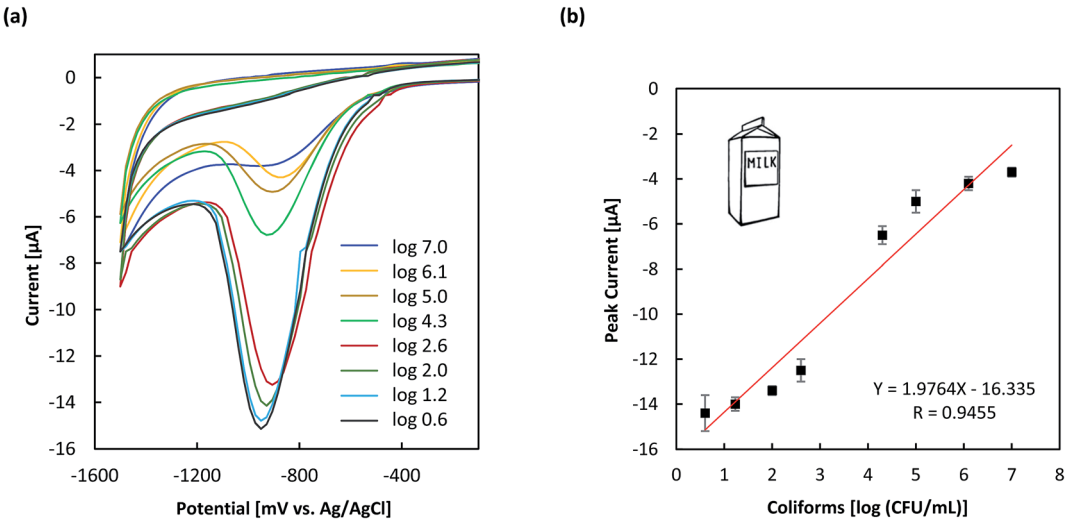

Fig. 4 Electrochemical measurement of coliforms in milk samples. (A) Cyclic voltammograms and (B) corresponding calibration curve to determine the relationships between the electrocatalytic oxygen peak current obtained from cyclic voltammograms and the TVCs obtained from the standard plate count method (Sanita-kun coliform count). The plot of current as a function of the concentration of coliforms with a linear trend line was calculated with regression analysis with an R-squared value of 0.9455 and a correlation coefficient of $r = 0.97$). The data are the average of three to seven independent measurements.

coliforms, and the average rates of reduction from the peak current value of the lowest detected concentration of coliform (0.6 log CFU mL^{-1}) were as follows: 97.2%, 93%, 86.8%, 45.1%, 34.7%, 29.2%, and 25.7% for 1.2, 2, 2.6, 4.3, 5, 6.1, and 7 log CFU mL^{-1}, respectively. In this case, multiple measurements were carried out, and the error bars indicate the standard deviation of three or more individual measurements. These results indicate that the effect of electrochemical reactions resulting from the oxygen consumption activity of coliforms can be correlated to the microbial density of total coliforms. The results confirm that the detection of coliforms in the range of 0.6 to 7.0 log CFU mL^{-1} in milk samples can be performed using the DEP-On-Go sensor within 4 h of incubation in selective broth media.

4. Conclusion

This study reports the high practical applicability and capability of the DEP-On-Go sensor as a portable, affordable and reliable bacteria-sensing system for the instant and quantitative detection of microbial loads in liquid and solid food samples. The sensor was able to instantly detect the TVC at an initial concentration of a few hundreds of CFU mL^{-1} and the coliform count at an initial concentration of a few tens of CFU mL^{-1} within 4 h, making it suitable for use in a wide range of food types. The TVCs in unexpired pasteurized packed milk in this study is unacceptably high, which could be a risk to public health and thus suggests that effective measures, *e.g.*, using the DEP-On-Go sensor, should be routinely performed to ensure safe milk and food for human consumption. This food-sensing technology has attractive advantages in field applications of food safety and is anticipated to have a significant impact on modern food industries.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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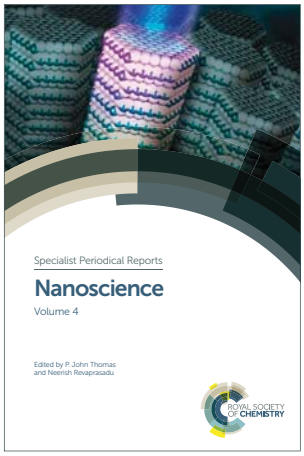

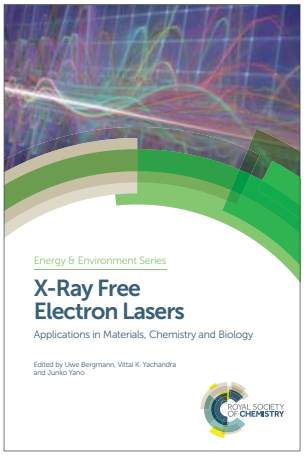

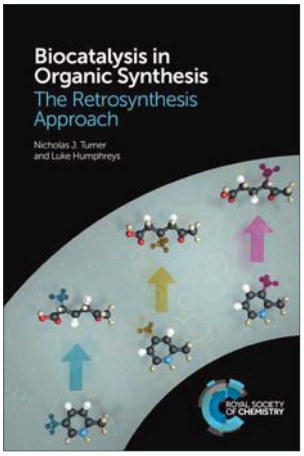

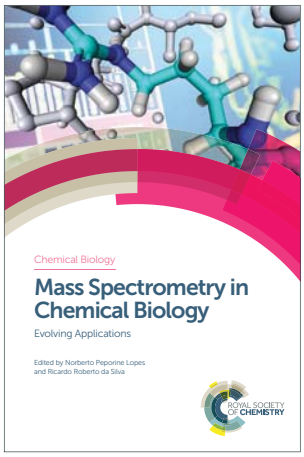

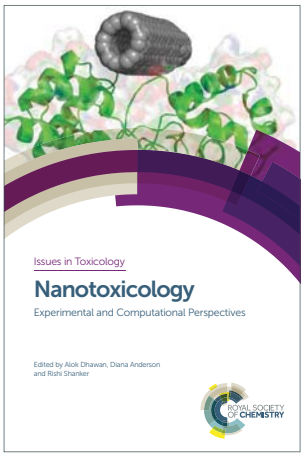

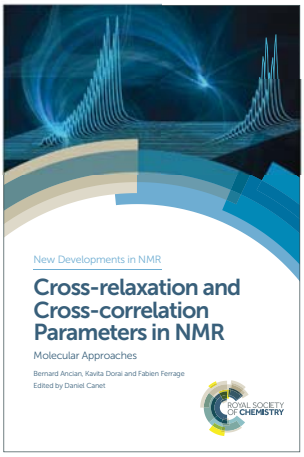

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