Evaluation of a Rapid Oral Bacteria Quantification System Using Dielectrophoresis and the Impedance Measurement

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To clarify the availability of the dielectrophoretic impedance measurement (DEPIM) system as the evaluator for oral care, we evaluated the usefulness of DEPIM system by comparison with the standard plate counting (SPC) method. First, the relationship between the DEPIM results and bacterial concentration measured by SPC was clarified. Next, the measurement of the microorganism number in a mixed suspension was evaluated with DEPIM and SPC. The bacterial counts with DEPIM strongly correlated with those with SPC (\(r^2=0.633-0.997\)) and this correlation was also shown in the measurement of a mixed bacterial suspension (ranging from \(10^5\) to \(10^8\) cfu/ml) of two bacterial species. Moreover, the experiments using dissociating enzymes to eliminate the influence of the size of the bacterial aggregates demonstrated that the microbial measurement results with DEPIM are unaffected by bacterial aggregates. This study demonstrated that bacterial counts with DEPIM strongly correlated with those with SPC and were unaffected by bacterial aggregates.

Key words: Dielectrophoresis / Impedance / Aspiration pneumonia/Oral care / Rapid microbial quantification.

Aspiration pneumonia-associated mortality is a very serious problem in elderly patients (Marik, 2001). It has been suggested that the oropharyngeal bacterial flora is a reservoir for respiratory pathogens, and patients with poor oral hygiene have a higher risk of respiratory disease (Scannapieco et al., 1992; Scannapieco and Mylotte, 1996). It has been also reported that oral health care for elderly patients in nursing homes reduces the incidence of bacterial pneumonia (Yoneyama et al., 1996; Yoneyama et al., 1999; Ishikawa et al., 2008).

To date, the standard plate counting (SPC) method has been used as a common method for the quantification of oropharyngeal microflora in our laboratory and others (Ishikawa et al., 2008; Hirota et al., 2010). However, the quantification of oropharyngeal bacteria from clinical samples with the SPC method takes a few days due to its culture-based approach.

To evaluate the efficacy of professional mechanical oral cleaning, the development of a quick and easy bacterial counter that can be used at the bedside would be beneficial. Suehiro et al. reported the dielectrophoretic impedance measurement (DEPIM) method based on dielectrophoresis (DEP) for bacteria detection (Suehiro et al., 1999; Suehiro et al., 2003a; Suehiro et al., 2003b; Suehiro et al., 2003c; Suehiro et al., 2005). Kikutani et al. recently reported the usefulness and clinical applicability of a novel rapid bacterial counter (Panasonic Healthcare Co., Ltd., Tokyo, Japan) for the simple and rapid quantification of oral bacteria (Kikutani et al., 2012). A rapid oral bacteria detection apparatus using the DEPIM method examined in this study as the principle of measurement has been also recently reported (Hamada et al., 2011).

In this study, we further characterized this same
DEPIM system as a reliable system for rapidly quantifying the microorganism number in a single and mixed aggregated bacterial suspension by comparison with the spiral inoculation system based on the standard plate counting (SPC) method. Especially, the microorganisms, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans, often isolated from the patients with aspiration pneumonia, were used for the counting the microorganism number in a mixed bacterial suspension. S. aureus was also used for the measurement of the aggregated and dispersed bacterial number toward clinical application. We further discussed the system’s usefulness as a quick and easy bacterial counter.

Microorganisms used in this study are shown in TABLE 1. Bacterial culture was suspended in sterile 0.1 M D-mannitol (Wako Pure Chemical Industries, Ltd., Japan) and centrifuged for 5 min at 12,000 × g at 4°C. The bacterial pellet was washed twice by suspending it in 0.1 M D-mannitol and centrifuged, then resuspended in 0.1 M D-mannitol. For the quantification experiments, the cells were suspended in 0.1 M D-mannitol to a final concentration of 1 × 10^7 cells/ml. Each bacterium was dispersed into 0.1 M D-mannitol by vortexing for 30 sec. In order to count the colony-forming units (CFUs), 50 µl of the bacterial solution was inoculated onto an agar plate using the SPC method (model D, Spiral System Co., Ohio, USA) as described before (Ishikawa et al., 2008). Each species of bacteria suspended in a 0.1M D-mannitol solution was put into the test cell at various diluted concentrations, and these samples were measured using the DEPIM apparatus as described above.

First, the relationship between the DEPIM measurement results by the apparatus and bacterial concentration determined by the SPC method was determined. Fig.1 shows the correlation between bacterial numbers determined by the DEPIM method and SPC method. Bacterial numbers measured by the two methods significantly correlated in the range of 10^5-10^8 CFU/ml (r²=0.633-0.996). Only C. albicans numbers significantly correlated in the range of 10^4 -10^7 CFU/ml (r²=0.997). However, for S. mutans DEPIM showed weak correlation with the SPC method.

**TABLE 1. Microorganisms used this study and culture conditions**

| Microorganism | Agar plate | Culture condition |
|---------------|------------|-------------------|
| Escherichia coli K12 | Trypticase soy | Aerobic incubation for 2 days at 37°C |
| Staphylococcus aureus FHSAT1 | Trypticase soy | Aerobic incubation for 2 days at 37°C |
| Pseudomonas aeruginosa PAO1 | Trypticase soy | Aerobic incubation for 2 days at 37°C |
| Candida albicans CAD1 | Sabouraud dextrose | Aerobic incubation for 3 days at 37°C |
| Streptococcus mutans ATCC25175 | Trypticase soy | Anaerobic incubation for 3 days at 37°C |
| Haemophilus influenzae | Blood TS | Anaerobic incubation for 3 days at 37°C |
| Capnocytophaga | Blood TS | Anaerobic incubation for 3 days at 37°C |
| Eikenella corrodens ATCC 23834 | Blood TS | Anaerobic incubation for 3 days at 37°C |
| Actinomyces viscosus ATCC 19246 | Blood TS | Anaerobic incubation for 3 days at 37°C |
| Fusobacterium nucleatum ATCC 25586 | Blood TS | Anaerobic incubation for 3 days at 37°C |
| Prevotella intermedia ATCC 25611 | Blood TS | Anaerobic incubation for 3 days at 37°C |
| Corynebacterium matruchotii K34 | Blood TS | Anaerobic incubation for 3 days at 37°C |
| Tannerella forsythia ATCC43037 | BF plates | Anaerobic incubation for 14 days at 37°C |

Blood TS, trypticase soy agar (Difco Laboratories, Detroit, MI) supplemented with 10% defibrinated horse blood, hemin (5.0 µg/ml) and menadione (1.0 µg/ml).

Anaerobically, 10% CO₂, 10% H₂, 80% N₂.

BF plates, described by K. Honma et al., 2001.
60 kD enzyme (kindly provided by Prof. M. Sugai, Hiroshima University, Hiroshima, Japan) in the reaction buffer (0.1 M NaPi and 0.1 M NaCl) for 50 min (Sugai et al., 1991). This enzyme is responsible for cell separation at the last stage of the division cycle of S. aureus (Sugai et al., 1991). After dispersion, the bacteria were collected and washed with 0.1 M D-mannitol. Finally, the bacterial number was determined with the DEPIM system and the SPC method, and the bacteria were Gram stained. As shown in Fig. 3, S. aureus usually grows in an auto-aggregated manner (a), and can be dispersed by the separation enzyme treatment (b). Fig. 3 (c) shows the bacterial number of aggregated and dispersed S. aureus determined by the DEPIM and SPC methods and demonstrated the relationship between both the aggregated and dispersed bacterial numbers detected by DEPIM and SPC methods. The regression curve of separation enzyme-treated cells shifted to the right from that of non-treated cells. This result means the enzymatic dispersal of S. aureus cells did not affect bacterial numbers detected by the DEPIM.
Oropharyngeal bacteria may translocate to lower respiratory tract and cause bronchitis and pneumonia. Oral care can reduce the oropharyngeal bacteria and then reduce the incidence of respiratory tract infection. To assess the effect of oral care, it is of importance to follow the change in oropharyngeal bacterial numbers. Counting bacterial numbers usually depends on the SPC method, which takes two or more days to give the results. A new method for the rapid quantification of oropharyngeal bacteria based on the DEPIM system has been developed, and its application to the counting of oropharyngeal bacteria was reported (Kikutani et al., 2012). This previous report showed that there was a high correlation between data from the DEPIM method and the conventional culture method using E. coli samples, as well as between the DEPIM method and both the culture and fluorescence microscopy methods using oral samples containing a mixture of various bacterial species taken from the tongues of elderly patients in nursing homes. The present results using the DEPIM system also correlated with those resulting from the SPC method examining a mixed microorganism suspension as well as a single culture of all tested species in the concentration range of $10^2$ - $10^5$ CFU/ml.

Interestingly, Ishikawa et al. (2008) evaluated the longitudinal prevalence of oropharyngeal bacteria in the institutionalized dependent elderly after a 5-month intervention of professional mechanical cleaning of the oral cavity and found that the levels of total oropharyngeal bacteria and streptococci decreased from $10^2$-$10^5$ to $10^2$-$10^6$ CFU/ml. Therefore, this rapid oral bacteria quantification (DEPIM) system is clinically useful because of this range for counting bacteria at the bedside. A clinical trial is required to confirm this.

Staphylococci and Candida species are drawing new attention because these microorganisms affect the emergence and establishment of antimicrobial resistance because of the high involvement of biofilms in chronic and systemic infections (Peters et al., 2010). *P. aeruginosa* and *S. aureus* are opportunistic pathogens and frequently co-infect the lungs of cystic fibrosis patients (Folescu et al., 2012). Recently, we also reported that the high detection rate of *P. aeruginosa* from the oropharyngeal microflora of cerebrovascular disease patients requiring daily nursing care is increased by the state of dysphagia, not catheter use, and suggested that concentrating on oropharyngeal microflora, especially *P. aeruginosa*, may be important in preventing aspiration pneumonia in cerebrovascular disease patients with dysphagia (Hirota et al., 2010). The DEPIM system can present bacterial numbers well correlated to that by the culture method even for mixed cultures of *S. aureus* and *P. aeruginosa* or *C. albicans* as shown in Fig. 2. This allows us to use the DEPIM system to determine the bacterial number from clinical samples.

*F. nucleatum* is the coaggregating partner with *P. gingivalis* and may be present with *P. gingivalis* in the oral cavity (Kinder and Holt, 1989). *P. gingivalis* and *F. nucleatum* are common isolates from aspiration pneumonia (Okuda, 2005; El-Solh et al., 2003). Our result with these bacterial species demonstrated that the DEPIM method is useful for counting the number of coaggregated bacterial samples better than the culture method (data not shown).

In conclusion, these results suggest that the DEPIM method is a potent tool for evaluating the microbial status of the oral cavity within one minute, and to know the risk of aspiration pneumonia.

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