those obtained by the culture method ($R = 0.852$, $p < 0.01$), as well as those obtained by the FM method ($R = 0.885$, $p < 0.01$). Moreover, there was a significant correlation between the data obtained by the culture method and those obtained by the FM method ($R = 0.934$, $p < 0.01$) (Figs 3–5).

Discussion

In the long-term care of elderly individuals, the incidence of aspiration pneumonia as a serious complication is high.[22,23] Oral health care is an important preventative measure against aspiration pneumonia in the elderly.[18,19] When aspiration pneumonia becomes serious, high medical costs related to hospital admission and medications are inevitable. To prevent aspiration pneumonia through oral health care maintains cost efficiency; lower-cost interventions in oral hygiene thus reduce some of the higher-cost outcomes of aspiration pneumonia.[24] People requiring or receiving care often cannot adequately maintain oral hygiene alone.[25,26] Therefore, caregivers and nursing staff are essential to maintain their oral environment, evaluation of oral conditions, planning of oral health care and oral health care by dental staff. Furthermore, to effectively design and conduct an oral health care plan, the state of oral health of individuals must first be clarified.

There are various bacterial species that cause aspiration pneumonia, with the major factor in the development of pneumonia being the amount rather than the type of oral bacteria aspirated.[15] Thus, measurement of the number of bacteria should be important in assessing the risk of aspiration pneumonia. In the field of dentistry, the amount of *Candida albicans* has been measured for the diagnosis of oral candidiasis, and the amounts of streptococci and lactobacilli have also been measured in plaque and saliva to evaluate the risk of caries. Methods to prevent these diseases and to reduce their risks have frequently been used in clinical practice. These methods include simple evaluations employing a colorimetric reagent that reacts with the acid metabolites of microorganisms[27] and the evaluation of colonies formed on selective media after culture.[28,29] On the other hand, as oral care parameters, observation of dental plaque accumulation[30,31] and tongue coating status[32,33] have been clinically applied, with both techniques found to be subjective.

The present apparatus using the DEPIM method, consisting of dielectrophoresis and impedance measurement, requires only 20 s for a measurement and can be readily performed in a short period of time by any examiner.[34] The results of this study showed a high correlation between data obtained by the DEPIM method and the conventional culture method using *E. coli* samples, as well as between the DEPIM method and both the culture and FM methods using oral samples containing a mixture of various bacterial species.

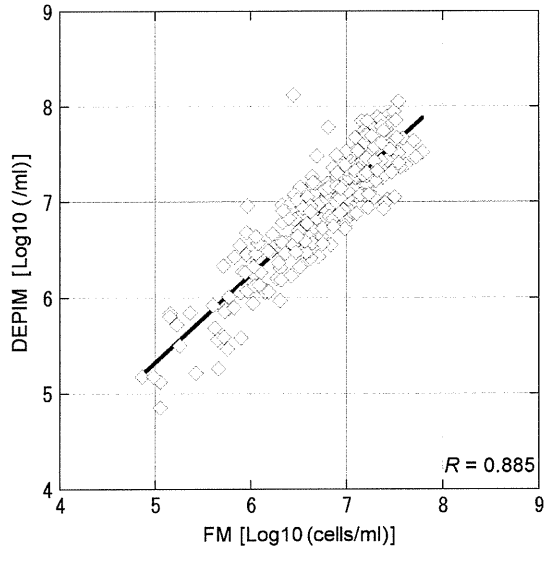

Figure 4 Relationship between dielectrophoretic impedance measurement (DEPIM) method and fluorescence microscopy (FM) method. There was a significant correlation between the DEPIM and FM methods ($R = 0.885$, $p < 0.01$).

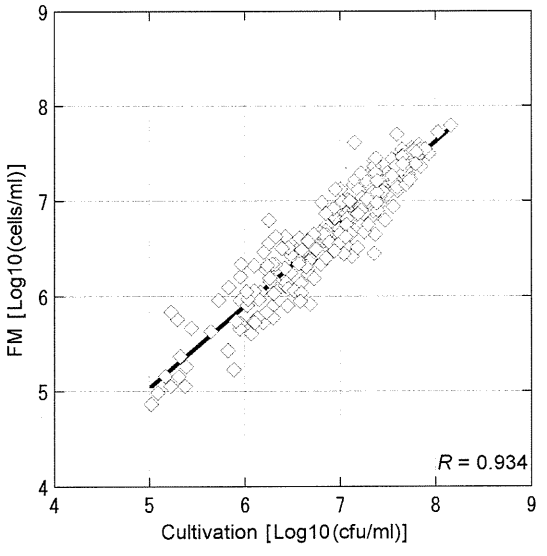

Figure 5 Relationship between culture method and fluorescence microscopy (FM) method. There was a significant correlation between the culture and FM methods ($R = 0.934$, $p < 0.01$).

Conclusion

The present apparatus is useful for measuring the number of oral bacteria and could be important in maintaining the quality of oral health care.

Acknowledgements

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References

1. **Scannapieco FA.** Pneumonia in nonambulatory patients. The role of oral bacteria and oral hygiene. *J Am Dent Assoc* 2006; **137**(Suppl): 21S–25S.
2. **Marik PE.** Aspiration pneumonitis and aspiration pneumonia. *N Engl J Med* 2001; **344:** 665–671.
3. **Loeb MB, Becker M, Eady A, Walker-Dilks C.** Interventions to prevent aspiration pneumonia in older adults: a systematic review. *J Am Geriatr Soc* 2003; **51:** 1018–1022.
4. **El Solh AA, Saliba R.** Pharmacologic prevention of aspiration pneumonia: a systematic review. *Am J Geriatr Pharmacother* 2007; **5:** 352–362.
5. **Yamaya M, Yanai M, Ohrui T, Arai H, Sasaki H.** Interventions to prevent pneumonia among older adults. *J Am Geriatr Soc* 2001; **49:** 85–90.
6. **Abe S, Ishihara K, Okuda K.** Prevalence of potential respiratory pathogens in the mouths of elderly patients and effects of professional oral care. *Arch Gerontol Geriatr* 2001; **32:** 45–55.
7. **Taylor GW, Loesche WJ, Terpenning MS.** Impact of oral diseases on systemic health in the elderly: diabetes mellitus and aspiration pneumonia. *J Public Health Dent* 2000; **60:** 313–320.
8. **Limeback H.** Implications of oral infections on systemic diseases in the institutionalised elderly with a special focus on pneumonia. *Ann Periodontol* 1998; **3:** 262–275.
9. **Finucane TE, Bynum JP.** Use of tube feeding to prevent aspiration pneumonia. *Lancet* 1997; **349:** 433.
10. **Scannapieco FA, Bush RB, Paju S.** Associations between periodontal disease and risk for nosocomial bacterial pneumonia and chronic obstructive pulmonary disease. A systematic review. *Ann Periodontol* 2003; **8:** 54–69.
11. **Paju S, Scannapieco FA.** Oral biofilms, periodontitis, and pulmonary infections. *Oral Dis* 2007; **13:** 508–512.
12. **Sumi Y, Miura H, Nagaya M, Michiwaki Y, Uematsu H.** Colonisation on the tongue surface by respiratory pathogens in residents of a nursing home – a pilot study. *Gerodontology* 2006; **23:** 55–59.
13. **Sumi Y, Miura H, Michiwaki Y, Nagaosa S, Nagaya M.** Colonization of dental plaque by respiratory pathogens in dependent elderly. *Arch Gerontol Geriatr* 2007; **44:** 119–124.
14. **Stoutenbeek CP, van Saene HK, Miranda DR, Zandstra DF.** The effect of selective decontamination of the digestive tract on colonisation and infection rate in multiple trauma patients. *Intensive Care Med* 1984; **10:** 185–192.
15. **Inglis TJ, Sherratt MJ, Sproat LJ, Gibson JS, Hawkey PM.** Gastroduodenal dysfunction and bacterial colonisation of the ventilated lung. *Lancet* 1993; **341:** 911–913.
16. **Hirota K, Yoneyama T, Ota M, Hashimoto K, Miyake Y.** Pharyngeal bacteria and professional oral health care in elderly people. *Nippon Ronen Igakkai Zasshi* 1997; **34:** 125–129 (in Japanese).
17. **Ishikawa A, Yoneyama T, Hirota K, Miyake Y, Miyatake K.** Professional oral health care reduces the number of oropharyngeal bacteria. *J Dent Res* 2008; **87:** 594–598.
18. **Yoneyama T, Yoshida M, Matsui T, Sasaki H.** Oral care and pneumonia. Oral Care Working Group. *Lancet* 1999; **354:** 515.
19. **Adachi M, Ishihara K, Abe S, Okuda K, Ishikawa T.** Effect of professional oral health care on the elderly living in nursing homes. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; **94:** 191–195.
20. **Yoneyama T, Yoshida M, Ohrui T *et al.* Oral Care Working Group** Oral care reduces pneumonia in older patients in nursing homes. *J Am Geriatr Soc* 2002; **50:** 430–433.
21. **Hamada R, Suehiro J, Nakano M, Kikutani T Konishi K.** Development of rapid oral bacteria detection apparatus based on dielectrophoretic impedance measurement method. *IET Nanobiotechol.*, 2011; **5:** 25–31.
22. **Marrie TJ, Blanchard W.** A comparison of nursing home-acquired pneumonia patients with patients with community-acquired pneumonia and nursing home patients without pneumonia. *J Am Geriatr Soc* 1997; **45:** 50–55.
23. **Mylotte JM, Goodnough S, Naughton BJ.** Pneumonia versus aspiration pneumonitis in nursing home residents: diagnosis and management. *J Am Geriatr Soc* 2003; **51:** 17–23.
24. **Terpenning M, Shay K.** Oral health is cost-effective to maintain but costly to ignore. *J Am Geriatr Soc* 2002; **50:** 584–585.
25. **Simons D, Kidd EA, Beighton D.** Oral health of elderly occupants in residential homes. *Lancet* 1999; **353:** 1761.
26. **Wårdh I, Berggren U, Andersson L, Sörensen S** Assessments of oral health carein dependent older persons in nursing facilities. *Acta Odontol Scand* 2002; **60:** 330–336.
27. **Camling E, Emilson CG.** Results with the caries activity test "Cariostat" compared to prevalence of

mutans streptococci and lactobacilli. *Swed Dent J* 1989; **13:** 125–130.
28. **Axéll T, Simonsson T, Birkhed D, Rosenborg J, Edwardsson S.** Evaluation of a simplified diagnostic aid (Oricult-N) for detection of oral candidoses. *Scand J Dent Res* 1985; **93:** 52–55.
29. **Asokan S, Rathan J, Muthu MS, Rathna PV Emmadi P, Raghuraman, Chamundeswari.** Effect of oil pulling on *Streptococcus mutans* count in plaque and saliva using Dentocult SM Strip mutans test: a randomized, controlled, triple-blind study. *J Indian Soc Pedod Prev Dent* 2008; **26:** 12–17.
30. **Silness J, Löe H.** Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964; **22:** 121–135.
31. **O'Leary TJ, Drake RB, Naylor JE.** The plaque control record. *J Periodontol* 1972; **43:** 38–41.
32. **Miyazaki H, Sakao S, Katoh Y, Takehara T.** Correlation between volatile sulphur compounds and certain oral health measurements in the general population. *J Periodontol* 1995; **66:** 679–684.
33. **Winkel EG, Roldan S, Van Winkelhoff AJ, Herrera D, Sanz M.** The clinical effects of a new mouthrinse containing chlorhexidine, cetylpyridinium chloride and zinc-lactate on oral halitosis. A dual-center, double-blind placebo-controlled study. *J Clin Periodontol* 2003; **30:** 300–306.
34. **Kikutani T, Tamura F, Sekino S *et al.*** Development and verification of a rapid oral bacteria detection system. In: *Proceedings of the 56th Annual Meeting of the Japanese Association for Dental Research.* Nagoya: School of Dentistry, Aichi Gakuin University, 2008: 61.

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Videoendoscopic assessment of swallowing function to predict the future incidence of pneumonia of the elderly

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SUMMARY **The purpose of the present study was to examine what dysphagic signs identified by videoendoscopy (VE) could predict the incidence of pneumonia and body weight loss in elderly patients living in nursing homes. This study was performed at six nursing care facilities in Japan from March 2007 to February 2009. The 148 subjects (85·1 ± 8·0 years, male/female: 43/105) were evaluated for their feeding and swallowing movements by clinical and VE examinations during the consumption of a regular meal. The VE examination items included the existence/absence of pharyngeal residue, laryngeal penetration, and aspiration of food and saliva. The patients were followed-up for 3 months with individualized feeding therapy based on the results of the clinical/VE examination at baseline, and the incidence of pneumonia was examined as the primary outcome. In patients without pneumonia, the body weight change was also measured as a secondary outcome. The risk factors for pneumonia and body weight loss (of 3% or more) were identified among the clinical/VE examination items by a Cox proportional hazard analysis. Even with elaborative feeding therapy, 12 (8·1%) of the 148 patients developed pneumonia during the 3 months follow-up period. The existence of signs of 'silent aspiration of saliva' or 'aspiration of saliva' detected by VE examination was a significant risk factor for both pneumonia and a body weight loss of 3% or more. This study shows that 'aspiration of saliva' detected by VE is a significant risk factor for both pneumonia and body weight loss in elderly patients living in nursing homes.**

KEYWORDS: **videoendoscopy, aspiration-related pneumonia, dysphagia, aspiration of saliva, body weight loss**

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Introduction

Dependent elderly patients are at high risk for feeding and swallowing disorders as a consequence of disease and/or aging (1–3). Studies done in long-term care facilities have shown a prevalence of such disorders ranging from 60% to 87% (4, 5). Among the various disorders, special attention has been given to dysphagia because it may lead to malnutrition with immune system compromise, dehydration, asphyxiation, or even aspiration pneumonia (1–3). Moreover, a previous follow-up study of patients with dysphagia in such care facilities revealed an incidence of pneumonia of 43% and a mortality rate of 45% at 1 year following the detection of their swallowing disorder (6). Therefore, clinicians should be able to identify dysphagia in order to predict those patients at risk of developing complications secondary to dysphagia, as well as to develop and implement a rehabilitation plan stressing prevention and compensation.

Videofluorography (VF) has been regarded as the most popular adjunctive instrument for the

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examination of patients with suspected oropharyngeal dysphagia. Previous studies have examined the use of VF as a means to predict those at risk for dysphagia and its complications (7, 8). For instance, Mann *et al.* (7) found that the single best independent predictor for chest infection following an acute stroke was a delayed or absent swallowing response in acute stroke patients. Teraoka *et al.* (8) found that the single best predictor of oral intake in post-stroke patients with dysphagia was the presence of aspiration detected by VF assessment. Nevertheless, one major disadvantage of VF for patients living in long-term care facilities is that the patients need to be transported to a hospital setting, which is sometimes inconvenient or may disorientate the patient because of the sudden change in the environment. Other disadvantages are related to the exposure to X-ray radiation and the risk of aspiration during VF assessment in some patients with severe physical or mental alterations (9).

On the other hand, videoendoscopic (VE) examination of swallowing allows for easy assessment of patients in their usual environment because the instrument is portable and does not require a radiology suite (10). Additionally, although VE is most useful for the examination of the integrity of the upper airway before and after a swallow response, it enables the evaluation of the tongue function during mastication and deglutition, as well as the detection of aspiration by the objective visualization of the airway (11, 12).

Videoendoscopic examination has been shown to successfully estimate the existence of accumulated oropharyngeal secretions, thus resulting in excellent prediction of aspiration (13, 14). In addition, Ota *et al.* (15) reported that the secretion scale based on the VE examination is a useful evaluation tool for predicting not only aspiration, but also pneumonia, in acute-phase dysphagic stroke patients. Furthermore, Link *et al.* (16) reported that there was a relationship between the VE-based pooled hypopharyngeal secretions, laryngeal penetration, aspiration and recurrent pneumonia with neurological disorders in pediatric patients. It is therefore evident that VE is the best tool to examine pooled hypopharyngeal secretions, laryngeal penetration, and aspiration. Therefore, even though the agreement rate between the VF and VE findings on dysphagia was shown to be high (90%) (17), VE examinations are becoming increasingly popular for examining the aspiration of saliva and food at the bedside and in long-term care facilities (17, 18).

In a prospective study with acute stroke patients, Lim *et al.* (19) found a strong association between aspiration detected by VE and the development of aspiration pneumonia. However, the predictors of aspiration pneumonia in dependent elderly patients with dysphagia in long-term care facilities have not been sufficiently investigated using VE. Therefore, the purpose of this prospective cohort study was to investigate whether the dysphagic signs identified by VE were risk factors for pneumonia and body weight loss in patients living in long-term care facilities.

Materials and methods

Subjects

Six hundred and forty-seven inpatients were initially identified from six nursing care facilities in Tokyo, Japan from March 2007 to February 2009 (Fig. 1). All patients, except for 28 subjects who were tube-fed, were screened for dysphagia by a check-list given to the patient's caregiver. The screening check-list contained 11 items: pooling of food, uncomfortable feeling in the throat, previous history of asphyxiation, previous history of aspiration, previous history of pneumonia, increased phlegm production, choking on saliva, choking on food, choking after a meal, prolongation of their eating time, and insufficient intake. The 171 patients who had at least one item checked positively by the caregiver were suspected to have dysphagia and comprised the intended sample population. However, 23 patients were excluded because of cognitive failure or refusal to participate in this study. Consequently, the final study population consisted of 148 patients (male/female: 43/105) with a mean age of 85·1 ± 8·0 years and an age range from 59 to 100 years. The protocol for this study was approved by the Ethics Committee of the Nippon Dental University School of Life Dentistry at Tokyo (#08-10).

Baseline measurements and feeding therapy

At the baseline measurement, a medical doctor assessed the patients' general health condition, and none of the patients fulfilled the Mann's criteria (7) for a diagnosis of pneumonia, that is, the presence of at least three of the following signs and symptoms: fever >38 °C, productive cough with sputum, tachypnea higher than 22 breaths per minute, inspiratory crackles,

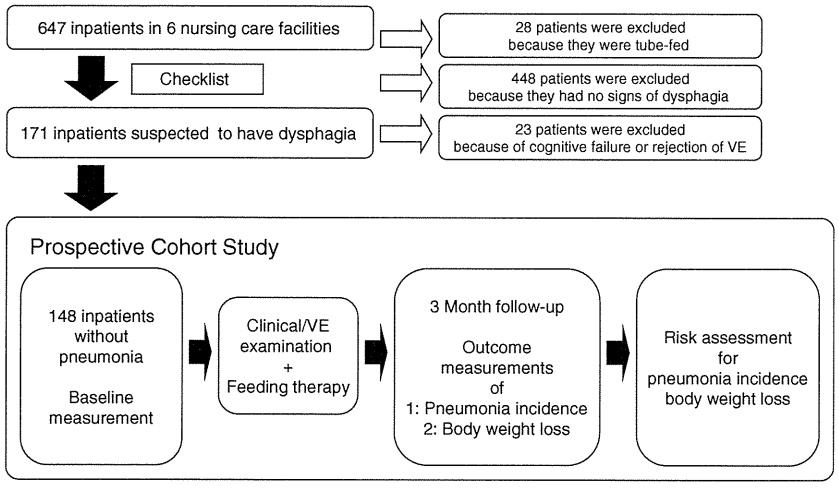

Fig. 1. The sampling process used for this study.

an abnormal chest x-ray, or positive gram staining and cultures.

All included subjects had their eating ability and dysphagic signs and symptoms evaluated clinically according to a clinical examination form regarding the signs and symptoms of dysphagia (spilling food, pooling food, oral food residue after a swallow, inability to open the mouth, choking/coughing, increased phlegm while eating, dyspnea, wet hoarseness, other), the hand and mouth coordination during the meal (feeding posture, prolongation of eating time) and the caregiver's technique used for feeding assistance.

In addition, each patient's swallowing function was examined by VE, which consisted of a flexible endoscope (ENF-V2*) connected to a high-intensity compact light source (CLH-SC*) and a video recorder (OTV-SC*).The endoscope was passed transnasally to the hypopharynx at a vantage point that provided a full view of the laryngeal vestibule, and was kept in place for a period of 10–15 min to assess the patient's eating ability, or saliva swallows when the patient was not consuming a meal. The patients were examined in their usual eating position, that is, the ambulatory patients were seating in the upright position, while the bed-bound patients were sitting on a bed. All swallows were recorded on videotapes for the further analyses by experienced physicians familiar with endoscopic swallowing studies and who were blinded to the intentions of the study. Each patient's video-recording data were reviewed for the presence or absence of pharyngeal residue, and penetration and aspiration of food or saliva. 'Penetration' was defined as a passage of material into the larynx that does not pass below the vocal folds, while 'aspiration' was defined as passage of material below the level of the vocal folds. In cases where the aspiration of food or saliva did not induce a cough, it was defined as 'silent aspiration' according to the criteria proposed by Rosenbek *et al.* (1996) (20). To assess the inter-rater reliability of the swallowing evaluations, the three investigators who were unaware of the original evaluation results, separately reviewed a random 10% sample of these evaluations. The overall agreement rate between investigators was substantial according to the Landis and Koch criteria (21) (kappa coefficient = 0·660).

On the basis of these aforementioned evaluations, the patients received various feeding therapies (22) during the follow-up period, for example, confirmation of feeding conditions [76 patients (51·4%) of 148 patients, multiple answers possible], appropriate feeding assistance [69 patients (46·6%)], food modification [32 patients (21·6%)], modification in feeding posture [19 patients (12·8%)] and modification in food intake [four patients (2·0%)] for 3 months. Food modification involved changing the dietary consistency. We modified the food and liquid texture individually according to the National Dysphagia Diet recommendations (23). Food intake and feeding assistance required modifications to accommodate the individual needs of the patients, such as changes in the rate and amount of the food consumed, appropriate utensils and the

*Olympus Corporation, Tokyo, Japan.

method used for self-feeding (22). Modifications in the feeding posture were applied in order to maximize the physical capabilities and improve swallowing, and involved strategies such as head-turn or chin-tuck maneuvers or whole body-positioning strategies including the patient tilting to the side or back, side-lying, or maintaining an upright posture (22). All patients received oral health care after every meal by the caregiver who was instructed once a week about the oral care procedures by a dental hygienist. Caregivers cleaned each patient's oral cavity using a toothbrush for approximately 5 min after each meal. The brushing was carried out as usual for daily tooth brushing without paste, and included brushing the palatal and mandibular mucosa and tongue dorsum. Dentures were also cleaned with a denture brush every day.

The 3 month follow-up and outcome measurement

The first outcome variable after 3 months of follow-up was the incidence of pneumonia diagnosed according to the same criteria applied at the baseline measurement. Once the patients received a diagnosis of pneumonia, they were sent to a local hospital for treatment, without exception. Consequently, their oral feeding was prohibited to prevent further aspiration pneumonia and their body weight typically decreased as a result (24). The incidence of pneumonia and body weight loss were therefore strongly correlated after the development of pneumonia. Thus, when pneumonia was identified, follow-up measurements of the patient's body weight were terminated.

The second outcome variable during the follow-up period was a change in body weight demonstrated by monthly measurements. Since there is a close relationship between pneumonia and body weight loss, the incidence of body weight loss of 3% or more was examined in patients who had not been diagnosed with pneumonia during the 3 months of follow-up. Once the patients developed a body weight loss of 3% or more, the patients received some form of nutrition therapy, and thus, the follow-up observation was terminated.

Statistical analysis

A survival curve of the patients who had not been diagnosed with pneumonia was drawn for a Kaplan–Meier analysis. According to the presence/absence of pneumonia during the 3 months of follow-up, we divided the final sample population into pneumonia and non-pneumonia sub-groups, and performed a *t*-test, chi-square analysis or Fisher's exact test to analyse the differences between the two groups.

Similarly, a survival curve of those patients who had not lost more than 3% of their body weight was drawn for a Kaplan–Meier analysis (outcome event: the incidence of body weight loss of 3% or more). Differences between the weight gain/no change sub-group (body weight gain, or a small weight loss of no more than 3% of the initial body weight) and the weight loss group (body weight loss of 3% or more (10, 25)) were analysed with the same statistical tests utilized for the incidence of pneumonia.

Additionally, a Cox proportional hazard analysis was performed to identify the risk factors for the incidence of pneumonia and the body weight loss of 3% or more. The analysed predictors were age, self-feeding ability, the Barthel activities of daily living (ADL) index, a body mass index (BMI) lower than 18·5, pharyngeal residue, laryngeal penetration, aspiration of food and aspiration of saliva. Regarding the aspiration of food or saliva, the data were handled as ordinal variables (negative, positive, positive as silent aspiration). The data were analyzed with the Statistical Package for the Social Sciences software program (SPSS version 15.0†). A *P*-value $<0{\cdot}05$ was considered to be statistically significant.

Results

Baseline condition of the patients

Examination of the medical conditions of the initial 148 patients showed the presence of a prior stroke in 83 (comorbidity admitted) (56·1%), dementia in 74 (50·0%), Parkinson's disease in 10 (6·8%), cardiovascular disease in 10 (6·8%), hypertension in 8 (5·4%), previous pneumonia in 5 (3·4%), diabetes mellitus in 3 (2·0%), fractures in 3 (2·0%) and other comorbidities in 14 patients (9·5%).

The clinical examination regarding the eating ability and signs and symptoms of dysphagia before the VE evaluation showed choking/coughing in 110 out of 148 patients (multiple choice admitted), pooling of food in 28, prolongation of the eating time in nine, inability to

†SPSS Japan Inc., Tokyo, Japan.

open the mouth in two, and spilling of food in one patient.

The VE evaluation detected pharyngeal residue in 97 (65·5%) out of the 148 patients, laryngeal penetration in 67 (45·3%), aspiration of food in 41 (27·7%), silent aspiration of food in 19 (12·8%), aspiration of saliva in 8 (5·41%), and silent aspiration of saliva in 10 (6·76%) patients (Table 1).

Risk factors for pneumonia and body weight loss

Even with elaborative feeding therapy, during the 3 months of follow-up after the baseline measurement, 12 (8·1%) of the 148 patients developed pneumonia (Fig. 2). In addition, among the non-pneumonia patients, 90 (66·2%) of them presented with weight gain, no change or weight loss of 3% or less (weight gain/no change group), while 46 patients (33·8%) lost 3% or more of their body weight (weight loss group) (Fig. 3).

The differences between the pneumonia and non-pneumonia groups concerning the clinical/demographic data and the dysphagic signs detected by VE are shown in Table 1. The unpaired *t*-test showed that there were no significant differences in the patient age ($P = 0·505$), gender ($P = 0·244$), self-feeding ability ($P = 0·419$), number of patients with a BMI lower than 18·5 ($P = 0·190$), and the Barthel Index ($P = 0·060$) between the subjects with and without pneumonia. On the other hand, there was a significant difference in the frequency of 'aspiration of saliva' between the pneumonia and non-pneumonia patients ($P = 0·026$). In contrast, a comparison between the body weight gain/no change and body weight loss groups showed that there were no significant differences concerning any of the analysed variables (Table 2).

The results of the Cox proportional hazard analysis revealed that a sign of the 'aspiration of saliva' detected by VE was a significant risk factor for pneumonia (Table 3) and for a body weight loss of 3% or more (Table 4).

Discussions

The presence of aspiration-related pneumonia is known to be associated with a high mortality rate in the elderly. Patients in nursing homes may have a higher incidence of pneumonia because of their multiple underlying diseases, which may lead to immunosuppression, excessive use of medications, generalized decreased functional status, as well as factors related to malfunctioning of the masticatory and orophraryngeal systems and inadequate oral care. In particular, dysphagia is known to be strongly associated with aspiration pneumonia. Teramoto *et al.* (26). reported

Table 1. The relationship between the clinical/VE signs and the incidence of pneumonia

	Total subjects	No pneumonia ($n = 136$)	Pneumonia ($n = 12$)	*P*-value
Age (mean ± s.d.)	148	85·0 ± 8·1	86·8 ± 5·4	0·505†
Male/female	148	38/98	5/7	0·244††
Self-feeding (yes/no)	148	47/89	5/7	0·419††
Barthel Index (mean ± s.d.)	116*	13·1 ± 18·1	7·2 ± 7·12	0·060†
BMI < 18·5**	118**	43/110 (39·1%)	5/8 (62·5%)	0·190††
Pharyngeal residue	148	88 (64·7%)	9 (75·0%)	0·354††
Laryngeal penetration	148	62 (45·6%)	5 (41·7%)	0·519††
Aspiration of food	148			0·326††
Silent aspiration	19	19	0	
Aspiration	41	38	3	
NA	88	79	9	
Aspiration of saliva	148			0·026††
Silent aspiration	10	7	3	
Aspiration	8	7	1	
NA	130	122	8	

*Of 116 patients, 107 were in the no pneumonia group and nine were in the pneumonia group.
**Of 118 patients, 110 were in the no pneumonia group and eight were in the pneumonia group.
†*T*-test.
††Chi-square test.

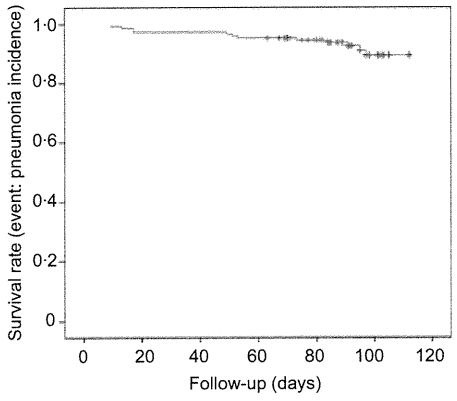

Fig. 2. The survival curve of the patients who did not suffer from pneumonia. The survival curve was drawn for a Kaplan–Meier analysis (outcome event: incidence of pneumonia).

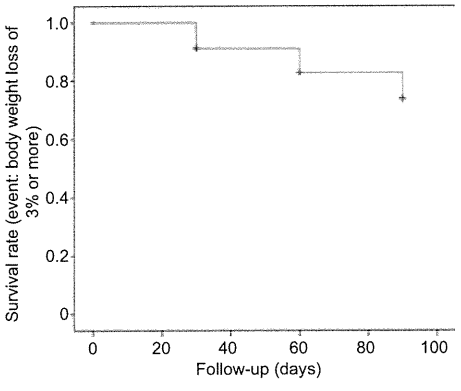

Fig. 3. The survival curve of the patients who did not suffer from a body weight loss of 3% or more. The survival curve was drawn for a Kaplan–Meier analysis (outcome event: incidence of body weight loss of 3% or more).

that 70% of the pneumonia in the elderly occurred due to aspiration, and Yamaya *et al.* (27). reported a high prevalence of silent aspiration in older persons leading to the deterioration of swallowing function due to cerebrovascular disease. In a previous study, Doggett *et al.* (28) estimated that approximately 43–54% of stroke patients have dysphagia and aspiration of food or saliva, and that approximately 37% of these patients would develop aspiration-related pneumonia.

In this present study, penetration and aspiration (apparent or silent) was observed in 67 subjects (45·2%) and 60 subjects (40·5%), respectively. The prevalence of aspiration found in this investigation was relatively high compared to previous studies utilizing VE examination (29%) (29), but was similar to the range observed in a previous review article where it was reported to occur in 15–39% of subacute dysphagic stroke patients (30). According to this review, the exact prevalence of aspiration remains unknown because of the differences in the size and methodology used in the existing studies.

The incidence of pneumonia was 12 (8·1%) among the 148 subjects (Table 1), which is in accordance with the study by Lim *et al.* (19), who reported that five patients (10%) developed pneumonia during their inpatient stay, and that all of them were at risk of aspiration of saliva or food as determined by a VE examination. On the other hand, Croghan *et al.* (6) reported that 55% of their nursing home patients presented with aspiration on VF examination, and 43% developed pneumonia.

One possible reason for such a discrepancy in the association of pneumonia and aspiration or penetration could be due to the technique (VE vs. VF) utilized to assess the swallowing disorders. Although a number of methods have been used to detect the symptoms of dysphagia, it is very difficult to evaluate 'silent aspiration of saliva' with a bedside clinical assessment alone, because it has been shown that it is missed in up to 40% of the patients aspirating silently (31, 32). At present, VF and VE are regarded as the best methods to evaluate swallowing function. In particular, VF has been used as a gold standard to evaluate swallowing because it can detect aspiration. However, it may not be as accurate in identifying 'silent aspiration of saliva', as compared to VE, because the latter enables direct visualization of the aspiration of saliva (18, 33, 34). Kelly *et al.* (35) reported that penetration and aspiration are perceived more sensitively in VE images than in VF images of the same swallows. It is also well known that VE can identify the microaspiration and aspiration of secretions with a high reliability, whereas VF cannot (36, 37). Additional advantages of VE are related to its application. Inpatients may become agitated or fatigued in the radiology suite or may not respond well to the taste of barium-coated boluses, or may even reject the radiation exposure, limiting the applications of VF. Videoendoscopy allows the patient's examination to be performed regardless of his/her altered mental status or immobility (38). Finally, Wu *et al.* (39) stated that VE is conclusively a safe, more efficient and sensitive method than VF for evaluating swallowing.

Another reason for the discrepancy could be the effect of the feeding therapy provided in this study, which could have reduced the symptoms of dysphagia,

Table 2. The relationship between the clinical/VE signs and the change in body weight

	Total subjects	Gain/no change (n = 90)	Weight loss (n = 46)	P-value
Age (mean ± s.d.)	136	84·6 ± 8·0	85·7 ± 8·6	0·464†
Male/female	136	25/65	13/33	0·553††
Self-feeding (yes/no)	136	29/61	16/30	0·454††
Barthel Index (mean ± s.d.)	107*	14·9 ± 18·7	9·6 ± 17·0	0·163†
BMI < 18·5	110**	30/74 (40·5%)	13/36 (36·1%)	0·655††
Pharyngeal residue	136	61 (67·8%)	27 (58·7%)	0·294††
Laryngeal penetration	136	44 (48·9%)	18 (39·1%)	0·2797††
Aspiration of food	136			0·975††
Silent aspiration	19	13	6	
Aspiration	38	25	13	
No aspiration	79	52	27	
Aspiration of saliva	136			0·342††
Silent aspiration	7	4	3	
Aspiration	7	3	4	
No aspiration	122	83	39	

Weight loss was diagnosed as the loss of 3% or more of the body weight from the baseline measurement.
*Of the 107 patients, 72 were in the gain/no change group and 35 were in the weight loss group.
**Of the 110 patients, 74 were in the gain/no change group and 36 were in the weight loss group.
†T-test.
††Chi-square test.

Table 3. The results of the Cox proportional hazard analysis for the possible predictors of the incidence of pneumonia

Predictors	B	P-value	HR	95% CI
Age	0·011	0·860	1·011	0·900–1·135
Self-feeding	0·105	0·909	1·111	0·182–6·785
Barthel Index	−0·010	0·769	0·990	0·927–1·057
BMI < 18·5	2·064	0·070	7·874	0·844–73·440
Pharyngeal residue	−0·621	0·615	0·537	0·048–6·067
Laryngeal penetration	0·571	0·642	1·771	0·160–19·644
Aspiration of food (negative/positive/ positive with SA)	−0·216	0·830	0·805	0·112–5·794
Aspiration of saliva (negative/positive/ positive with SA)	1·290	0·025	3·634	1·174–11·242

HR, hazard ratio; CI, confidence interval; SA, silent aspiration.

Table 4. The results of the Cox proportional hazard analysis for the possible predictors of a body weight loss of 3% or more

Predictors	B	P-value	HR	95% CI
Age	0·019	0·448	1·019	0·971–1·070
Self-feeding	0·530	0·228	1·698	0·718–4·014
Barthel Index	0·000	0·992	1·000	0·976–1·025
BMI < 18·5	0·859	0·032	2·362	1·074–5·191
Pharyngeal residue	−0·060	0·896	0·942	0·381–2·325
Laryngeal penetration	0·019	0·970	1·019	0·374–2·780
Aspiration of food (negative/positive/ positive with SA)	−0·203	0·569	0·816	0·405–1·644
Aspiration of saliva (negative/positive/ positive with SA)	1·186	0·000	3·275	1·828–5·866

HR, hazard ratio; CI, confidence interval; SA, silent aspiration.

pharyngeal residue, laryngeal penetration, and aspiration of food, as demonstrated by the fact that 66% of the subjects were able to increase their body weight or keep the body weight loss to within 3%. Nevertheless, a detailed analysis of the effectiveness of feeding therapy on the reduction of the symptoms of dysphagia could not be performed, because it was beyond the scope of this study.

Additionally, the differences in the target populations and their respective medical conditions could also have affected the overall incidence of pneumonia. This study gathered a heterogeneous patient population consisting of patients presenting with well-known disorders/diseases associated with the symptoms of dysphagia (e.g. stroke, Parkinson's disease, dementia) as well as other non-debilitating diseases/disorders (hypertension, fractures). On the other hand, a strong point in this study was the inclusion of a relatively high number of subjects from six nursing care facilities, which was large compared to other follow-up studies. Therefore,

the incidence of pneumonia may have been relatively lower in such a large heterogeneous study sample.

Regarding the risk factors associated with the development of pneumonia, some of them were reported to be age, primary disease, consciousness disorders, nutritional status, poor ADL, poor oral status, and swallowing dysfunction (40, 41). In the present study, among the analysed predictors, the 'aspiration of saliva' detected by VE was the only significant risk factor for pneumonia. In cases of bad oral health, saliva contains numerous bacteria. Therefore, patients with silent aspiration of saliva (without a cough reflex) are aspirating bacteria, which may be the main factor responsible for increasing the risk of pneumonia.

Additionally, even with the elaborative feeding therapy provided in this study, the control of aspiration of saliva or silent aspiration of saliva was generally difficult. In the present study, there was also a tendency for there to be a higher incidence of pneumonia in poor ADL patients. Langmore *et al.* (42) also reported that severely dependent functional status was an especially potent predictor of aspiration pneumonia. Riquelme *et al.* (40) reported that there was a significant relationship between the ADL and mortality rate. It was also observed that patients with a BMI < 18·5 had a higher tendency to develop pneumonia ($P = 0{\cdot}070$) compared with those with a poor ADL ($P = 0{\cdot}769$). It is well known that a lower nutrition condition affects the host immunological function, thus making the subjects more susceptible to pneumonia (43).

On the other hand, aspiration of saliva was also detected as a significant risk factor for body weight loss in this study. This finding could be explained by the possible presence of subclinical aspiration-related pneumonia in those subjects with a body weight loss of 3% or more.

The overall findings in this study demonstrated that it is still very difficult to prevent aspiration of saliva even if physicians provide elaborative feeding therapy and even if patients do not eat and drink anything through the mouth. Effective strategies to prevent the silent aspiration of saliva will therefore be an important target for future research.

Conclusion

The results of this study showed that, even with elaborative feeding therapy, 'aspiration of saliva' as detected by videoendoscopic examination was found to be a significant risk factor for pneumonia and a body weight loss of 3% or more in elderly patients living in nursing homes.

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References

1. Ekberg O, Feinberg MJ. Altered swallowing function in elderly patients without dysphagia: radiologic findings in 56 cases. AJR Am J Roentgenol. 1991;156:1181–1184.
2. Sheth N, Diner W. Swallowing problems in the elderly. Dysphagia. 1988;3:209–215.
3. Tibbling L, Gustafsson B. Dysphagia and its consequences in the elderly. Dysphagia. 1991;6:200–202.
4. Siebens H, Trupe E, Siebens A, Cook F, Anshen S, Hanauer R *et al.* Correlates and consequences of eating dependency in the institutionalized elderly. J Am Geriatr Soc. 1986;34:192–198.
5. Steele CM, Greenwood C, Ens I, Robertson C, Seidman-Carlson R. Mealtime difficulties in a home for the aged: not just dysphagia. Dysphagia. 1997;12:43–50.
6. Croghan JE, Burke EM, Caplan S, Denman S. Pilot study of 12 month outcomes of nursing home patients with aspiration on videofluoroscopy. Dysphagia. 1994;9:141–146.
7. Mann G, Hankey GJ, Cameron D. Swallowing function after stroke: prognosis and prognostic factors at 6 months. Stroke. 1999;30:744–748.
8. Teraoka F, Nishi M, Yoshizawa T, Momose M, Hirashima Y, Ichikawa T. Outcome of dysphagia in stroke patients: predictive factors for the resumption of a regular diet (in Japanese). Jpn J Rehabil Med. 2004;41:421–428.
9. Schroter-Morasch H, Bartolome G, Troppmann N, Ziegler W. Values and limitations of pharyngolaryngoscopy (transnasal, transoral) in patients with dysphagia. Folia Phoniatr Logop. 1999;51:172–182.
10. Kikutani T, Takahashi N, Fukui T, Katagiri H, Tohara T, Tamura F *et al.* Nourishment support in the nursing care facility for the elderly through implemented conferencing for feeding support (in Japanese). Jpn J Gerodontol. 2008;22:371–376.
11. Takahashi N, Kikutani T, Tamura F, Suda M, Fukui T, Katagiri H *et al.* Evaluation of tongue motor function using videoendoscopic evaluation system for patients with mastication disorders with motor dysfunction (in Japanese). Jpn J Gerodontol. 2009;24:20–27.
12. Abe R, Furuya J, Suzuki T. Videoendoscopic measurement of food bolus formation for quantitative evaluation of masticatory function. J Prosthodont Res. 2011;55:171–178.

13. Murray J, Langmore S, Ginsberg S, Dostie A. The significance of accumulated oropharyngeal secretions and swallowing frequency in predicting aspiration. Dysphagia. 1996;11:99–103.
14. Donzelli J, Brady S, Wesling M, Craney M. Predictive value of accumulated oropharyngeal secretions for aspiration during video nasal endoscopic evaluation of the swallow. Ann Otol Rhinol Laryngol. 2003;112:469–475.
15. Ota K, Saitoh E, Baba M, Sonoda S. The secretion severity rating scale: a potentially useful tool for management of acute-phase fasting stroke patients. J Stroke Cerebrovasc Dis. 2011;20:183–187.
16. Link DT, Willging JP, Miller CK, Cotton RT, Rudolph CD. Pediatric laryngopharyngeal sensory testing during flexible endoscopic evaluation of swallowing: feasible and correlative. Ann Otol Rhinol Laryngol. 2000;109:899–905.
17. Langmore SE, Schatz K, Olson N. Endoscopic and videofluoroscopic evaluations of swallowing and aspiration. Ann Otol Rhinol Laryngol. 1991;100:678–681.
18. Bastian RW. The videoendoscopic swallowing study: an alternative and partner to the videofluoroscopic swallowing study. Dysphagia. 1993;8:359–367.
19. Lim SHB, Lieu PK, Phua SY, Seshadri R, Venketasubramanian N, Lee SH *et al.* Accuracy of bedside clinical methods compared with fiberoptic endoscopic examination of swallowing (FEES) in determining the risk of aspiration in acute stroke patients. Dysphagia. 2001;16:1–6.
20. Rosenbek JC, Robbins J, Roecker EB, Coyle JL, Wood JL. A penetration-aspiration scale. Dysphagia. 1996;11:93–98.
21. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159–174.
22. Crary M, Groher M (eds). The introduction to adult swallowing disorders. Woburn, MA: Butterworth-Heinemann; 2003.
23. National Dysphagia Diet Task Force. The National Dysphagia Diet: standardization for optimal care. Chicago, IL: American Dietetic Association; 2002.
24. Cabre M, Serra-prat M, Palomera E, Almirall J, Pallares R, Clave P. Prevalence and prognostic implications of dysphagia in elderly patients with pneumonia. Age Ageing. 2010;39:39–45.
25. Blackburn GL, Bistrian BR, Maini BS, Schlamm HT, Smith MF. Nutritional and metabolic assessment of the hospitalized patient. J Parenter Enteral Nutr. 1977;1:11–22.
26. Teramoto S, Fukuchi Y, Sasaki H, Sato K, Sekizawa K, Matsuse T. High incidence of aspiration pneumonia in community- and hospital-acquired pneumonia in hospitalized patients: a multicenter, prospective study in Japan. J Am Geriatr Soc. 2008;56:577–579.
27. Yamaya M, Yanai M, Ohrui T, Arai H, Sasaki H. Interventions to prevent pneumonia among older adults. J Am Geriatr Soc. 2001;49:85–90.
28. Doggett DL, Tappe KA, Mitchell MD, Chapell R, Coates V, Turkelson CM. Prevention of pneumonia in elderly stroke patients by systematic diagnosis and treatment of dysphagia: an evidence-based comprehensive analysis of the literature. Dysphagia. 2001;16:279–295.
29. Leder SB, Sasaki CT, Burrell MI. Fiberoptic endoscopic evaluation of dysphagia to identify silent aspiration. Dysphagia. 1998;13:19–21.
30. Ramsey D, Smithard D, Kalra L. Silent aspiration: what do we know? Dysphagia. 2005;20:218–225.
31. Linden P, Siebens A. Dysphagia: predicting laryngeal aspiration. Arch Phys Med Rehabil. 1983;64:281–284.
32. Logemann JA. Evaluation and treatment of swallowing disorders. San Diego: College-Hill Press; 1983.
33. Kidder TM, Langmore SE, Martin JW. Indications and techniques of endoscopy in evaluation of cervical dysphagia: comparison with radiographic techniques. Dysphagia. 1994;9:256–261.
34. Broniatowski M. Fiberoptic endoscopic evaluation of dysphagia and videofluoroscopy. Dysphagia. 1998;13:22–23.
35. Kelly A, Drinnan M, Leslie P. Assessing penetration and aspiration: how do videofluoroscope and fiberoptic endoscopic evaluation of swallowing compare? Laryngoscope. 2007;117:1723–1727.
36. Gerek M, Atalay A, Cekin F, Ciyiltepe M, Ozkaptan Y. The effectiveness of fiberoptic endoscopic swallow study and modified barium swallow study techniques in diagnosis of dysphagia. Kulak Burun Bogaz Ihtis Derg. 2005;15:103–111.
37. Tohara H, Nakane A, Murata S, Mikushi S, Ouchi Y, Wakasugi Y *et al.* Inter- and intra-rater reliability in fibroptic endoscopic evaluation of swallowing. J Oral Rehabil. 2010;33:884–891.
38. Staff DM, Shaker R. Videoendoscopic evaluation of supraesophageal dysphagia. Curr Gastroenterol Rep. 2001;3:200–205.
39. Wu CH, Hsiao TY, Chen JC, Chang YC, Lee SY. Evaluation of swallowing safety with fiberoptic endoscope: comparison with videofluoroscopic technique. Laryngoscope. 1997;107:396–401.
40. Riquelm R, Torres A, El-Ebiary M, De La Bellacasa JP, Estruch R, Mensa J *et al.* Community-acquired pneumonia in the elderly: a multivariate analysis of risk and prognostic factors. Am J Respir Crit Care Med. 1996;154:1450–1455.
41. Splaingard M, Hutchins B, Sulton L, Chaudhuri G. Aspiration in rehabilitation patients: videofluoroscopy vs bedside clinical assessment. Arch Phys Med Rehabil. 1988;69:637–640.
42. Langmore SE, Terpenning MS, Schork A, Chen Y, Murray JT, Lopatin D *et al.* Predictors of aspiration pneumonia: how important is dysphagia? Dysphagia. 1998;13:69–81.
43. Rothan-Tondeur M, Meaume S, Girard L, Weill-Engerer S, Lancien E, Abdelmalak S *et al.* Risk factors for nosocomial pneumonia in a geriatric hospital: a control-case one-center study. J Am Geriatr Soc. 2003;51:997–1001.

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Tooth loss as risk factor for foreign-body asphyxiation in nursing-home patients

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ABSTRACT

Foreign body asphyxiation causes severe medical conditions including pneumonia in the elderly requiring nursing care. The objective of this study was to elucidate the relationships between insufficient occlusal support due to tooth loss and the onset of asphyxiation accidents, and determine preventive measures for such accidents in nursing homes in Japan. The subjects were 437 elderly (110 men and 327 women) requiring nursing care. The frequency and risk factors for asphyxiation accidents and the food causing asphyxiation were examined in these subjects for 2.5 years, from June 2006 to December 2008. During the study period, 51 of the 437 subjects suffered asphyxiation. Self-feeding ability and loss of occlusal support were associated with a covariate-adjusted relative ratio for asphyxiation of 3.1 (95% confidence interval (CI) = 1.50–6.44) and 1.7 (95% CI = 1.12–2.74), respectively. To prevent asphyxiation in elderly people, it was found that maintaining or restoring occlusal support may be required. It was concluded that self-feeding ability and loss of occlusal support are significant risk factors for foreign-body asphyxiation among elderly people requiring nursing care.

1. Introduction

Asphyxiation occurs when any object is aspirated into the larynx or tracheobronchial tree, and causes airway obstruction: this obstruction can result in inability to breathe, with the need for rapid intervention to prevent asphyxial death (Ekberg and Feinberg, 1992). Therefore, asphyxiation cases are identified by signs and symptoms of dyspnea, abnormal respiratory rate, rhythm/depth of breathing, restlessness and cyanosis. Asphyxia is reported to be a common cause of death not only in the general population (Feinberg et al., 1992), but also in infants (<1 year of age) and the elderly. To date, a high frequency (incidence: 0.66 fatalities/100,000) of asphyxia has been reported in the general population each year (Fioritti et al., 1997). In Japan, deaths from asphyxiation have increased since the 1980s. Moreover, the mortality from asphyxiation incidents in infants has decreased by more than 60% in the past 30 years. However, mortality from asphyxiation in the elderly has increased rapidly (Ichikawa and Marui, 2000). This represents an important warning regarding asphyxiation to both elderly receiving care and their caregivers.

Samuels and Chadwick (2006) reported rapid eating, cramming of food into the mouth, and premature transfer of food into the pharynx as possible causes of asphyxiation in the elderly. Several reports have also suggested that oral stage dysfunction and cognitive impairment contribute to asphyxiation (Carter and Jancar, 1984; Feinberg et al., 1992; Finestone et al., 1998). A huge number of elderly people, including those requiring nursing care, lose teeth and/or occlusal support, resulting in decreased oral function (Hatch et al., 2001). Since wearing dentures is related to oral function, speech function and independent activities of daily living (Minakuchi et al., 2006), elderly people who lose occlusal support must wear dentures. However, it is sometimes difficult for elderly people requiring nursing care to wear dentures, for many reasons including mismanagement of dentures, losing dentures, and shortage of oral-care services. There have been few reports on the relationship between asphyxiation accidents and insufficient occlusal support due to tooth loss or failure to restore occlusal support by means of dentures. Haddon suggested that it is possible to eliminate these risk factors related to accidental death. Even when accidents do occur, the worst outcome can be avoided by thorough application of appropriate measures during and after an accident (Haddon, 1980).

In the present study, we examined the risk factors for asphyxiation accidents among the elderly in nursing homes, and determined the relationships among insufficient occlusal support caused by tooth loss, restoration of occlusal support by means of dentures, and asphyxiation accidents.

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2. Materials and methods

2.1. Participants

The survey was conducted in 486 individuals (mean age: 85.0 ± 8.5 years), whose guardians gave consent to their participation in the present study, among elderly people requiring care in 13 nursing homes in Japan. Of them, those who were discharged from nursing homes ($n = 49$) because of other reasons than asphyxiation were excluded from this study, since we could no longer peruse them. The study was performed on 437 subjects (110 men and 327 women; mean age, 80.8 ± 8.3 years for men and 86.4 ± 8.1 years for women; Barthel index, 25.1 ± 25.0).

2.2. Survey of asphyxiation

Asphyxiation occurring while eating food over a period of 2 years and 6 months from June 2006 to December 2008 was examined, and the outcomes were also determined. In this study, asphyxiation accidents were limited to those caused by food.

Asphyxiation cases were identified by signs and symptoms such as dyspnea, abnormal respiratory rate, rhythm/depth of breathing, restlessness and cyanosis as reported by the North American Nursing Diagnosis Association (2003).

2.3. Examination of risk factors for asphyxiation

The following six items were assessed to examine the risk factors for asphyxiation. In terms of the oral environment and oral function, the procedures used by dentists and physicians in each nursing home were studied.

2.3.1. Self-feeding ability

Subjects who could feed themselves at least partly without any help were assigned to the "independent group" and those who were able to eat only with assistance were assigned to the "dependent group".

2.3.2. Activity of daily living (ADL)

ADL in these subjects was evaluated using the Barthel Index (Mahoney and Barthel, 1965). When the index was 45 points or higher, ADL was considered to be maintained, and when the index was less than 40 points, ADL was considered to be decreased.

2.3.3. Cognitive function

The severity of senile dementia was evaluated according to "ADL independence of demented elderly", designed by the Ministry of Health, Labour and Welfare of Japan (http://www.mhlw.go.jp:80/topics/kaigo/kentou/15kourei/sankou4.html; Hirakawa et al., 2008). Cognitive impairment was identified at rank 2 and higher of this scale (Table 1).

Table 1
ADL independence of demented elderly (Ministry of Health, Labour and Welfare of Japan).

Rating criteria	Description
Rank 0	Clear mentality
Rank 1	Although demented, the subject is almost independent in ADL at home or elsewhere
Rank 2	The subject shows slight impairment of cognition, but is independent under a carer's observation
Rank 3	The subject sometimes shows impairment of cognition, thus a carer is required
Rank 4	The subject often shows impairment of cognition, thus a carer is required all time
Rank 5	The subject shows serious mental symptoms or problematic behavior, thus specific medical care is required

2.3.4. Tongue coating

Tongue coating was visually evaluated according to the report by Miyazaki et al. (1995). We divided the scores into two groups (no, score 0 and 1; yes, score 2 and 3).

2.3.5. Food residue

We assessed food residue in the oral region after a meal (Ono et al., 2007).

2.3.6. Xerostomia

The presence or absence of xerostomia was examined. The categories reported by Kakinoki et al. (2004) were dry, mildly dry, wet (normal) and wet (high). The categories of dry and mildly dry were considered to indicate xerostomia.

2.4. Assessment of oral function

Assessment of oral function was performed based on the current number of teeth, occlusal condition and presence or absence of swallowing disorder.

2.4.1. Assessment of occlusal condition

With regard to the occlusal condition, the Eichner classification of occlusal support regions (Eichner, 1955) was used for reference. Subjects with an Eichner occlusal support classification of A1–B1, who had occlusal support in at least three sites in the molars, were assigned to the "natural occlusal support group". Those in whom occlusal support was restored with removable dentures were assigned to the "denture occlusal support group". Those with occlusal support in two or fewer sites with an Eichner classification of B2–C3, with no occlusal support in the molars, and unrestorable occlusal support using removable dentures were assigned to the "occlusal support disruption group".

2.4.2. Swallowing disorder

Swallowing disorder was defined as cases in which choking or accidental aspiration occurred, and cases that showed a gurgling sound on auscultation of the neck region (Takahashi et al., 1994) after swallowing 3 ml of water.

2.5. Survey of diagnosis

The presence or absence of general conditions that might have affected swallowing function was determined.

2.6. Survey of concomitant medication

Medication that might have affected oropharyngeal function (e.g., psychotropic agents, antidepressants) (Carl and Johnson, 2006) was investigated.

2.7. Statistical analysis

Chi-squared test was used to determine the independence of each group in two-group comparisons. Risk factors were screened by logistic analysis of variance using the presence or absence of a history of asphyxiation as a dependent variable and the presence of significant factors as an independent variable. The stepwise method (backward elimination method) was used for variable selection. Windows Japanese version SPSS (Ver. 16) was used for statistical analysis, and the level of significance was a p value of <0.05.

Table 2
Univariate analysis of subjects' demographics.

		Asphyxiation ($n=51$)	No asphyxiation ($n=386$)	Relative risk (95% CI)	p value
Sex	Male	10	100	1.43 (0.69–2.97)	0.21
	Female	41	286		
Self feeding	Independent	41	209	3.47 (1.69–7.13)	<0.001
	Dependent	10	177		
ADL	Maintained	34	312	2.11 (1.12–3.98)	0.02
	Decreased	17	74		
Cognitive function	Maintained	28	117	2.80 (1.55–5.06)	<0.001
	Decreased	23	269		
Tongue coating	Yes	20	135	1.20 (0.66–2.19)	0.55
	No	31	251		
Food residue	Yes	20	157	0.93 (0.51–1.69)	0.32
	No	31	222		
Xerostomia	Yes	15	132	0.80 (0.42–1.52)	0.44
	No	36	254		
Occlusal support	Natural occlusal support	5	83		
	Denture occlusal support	16	153		0.02
	Occlusal support disruption	30	150		
Swallowing disorder	Yes	27	124	2.38 (1.32–4.29)	0.03
	No	24	262		
Previous stroke	Yes	19	212	0.72 (0.40–1.32)	0.18
	No	32	174		
Drug administration	Yes	16	110	1.15 (0.61–2.16)	0.39
	No	35	276		

3. Results

3.1. Incidence of asphyxiation

Fifty-one subjects suffered asphyxiation due to food (10 men and 41 women; mean age, 85.6 ± 7.1 years). The annual incidence of asphyxiation accidents was 4.7%. Four subjects had two or more episodes of asphyxiation during the period (four times: one subject, three times: two subjects, two times: one subject). Death caused by asphyxiation occurred in two subjects.

The food causing asphyxiation was fruit in seven subjects, vegetables in four, meat in four, fish in four, rice in three, bread in one, and others in six. There were 29 unclear cases where several foods were involved. There could be multiple causes in those subjects. After the onset of asphyxiation, 13 subjects (25.5%) were transferred to an emergency clinic or hospitalized, but two of them died in hospital within 24 h.

3.2. Risk factors

Factors showing a significant relationship with the onset of asphyxiation were self-feeding [$p < 0.001$, relative risk = 3.47 (1.691–7.131)], ADL [$p = 0.02$, relative risk = 2.11 (1.12–3.98)], and cognitive function [$p < 0.001$, relative risk = 2.80 (1.55–5.06)]. Among 180 subjects who had lost occlusal support with their natural teeth and did not regain occlusion, 30 subjects (16.7%) suffered asphyxiation. However, among 169 subjects whose occlusal support was restored with dentures, 16 subjects (9.5%) suffered asphyxiation, and among 88 subjects with occlusal support with their natural teeth, 5 subjects (5.7%) suffered asphyxiation. The incidence of asphyxiation showed a significant difference ($p = 0.016$) among the three groups (Table 2) (Fig. 1).

3.3. Survey of diagnosis

The presence or absence of general conditions that might have affected swallowing function was determined, and found out that none of them had affected swallowing function (Table 3).

3.4. Results of logistic analysis

Risk factors were screened by logistic analysis of variance using the presence or absence of a history of asphyxiation as a dependent variable and the presence of significant factors in univariate analysis as an independent variable. The stepwise method (backward elimination method) was used for variable selection. As a result, "self-feeding" ($p < 0.001$, relative risk = 3.11, 95% CI:

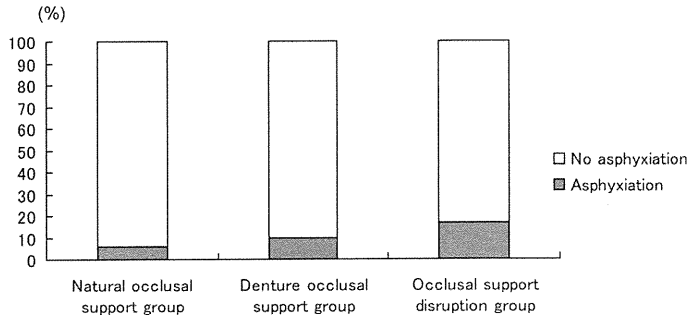

Fig. 1. Relationship between dental status and incidence of asphyxiation. Among 171 subjects who had lost occlusal support with their natural teeth and did not regain occlusion with dentures, 30 subjects (17.5%) suffered asphyxiation. Among 215 subjects whose occlusal support was restored by means of dentures, 21 subjects (9.8%) suffered asphyxiation. Among 113 subjects with occlusal support with their natural teeth, 5 subjects (4.4%) suffered asphyxiation ($p = 0.016$, chi-squared test).

Table 3
Univariate analysis of subjects' general conditions.

		Asphyxiation (n=51)	No asphyxiation (n=386)	Relative risk (95% CI)	p value
Cerebrovascular disease	Yes	19	212	0.72 (0.40–1.32)	0.180
	No	32	174		
Neuromuscular disease	Yes	5	25	1.57 (0.57–4.30)	0.264
	No	46	361		
Cardiac disease	Yes	4	48	0.60 (0.21–1.74)	0.242
	No	47	338		
Respiratory disease	Yes	4	18	1.74 (0.57–5.36)	0.247
	No	47	368		
Diabetes	Yes	1	5	1.52 (0.17–13.31)	0.527
	No	50	381		
Bone and joint disease	Yes	16	97	1.36 (0.72–2.57)	0.395
	No	35	289		

1.50–6.44) and "occlusal support" (p = 0.01, relative risk = 1.75, 95% CI: 1.12–2.73) were selected as significant explanatory variables (Table 4).

4. Discussion

The annual incidence per capita of asphyxiation accidents in the present study was 4.7 per 1000 population in Japan; this is lower than the results from previous research carried out at day-care facilities for elderly people (Takahashi et al., 1994). The incidence of asphyxiation among elderly people in care facilities for the aged was clearly higher than that of elderly people living at home. The elderly people in care facilities appeared to be frailer than those who received care at home. Although the frequency was generally low, accidents were often fatal. Our results show that many individuals were hospitalized after asphyxiation accidents and some of them died. Among the subjects with asphyxiation, approximately 8% had several asphyxiation episodes during the study period, which is four times higher than that reported previously (Suda et al., 2008). Those who suffered several asphyxiation accidents were considered to be at higher risk of death.

We demonstrated that factors related to asphyxiation accidents include self-feeding, ADL, cognitive function, occlusal support of molars, and swallowing disorders. Of these, there was a strong correlation between occlusal support of molars and the incidence of asphyxiation. Many elderly people lose their teeth because of dental caries or periodontal disease, and many of those in the present study had lost occlusal support with their natural teeth. This may lead to reduced chewing ability in elderly people (Hatch et al., 2001). However, the rate of use of dentures, especially among frail individuals, is known to be low. The ability to use dentures is reduced by impaired cognitive function, apraxia, and spatial cognition disorders and is known to be affected by a decrease in ADL (Carter and Jancar, 1984).

We demonstrated that cognitive function is one of the risk factors for asphyxiation. In elderly people with reduced cognitive function requiring nursing care, it has been reported that swallowing without chewing as well as cramming food into the mouth often occurs (Samuels and Chadwick, 2006). These people have also been reported to show symptoms of fast-eating syndrome (Bazemore et al., 1991). In fact, many elderly people with dementia die because of accidental swallowing or asphyxiation (Brunnström and Englund, 2009). In comparison with patients with cerebrovascular dementia, patients with frontotemporal dementia are known to have abnormal eating habits, including cramming food and eating fast (Bathgate et al., 2001), which makes it necessary to take measures to prevent asphyxiation in accordance with the type of dementia.

Table 4
Independent predictors of asphyxiation.

	Coefficient (±S.E.)	p value	Relative risk	95% CI	
				Lower	Upper
Self feeding	1.13 (±0.37)	<0.001	3.11	1.50	6.44
Occlusal support	0.56 (±0.23)	0.01	1.75	1.12	2.73

S.E.: standard error.

It is interesting that the incidence of asphyxiation showed a strong association with the ability to self-feed. The ability to understand the use of eating utensils, a sufficient range of arm motion, and coordination of both arms and oral function are necessary for self-feeding. Good management of self-feeding ability is an important factor in maintaining quality of life of the elderly. According to Volicer et al. (1987), (50)% of patients with Alzheimer disease lose the ability to self-feed within 8 years after diagnosis. Apraxia and spatial-cognitive disorders cause problems in self-feeding ability. Many diseases associated with dementia are considered to impair self-feeding ability. To improve or maintain self-feeding ability, it is necessary to undertake very complex measures based on an understanding of one's own chewing function and swallowing function, and on selection of food in accordance with those functions. If selection of food is necessary, reprocessing of food could be undertaken such as by subdividing, cutting or mixing, to make the food match the functions mentioned above. Every individual must consider the pace of eating by coordinating the amount of food in each bite to prevent accidents.

The results of this study suggest that absence of occlusal support is a risk factor for asphyxiation. Individuals who had lost occlusal support with their natural teeth showed a higher risk of asphyxiation than those with occlusal support. These results suggest that restoration of occlusal support with dentures might be an effective procedure to prevent asphyxiation. If dentists undertook measures based on continuous dental management for frail elderly people, more people might become able to wear dentures (Kawana et al., 2010). Prevention of dental caries and periodontal diseases that cause tooth loss is, of course, essential to prevent loss of occlusal support, and should be included in the management plan. Maintenance of occlusal support for frail elderly people by means of continuous management by dentists is also effective to prevent asphyxiation.

To further prevent asphyxiation and eat safely in elderly people with little ability to control the speed and amount of food, it is important to assist such people while taking food, rather than encourage them to improve their self-feeding ability.

Conflict of interest statement

None.

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References

Bathgate, D., Snowden, J.S., Varma, A., Blackshaw, A., Neary, D., 2001. Behaviour in frontotemporal dementia, Alzheimer's disease and vascular dementia. Acta Neurol. Scand. 103, 367–378.

Bazemore, P.H., Tonkonogy, J., Ananth, R., 1991. Dysphagia in psychiatric patients: clinical and videofluoroscopic study. Dysphagia 6, 2–5.

Brunnström, H.R., Englund, E.M., 2009. Cause of death in patients with dementia disorders. Eur. J. Neurol. 16, 488–492.

Carl, L.L., Johnson, P.R., 2006. Drugs and Dysphagia: How Medications Can Affect Eating and Swallowing. Pro-Ed, Austin, TX.

Carter, G., Jancar, J., 1984. Sudden deaths in the mentally handicapped. Psychol. Med. 14, 691–695.

Eichner, K., 1955. Über eine Gruppeneinteilung der Lunckengebisse für die Prothetic. Dtsch. Zahnarztl. Z. 10, 1831–1834 (in German).

Ekberg, O., Feinberg, M., 1992. Clinical and demographic data in 75 patients with near-fatal choking episodes. Dysphagia 7, 205–208.

Feinberg, M.J., Ekberg, O., Segall, L., Tully, J., 1992. Deglutition in elderly patients with dementia: findings of videofluorographic evaluation and impact on staging and management. Radiology 183, 811–814.

Finestone, H.M., Fisher, J., Greene-Finestone, L.S., Teasell, R.W., Craig, I.D., 1998. Sudden death in the dysphagic stroke patient—a case of airway obstruction caused by a food bolus: a brief report. Am. J. Phys. Med. Rehabil. 77, 550–552.

Fioritti, A., Giaccotto, L., Melega, V., 1997. Choking incidents among psychiatric patients: retrospective analysis of thirty-one cases from the west Bologna psychiatric wards. Can. J. Psychiatry 42, 515–520.

Haddon Jr., W., 1980. Advances in the epidemiology of injuries as a basis for public policy. Public Health Rep. 95, 411–421.

Hatch, J.P., Shinkai, R.S., Sakai, S., Rugh, J.D., Paunovich, E.D., 2001. Determinants of masticatory performance in dentate adults. Arch. Oral Biol. 46, 641–648.

Hirakawa, Y., Kuzuya, M., Enoki, H., Hasegawa, J., Iguch, A., 2008. Caregiver burden among Japanese informal caregivers of cognitively impaired elderly in community settings. Arch. Gerontol. Geriatr. 46, 367–374.

Ichikawa, M., Marui, E., 2000. Mortality of unintentional injuries in childhood and later adulthood in Japan: 1968–1997. Jpn. J. Health Hum. Ecol. 66, 126–136.11.

Kakinoki, Y., Nishihara, T., Arita, M., Shibuya, K., Ishikawa, M., 2004. Usefulness of new wetness tester for diagnosis of dry mouth in disabled patients. Gerodontology 21, 229–231.

Kawana, H., Kikutani, T., Takahashi, N., Hirabayashi, M., Tashiro, H., Fukui, T., Tamura, F., 2010. Effect of continuous management of oral function in the nursing home for denture-wearing elderly under long-term care. J. Gerodont. 25, 3–10 (in Japanese).

Mahoney, F.I., Barthel, D.W., 1965. Functional evaluation: the Barthel index. Md. State Med. J. 14, 61–65.

Minakuchi, S., Takaoka, S., Ito, J., Shimoyama, K., Uematsu, H., 2006. Factors affecting denture use in some institutionalized elderly people. Spec. Care Dentist 26, 101–105.

Ministry of Health, Labour and Welfare of Japan, 2011. Activity of Daily Living Independence of Demented Elderly. Available at: http://www.mhlw.go.jp:80/topics/kaigo/kentou/15kourei/sankou4.html (accessed on May 29, 2011, in Japanese).

Miyazaki, H., Sakao, S., Katoh, Y., Takehara, T., 1995. Correlation between volatile sulphur compounds and certain oral health measurements in the general population. J. Periodontol. 66, 679–684.

NANDA International, 2003. NANDA Nursing Diagnosis: Definitions and Classification 2003–2004. North American Nursing Diagnosis Association, Philadelphia, p. 256.

Ono, T., Kumakura, I., Arimoto, M., Hori, K., Dong, J., Iwata, H., Nokubi, T., Tsuga, K., Akagawa, Y., 2007. Influence of bite force and tongue pressure on oro-pharyngeal residue in the elderly. Gerodontology 24, 143–150.

Samuels, R., Chadwick, D.D., 2006. Predictors of asphyxiation risk in adults with intellectual disabilities and dysphagia. J. Intellect. Disabil. Res. 50, 362–370.21.

Suda, M., Kikutani, T., Tamura, F., Yoneyama, T., 2008. Associated factors for suffocation accidents in the disabled elderly at home. J. Gerodont. 23, 3–11 (in Japanese).

Takahashi, K., Groher, M.E., Michi, K., 1994. Methodology for detecting swallowing sounds. Dysphagia 9, 54–62.

Volicer, L., Seltzer, B., Rheaume, Y., Fabiszewski, K., Herz, L., Shapiro, R., Innis, P., 1987. Progression of Alzheimer-type dementia in institutionalized patients: a cross-sectional study. J. Appl. Gerontol. 6, 83–94.

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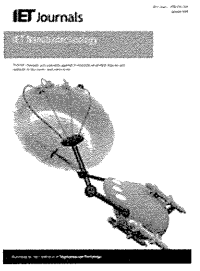

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Development of rapid oral bacteria detection apparatus based on dielectrophoretic impedance measurement method

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Abstract: In this study, a bacteria detection apparatus based on dielectrophoretic impedance measurement (DEPIM) method was demonstrated for rapid evaluation of oral hygiene. The authors integrated a micro electrode chip on which bacteria were captured by dielectrophoresis (DEP), an AC voltage source to induce DEP force, and an impedance measurement circuit to a portable instrument that enables rapid and automated oral bacterial inspection in hospitals and clinics. Special considerations have been made on effects of high electrical conductivity of oral samples on DEP force and DEPIM results. It was shown experimentally and theoretically that using a higher electric field frequency for the DEP bacteria trap and the impedance measurement could realise DEPIM application to bacteria inspection from oral samples with higher conductivity. Based on these investigations, the authors optimised the frequency condition of the DEPIM suitable for inspecting an oral sample along with the design and development of a portable DEPIM apparatus for on-site inspection of oral bacteria. Under the optimised frequency condition, DEPIM results were in good agreement with the conventional culture method showing significant applicability of the DEPIM apparatus for practical rapid oral bacteria inspection.

1 Introduction

Detection of pathogenic microorganisms is a crucial process in medical diagnosis for confirming the existence of a particular disease. Microbiological infectious disease of the oral cavity is an important matter to be concerned since the relationship between periodontal disease, caries, pneumonia, influenza and oral bacteria has been established [1–3]. Recently, aspiration pneumonia has become a focus of attention with regard to patients staying at intensive care units [4] and elderly people in nursing homes [5] because of their high mortality rate, longer length of stay in hospital and increased medical cost [6]. Yoneyama *et al.* [7] investigated the onset of pneumonia and its mortality rate among older patients in a nursing home, obtaining values of 19 and 16%, respectively, within the investigation period. Aspiration pneumonia is thought to be because of the mis-swallowing of bacteria that inhabit the oral cavity and these bacteria reaching the lower respiratory tract [8]. In addition, El-Solh *et al.* [9] suggested that dental plaque may serve as a reservoir for respiratory pathogens. Inglis *et al.* reported that the development of pneumonia depends on the amount of bacteria aspirated into the lungs from saliva being the medium that carries oral bacteria from dental plaque [10]. Influenza is a respiratory infection disease caused by the influenza viruses and has a high mortality rate in the elderly, and prevention of pandemic of influenza is a serious matter especially after a global outbreak of a new strain of H1N1 virus in 2009. Poor oral hygiene may result in increased susceptibility to influenza because bacteria enzymes may injure the oral mucosa and possibly accelerate the onset of viral infections [11]. Abe *et al.* suggest that it is necessary to accurately evaluate the amount of oral bacteria as a level of oral hygiene in order to prevent aspiration pneumonia [12] and influenza [3].

Conventionally, cultivation and colony counting techniques have been performed to evaluate oral hygiene [13] because cultivation is the most established method for inspecting the amount of bacteria not only for samples from the oral cavity but also for various samples from biogenics including humans, food, the environment etc. However, the cultivation method cannot provide a fast evaluation result since it requires rather a long time for bacteria incubation until the appearance of a visible colony on the culture medium (typically a few days); furthermore, it needs to be implemented by a specialist. Therefore in spite of this need,

evaluation of oral hygiene through the amount of oral bacteria has not been commonly used in clinical application except for research purposes. To solve these problems, several alternative bacteria counting methods have been developed. Adenosine TriPhosphate (ATP) bioluminescence is a rapid assay that detects luminescence caused by the enzyme reaction of ATP contained in various bacteria [14]. ATP bioluminescence is useful for on-site monitoring of bacterial contamination because the method does not require a culturing step, and compact equipment has been developed. However, it requires a reagent, which must be stored in a low temperature environment (typically 2 to 8°C) and must be used at room temperature. The direct-count technique using epifluorescence microscopy (EFM) is a highly sensitive bacteria detection method that requires a process of staining bacteria with fluorescent material and observation under a fluorescence microscope [15]. The measurement procedures are tedious, and inspectors need to have special skills in membrane filtration and microscopy.

Suehiro *et al.* proposed a biological cell detection technique called dielectrophoretic impedance measurement (DEPIM) based on dielectrophoresis (DEP) [16]. The DEPIM can also realise highly sensitive detection combined with electropermeabilisation [17, 18], and selective detection of biological cells according to their viability [19] or species by combining with an antigen–antibody reaction [20]. DEP is the electrokinetic motion of dielectrically polarised particles in non-uniform electric fields and is currently an active area of research in several laboratories [21, 22]. As most biological cells and macro molecules behave as dielectric particles in external AC electric fields, DEP has found many useful biotechnological applications. The DEPIM utilises positive DEP, which attracts polarised particles to a high field region, in order to capture biological cells onto an interdigitated electrode chip in the form of pearl chains. Higher cell population results in faster formation of the pearl chains, which bridge over the electrode gap and hence increase the admittance between the electrodes. By monitoring the temporal variation of the electrode impedance or admittance, the cell population can be quantitatively evaluated. By utilising positive DEP, it is possible to enrich the cell population on the microelectrode beyond that in bulk, realising highly sensitive detection of bacteria suspended in the aqueous medium. In addition, DEPIM can realise fast and simple bacteria inspection using only electrical phenomena and instruments without any preliminary chemical treatment.

The aim of this study was to adapt the DEPIM method to the detection of bacteria sampled from the oral cavity and to provide a new rapid, simple operation and on-site inspection method for the evaluation of oral hygiene through the amount of bacteria inhabiting the oral cavity. The bacterial inspection apparatus that utilises the DEPIM method should be applicable to a sample solution with high electrical conductivity. For example, a sample obtained from the oral cavity may include saliva that contains a large amount of electrolytic ions. In general, the positive DEP force becomes weak in a suspension medium with higher electrical conductivity. In order to realise DEPIM-based oral bacteria inspection, special attention was paid to the influence of suspension conductivity as well as electric field frequency on the DEP bacteria trapping process. Based on the experimental results, the DEP condition was optimised for oral bacteria detection and a hand held DEPIM apparatus was newly designed and developed, aiming at the practical application of DEPIM for the rapid and automated inspection of oral bacteria in hospitals or clinics. In addition, bacteria samples obtained from the oral cavity were inspected to validate the effectiveness of the optimised DEPIM condition and the newly developed DEPIM apparatus.

2 Material and methods

2.1 Electrode

Two different electrode configurations were used. A smooth interdigitated electrode system was employed in all the DEPIM experiments because this type of electrode configuration is suitable for accurate impedance measurement [16]. The smooth interdigitated electrode arrays of gold were patterned on a polycarbonate substrate by a laser ablation technique. Each microelectrode strip had a 5 μm gap in which cells were trapped and formed into pearl chains by positive DEP. On the other hand, a castellated electrode configuration [23] was employed for the visual observation of the cell collection process using positive DEP. The castellated electrode arrays of chrome were patterned on a glass substrate by the photolithography technique, and the microelectrode was surrounded by a silicon rubber spacer to form a chamber in which 22 μl of cell suspension liquid was stored.

2.2 DEP observation equipment

The details of the DEP observation apparatus have been described before [16]. The cell suspension liquid was stored in a reservoir tank and circularly fed to the test chamber using a peristaltic pump. Sinusoidal AC voltage was generated by a function generator (WF 1945, NF Corporation, Japan) and applied to the electrode system. Visual observation of DEP was conducted using an inverted microscope (BX-51, OLYMPUS, Japan) and a CCD digital camera (C-5060Z, OLYMPUS, Japan). The flow rate of the cell suspension liquid fed by the peristaltic pump was 2.1 ml/min, and the amplitude of the applied voltage was 10.0 V peak–peak, respectively, which were found to be appropriate conditions for the observation of positive DEP in the preliminary tests.

2.3 DEPIM equipment

Fig. 1 shows a block diagram and a photograph of the newly designed and developed DEPIM apparatus. To enable rapid and automated bacterial inspection in hospitals and clinics, the apparatus was designed as a portable instrument to enable stand-alone measurement without any other instrument or cable. The apparatus consists of two main blocks, one is the 'circuit block' which has a measurement instrument function, and the other is the 'cell block' that includes the electrode and test cell.

All the necessary functions for the electrical measurement of DEPIM are installed in the circuit block. The AC voltage source generates AC voltage, which energises the interdigitated electrode to generate positive DEP force. AC current flowing through the electrode is measured by the current detector. The current is converted from analogue to digital, and is then transferred to a digital processor. The processor calculates the electrode capacitance from the amplitudes of the applied AC voltage and detected current, and the phase difference between the two components. The sequential measurement is carried out for 20 s, and temporal variation of the electrode capacitance is stored,

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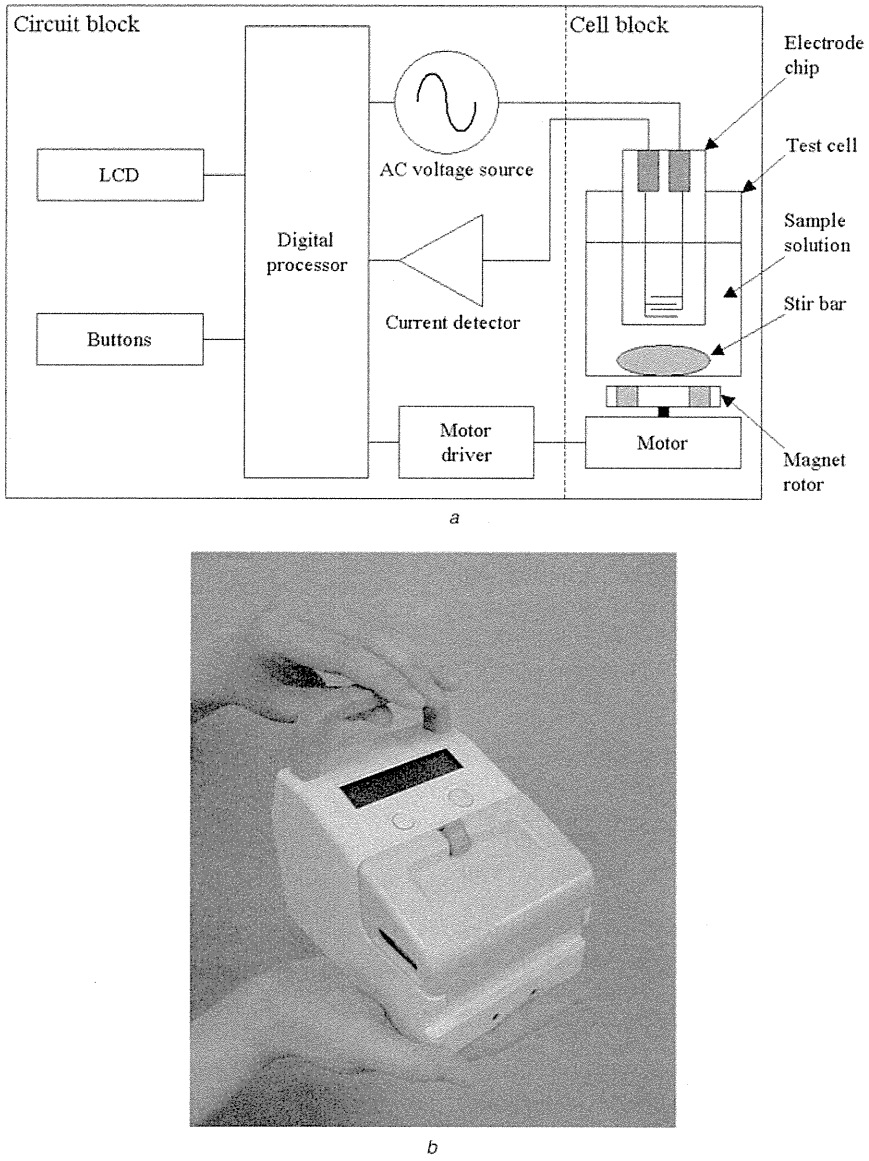

Fig. 1 *Portable DEPIM apparatus*
a Block diagram
b Photograph

then a tangent slope of capacitance change is calculated in order to estimate bacteria concentration, which has a linear relationship with the slope. According to DEPIM theory, bacteria concentration can be estimated by the increase rate of the capacitance as well as by the increase rate of the conductance [16]. In this study, the capacitance was preferred because the conductance change was expected because of the high ion concentration of oral samples and using the conductance change might have resulted in decreased accuracy regarding DEPIM. The processor also controls an LCD for the indication of the measurement results, some operation buttons, as well as a motor driver for driving a motor in the cell block to stir the sample solution.

In the cell block, 5 ml of bacterial suspension is stored in a test cell, in which the smooth interdigitated electrode is immersed. The electrode chip is connected to the AC voltage source and current detector in the circuit block. A magnetic stirrer continuously generates a circular flow in the test cell to enhance the DEP trapping of bacteria [16]. Impedance values measured by the DEPIM apparatus were calibrated using a dummy load (a parallel connection of resistance and capacitance with known values), as well as a buffer with known conductivity.

2.4 Bacteria samples

2.4.1 Optimisation of DEP condition: El-Solh *et al.* reported that *Escherichia coli* (*E. coli*) was often found in the oral cavities of elderly people and could cause pneumonia [9]. Accordingly, for observation of the DEP trapping process and preliminary optimisation of DEPIM measurement conditions, *E. coli* strain K-12 (NBRC3301), which has a high growth rate and has been successfully employed in previous work [16–20], was employed as a representative of oral bacteria in order to improve the efficiency of the experiments. *E. coli* were incubated at 30°C on agar plates for 24 h. Before each measurement, cells were harvested from the agar and suspended in a 0.1 M mannitol solution. After several washings by centrifugation, they were finally resuspended in a 0.1 M mannitol solution