

表2 各種の舌苔における菌種の分布状況²⁾

舌苔の種類	症例数	緑色レンサ球菌	ナイセリア属と近縁種	四連球菌	グラム陰性桿菌	表皮由来球菌	真菌
薄白苔	9	7	4	ND	ND	ND	ND
薄白膩苔	6	6	5	2	ND	1	1
厚白膩苔	8	6	4	3	ND	3	1
薄黄膩苔	7	6	3	3	ND	ND	ND
厚黄膩苔	27	19	13	3	5	4	6
黒苔	4	2	1	2	1	ND	ND
光剥苔	5	3	2	ND	1	1	2

注：数字は菌が検出された症例数，ND：未検出。

傾向を示している。厚白膩苔および薄白膩苔の色調は、ナイセリア属から独立した近縁菌で、シュードモナス属とも共通点の多い *Moraxella catarrhalis* の影響が大きい。この細菌は淡緑色色素を産生し、灰白色のコロニーを形成する。これに表皮由来球菌の白色および黄色のコロニーが混在することで、独特の白色系統の色を呈するものと考えられている。

薄黄膩苔および厚黄膩苔は、感染性の発熱性疾患の際にしばしば観察される粘着性の黄色の舌苔で、薄白膩苔および厚白膩苔からの移行が多いとされている。緑色レンサ球菌、ナイセリア属とその近縁菌、四連球菌、および表皮由来球菌が優位である。この舌苔の色調は、*M. catarrhalis* の灰白色のコロニーに、四連球菌および表皮由来球菌の産生する黄色およびオレンジ色のコロニーが多量に混合することによるものと考えられている。

黒苔は、前述の白苔や黄苔を呈する病態が悪化した際にしばしば認められ、舌苔が黒色を示すようになったものである。糸状乳頭が異常に伸張し、この間隙に堆積した微生物や壊死した上皮細胞が硫化水素 (H₂S) を生じ、ヘモグロビンなどと結合して硫化鉄 (Fe₂S₃) となった結果、黒色を呈するといわれている³⁾。従来は *Candida albicans* の増殖によって生ずると考えられていたが、この報告では真菌類

表3 各種の舌苔における検出菌種²⁾

舌苔の種類	例数	単一菌種 検出頻度 (%)	例数	複数菌種 検出頻度 (%)
薄白苔	7	77.8	2	22.2
白膩苔	2	14.3	12	85.7
黄膩苔	7	20.6	27	79.4*
黒苔	2	50.0	2	50.0**
光剥苔	1	20.0	4	80.0**

* : p < 0.05, ** : p < 0.01 (vs 薄白苔)

は検出されず、口腔常在菌であるグラム陽性菌が多く検出されている。この報告とは別に、*Bacillus subtilis* が優位であり、これによって黒色色素が産生される結果として舌が黒色を呈するという報告もあり⁴⁾、黒色色素の由来についてはいまだ定説が得られていない。

光剥苔は、糸状乳頭が限局的に消失して舌苔そのものが完全に剥落したもので、いわゆる鏡面舌の状態となったものである。胃疾患や肝疾患、その他の慢性疾患を有する場合に起こりやすいといわれている。微生物叢としては、緑色レンサ球菌、ナイセリア属とその近縁菌、真菌などが優位で、四連球菌や表皮由来球菌が少ないことが特徴である。

表4 舌苔の形成が認められた患者の唾液 pH²⁾

舌苔の種類	症例数	口腔内 pH (平均±SD)
薄白苔	133	7.272±0.262
白厚苔	29	6.614±0.332*
薄黄苔	23	7.157±0.278
黄厚苔	40	6.650±0.386*
薄膩苔	15	7.170±0.442
光紅苔	22	6.623±0.342*

* : p < 0.01 (vs 薄白苔)

表5 唾液分泌量に影響を及ぼす疾患と薬剤

疾患	糖尿病, サルコイドーシス, Sjögren 症候群, パーキンソン病, ウイルス感染, 頭頸部の放射線治療, 唾液腺摘出術, 唾液腺の先天的欠如, 精神的ストレス, など.
薬剤	催眠鎮痛薬, 抗炎症薬, 抗不安薬, 抗うつ薬, 抗てんかん薬, 鎮痙薬, 抗パーキンソン薬, 骨粗鬆症治療薬, 抗不整脈薬, 降圧薬, 抗コリン薬, 抗ヒスタミン薬, 鎮咳薬, 利尿薬, 気管支拡張薬, 消化器潰瘍治療薬, など.

IV 微生物叢に影響を及ぼす宿主要因と舌苔形成との関連

このような種々の舌苔形成には、宿主要因も大きく関与していると考えられている。たとえば、口腔内 pH も舌苔の微生物叢の構成変化に影響を及ぼす。

各種の舌苔形成が認められた患者の唾液 pH を比較検討したところ、白厚苔、黄厚苔、および光紅苔が形成されている患者では、生理的な範囲内の舌苔である薄白苔の場合よりも有意に pH が低下し、口腔内が弱酸性の環境に傾いているという結果が報告されている²⁾ (表4)。さらに、酸性環境では角化上皮細胞の正常な脱落が阻害された結果、角質層が厚くなり、糸状乳頭が異常に伸張して、ここに食物残渣、壊死した上皮細胞、および微生物が堆積して病的な舌苔形成に至ったとした上で、そこでの微生物の代謝によって口腔内環境が弱酸性に維持されているのではないかと記載されている。

また、前述の舌苔の微生物叢に関する報告では、口腔内が酸性環境にある白厚苔や黄厚苔などでは、他の舌苔形成症例では認められない真菌の検出が認められている (表2)。口腔内真菌として代表的な *C. albicans* は、健康成人の舌の微生物叢で優位な

口腔内常在細菌と異なり、中性よりも弱酸性のほうが増殖が盛んであるといわれている⁴⁾。弱酸性環境を舌表面に作り出す白厚苔や黄厚苔などで真菌類の検出率が高かったのは、このことに起因している可能性が高い。

その他、唾液分泌量が低下する各種の疾患や薬剤 (表5) の服用時には、多くのケースで舌苔形成が認められることが知られている。白血球数、ヘモグロビン値、ヘマトクリット値などは舌苔の厚さと正の相関を示すが、アミラーゼ値は逆に負の相関を示すことが報告されている⁵⁾。一方、喫煙の習慣も舌苔形成に促進的に作用することが明らかになっている⁶⁾。

V 歯周病が舌苔の微生物叢に及ぼす影響について

これまで総論的に舌苔と口腔内微生物叢との関係を述べてきたが、ここでは成人に多く見られる歯周病との関連について考察する。

現在、歯周病細菌として *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola* などがクローズアップされている。高橋

らは、歯周組織の健全な若年成人および高齢者の舌苔からは歯周病細菌がほとんど検出されないという論文を発表している⁷⁾。一方で、舌苔の発生部位である舌背が歯周病細菌の受け皿になり得るという報告⁸⁾も見られ、その因果関係については不明な点が多く残されている。

また、唾液分泌減少による口腔乾燥症や、口腔ケアの不十分な高齢者においては、口腔内の自浄作用の低下とともに口腔内環境が悪化する。あわせて、このような口腔では口臭が大きな問題となる。舌苔中の細菌はケラチンを分解して口臭の原因となる揮発性硫黄化合物を発生することが知られている。さらに高齢者が歯周病に罹患すると、歯周ポケット内の細菌が豊富な滲出液中のタンパク質を栄養源として消費し、その過程で産生されるメチルメルカプタン(CH₄S)が口臭の原因となると考えられている⁹⁾。事実、Moritaら^{10, 11)}により、歯周病患者における口臭と歯周病の関連性が疫学的に証明されている。

おわりに

このように、舌苔の微生物叢は患者の病態や口腔内状態によって、その構成がかなり異なることが明らかになりつつある。要介護高齢者では、口腔乾燥

の症状とともに口腔内清掃が十分になされず、自浄作用の低下に伴い舌苔が生じる。舌苔形成が誤嚥性肺炎や強い口臭の原因になっているという指摘もあり、高齢化社会となった現在、この分野での研究の進展が望まれる。

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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 electroporation [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.

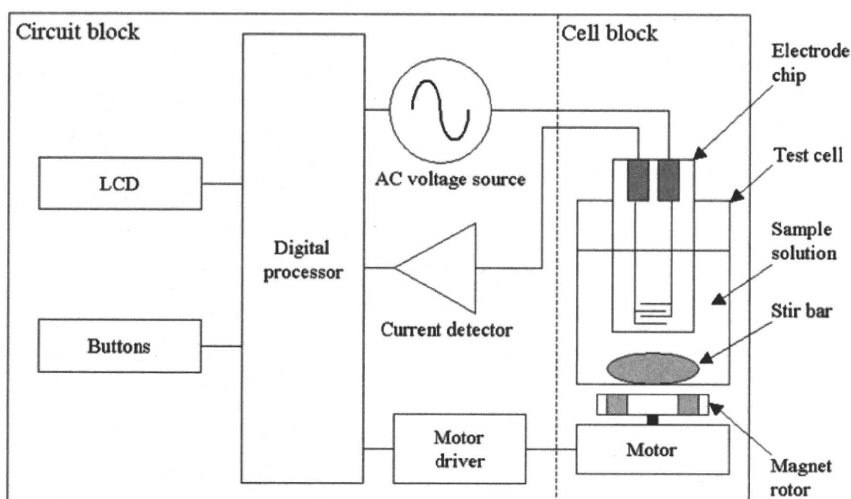
2.2 DEP observation equipment

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,



a



b

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

(conductivity $1 \mu\text{S cm}^{-1}$) at various diluted concentrations as determined by a colony counting method.

The oral cavity harbours a complex microbiota, with over 300 named species of bacteria so far isolated [6]. Abe *et al.* reported that bacteria exist in human saliva at a concentration ranging from 2.7×10^7 (pneumonia low-risk group) to $4.7 \times 10^8 \text{ cm}^{-3}$ (pneumonia high-risk group) [24]. Given that DEPIM can detect bacteria at a minimum concentration of 10^5 cm^{-3} [16], which is almost 1% of the lowest bacteria concentration expected in human saliva, the saliva can be diluted 100 times with deionised water so that the electrical conductivity is lowered to be as low as possible. According to Neyraud *et al.*, the conductivity of human saliva (mean \pm SD among seven healthy subjects) were $4115 \pm 1181 \mu\text{S cm}^{-1}$, and the maximum value was $7474 \mu\text{S cm}^{-1}$ [25]. This means that the maximum conductivity of the DEPIM sample after dilution 100 times becomes about $100 \mu\text{S cm}^{-1}$. Considering these conditions, the DEPIM experiments were conducted in the conductivity range from $5\text{--}150 \mu\text{S cm}^{-1}$. The conductivity of each sample solution was adjusted by dissolving sodium chloride in 0.1 M mannitol solution and checked by a conductivity meter (B-173, HORIBA, Japan).

2.4.2 Verification of testing oral samples: In order to ensure applicability of DEPIM to oral bacteria detection, samples were taken from 49 elderly residents in a care facility for elderly people requiring long-term care (This study was approved by the Ethics Committee of The Nippon Dental University, School of Life Dentistry in Tokyo.). Samples were collected from the median area of the tongue in contact with the mandibular first molar using a sterilised swab (1A754S, JCB, Japan). To avoid errors caused by an inadequate sampling process, the swab was pressed against the tongue with a constant force of 0.2 N by using a special device. The swab swiped the tongue surface twice (1 cm long for each swipe). The obtained samples were suspended in 7 ml of 0.1 M mannitol, and 5 ml was used as a sample for testing with DEPIM equipment, whereas the remaining solution was employed as a sample for the conventional culture method. For the culture method, samples were appropriately diluted in 0.1 M mannitol, spread over blood agar plates (each sample, 1–2 plates) using a spiral plating apparatus (Autoplate 4000; Spiral Biotech), followed by anaerobic culture at 37°C for 48 h. These operations were executed within several hours to prevent loss of viability of the bacteria. All visible colonies grown on the plate were counted using a colony counter (Acolyte; Synbiosys), and bacterial concentrations in the samples were determined. Correlations in the data obtained by these methods were then evaluated.

3 Results

3.1 Observation of DEP trapping process of bacteria

Photographs of the DEP collection of *E. coli* are shown in Fig. 2. The DEP collection observations were made at two different electric field frequencies of 100 and 800 kHz, and two different conductivities of the suspending medium of 1 and $50 \mu\text{S cm}^{-1}$. In the case of $1 \mu\text{S cm}^{-1}$, bacteria were trapped around the electrode corner because of positive DEP (Figs. 2a and b) at frequencies of 100–800 kHz. On the other hand, in the case of higher conductivity of $50 \mu\text{S cm}^{-1}$, bacteria were not trapped at 100 kHz. When forced flow was stopped, cells were focused at a weak

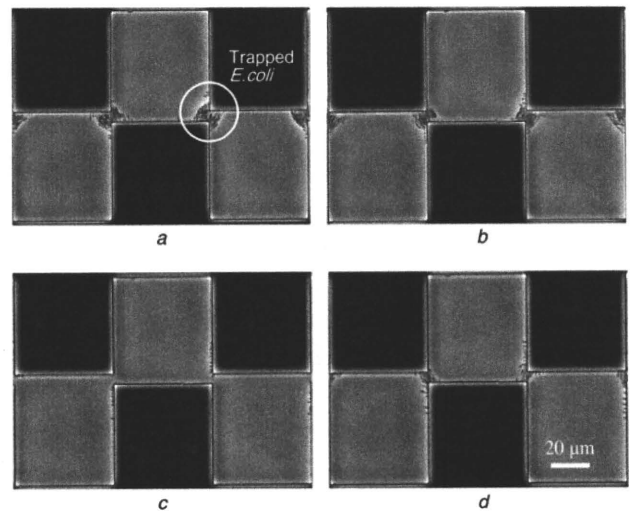


Fig. 2 DEP collection process of *E. coli*

AC signal of amplitude 10 V peak–peak was applied to a crenellated electrode for 15 s

- a At 100 kHz, $1 \mu\text{S cm}^{-1}$: bacteria were trapped by positive DEP around the electrode corner where the electric field strength becomes higher
- b At 800 kHz, $1 \mu\text{S cm}^{-1}$: the captured bacterial quantity was almost equal to a
- c At 100 kHz, $50 \mu\text{S cm}^{-1}$: hardly any bacteria were captured
- d At 800 kHz, $50 \mu\text{S cm}^{-1}$: the captured bacteria were slightly fewer than a and b

electric field region at the centre of the electrode gap surrounded by the electrode edge, showing that negative DEP became dominant under this condition (not shown in the picture). Some bacteria were captured at 800 kHz, although the number of trapped cells was slightly decreased compared to the case of the $1 \mu\text{S cm}^{-1}$ conductivity (Figs. 2c and d). These observation results suggest that positive DEP force exerted on the bacteria becomes weak with increased conductivity at the 100 kHz frequency.

3.2 DEPIM measurement using *E. coli* samples

DEPIM experiments were conducted in this range of conditions for $1\text{--}135 \mu\text{S cm}^{-1}$. Fig. 3 shows temporal variation of the electrode capacitance increment measured with *E. coli* at $5 \times 10^6 \text{ CFU/ml}$ (at 100 kHz) and $2 \times 10^7 \text{ CFU/ml}$ (at 800 kHz). As explained in the previous work [16], the capacitance increase is because of the presence of bacteria that are trapped and enriched in the electrode gap. It was found that the capacitance increase rate at $t = 0$ was directly proportional to the cell population that was quantitatively evaluated by the DEPIM method.

At a frequency of 100 kHz, the capacitance increase rate in the case of $25 \mu\text{S cm}^{-1}$ was obviously lowered in comparison with $1 \mu\text{S cm}^{-1}$ (Fig. 3a). However, at a higher frequency of 800 kHz, the temporal change of capacitance was almost the same for both the conductivities of 1 and $50 \mu\text{S cm}^{-1}$ (Fig. 3b).

4 Discussion

The DEP force acting on a spherical particle of radius r suspended in a medium of permittivity ϵ_s is given by [26]

$$F_{\text{DEP}} = 2\pi r^3 \epsilon_s \text{Re}[K(\omega)] \nabla E^2 \quad (1)$$

where E is the magnitude root mean square of the applied field and $\text{Re}[K(\omega)]$ is the real component of the Clausius–Mossotti

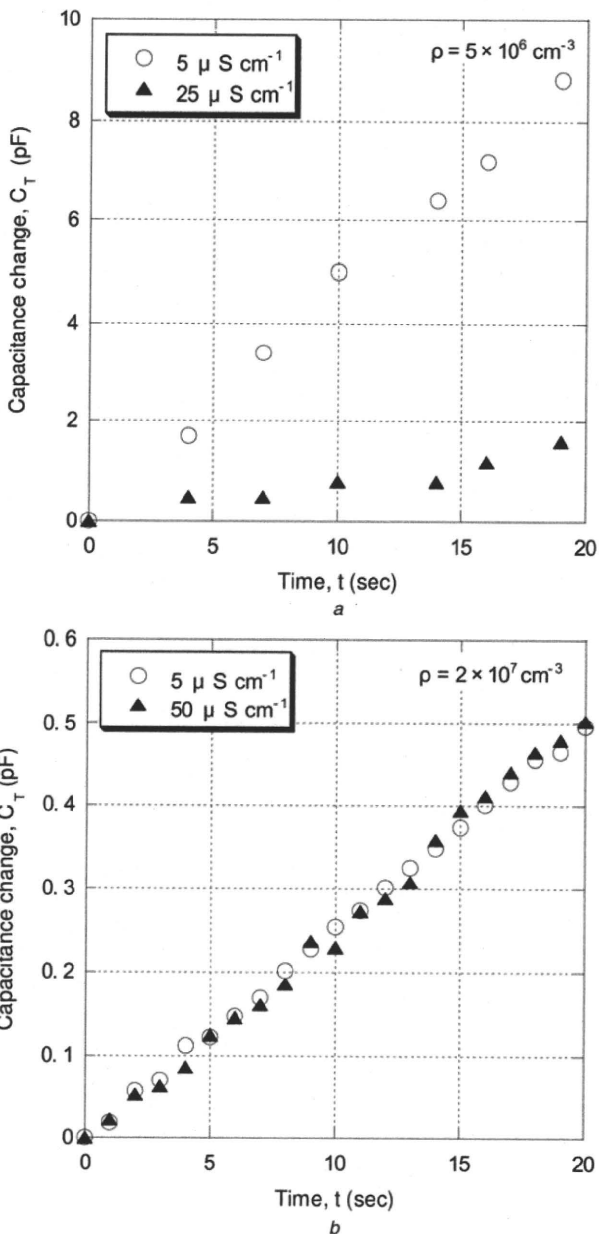


Fig. 3 DEPIM results measured with *E. coli*

a At a frequency of 100 kHz, and conductivity of $5 \mu\text{S cm}^{-1}$ (○), the capacitance increased almost linearly with time. The capacitance increase implies that bacteria were trapped and enriched under positive DEP at the electrode gap. With higher conductivity of $25 \mu\text{S cm}^{-1}$ (▲), the capacitance change became less remarkable with lower medium conductivity *b* At 800 kHz, the rate of capacitance increase with time was almost the same as for the medium conductivity of $5 \mu\text{S cm}^{-1}$ (○) and $50 \mu\text{S cm}^{-1}$ (▲) AC signal of amplitude 5 V peak-peak was applied to a smooth interdigitated electrode

factor given by

$$K(\omega) = \frac{\epsilon_p^* - \epsilon_s^*}{\epsilon_p^* + 2\epsilon_s^*} \quad (2)$$

where ϵ_p^* and ϵ_s^* are the complex permittivity of the particle and surrounding medium, respectively. For a real dielectric, the complex permittivity is defined as

$$\epsilon^* = \epsilon - j\frac{\sigma}{\omega} \quad (3)$$

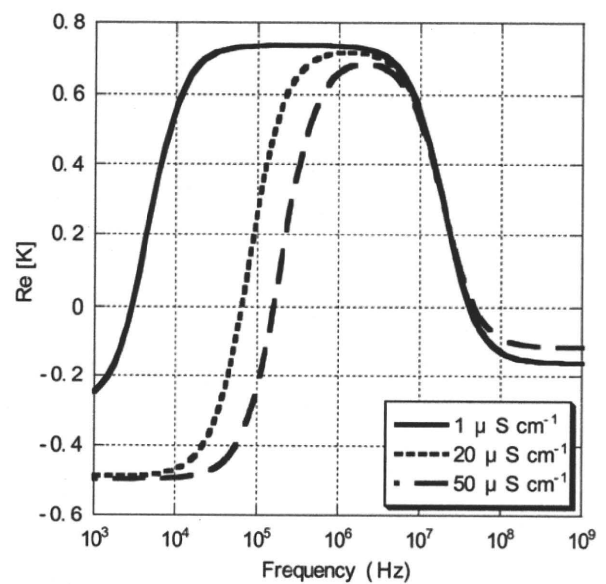


Fig. 4 Theoretical prediction of the external medium conductivity σ_s dependency of $\text{Re}[K]$ spectra

E. coli cells are modelled as a dielectric sphere covered by two shells. Parameters used in calculation are listed in Table 1

where ϵ is the permittivity and σ is the conductivity of the dielectric and ω is the angular frequency of the applied field. The effects of the conductivity of the surrounding medium on DEP force have been attributed to the modification of parameter $\text{Re}[K(\omega)]$. In general, positive-DEP force acting on bacteria decreases with a rise in the conductivity of the surrounding medium. Muller *et al.* [27] explained that DEP force is dependent on the relative magnitude of the conductivity and permittivity of cells and that of the media. From (2) and (3), one can understand that the positive DEP force decreases together with a rise in the conductivity of the medium if the conductivity of the cell and the permittivity of the cell and medium are constant.

An example of a theoretical prediction of the suspension medium conductivity dependency of parameter $\text{Re}[K(\omega)]$ is shown in Fig. 4. One *E. coli* cell is modelled as a dielectric sphere covered by shells. The shells represent the cytoplasmic membrane and the sphere covered by the shells represents the cytoplasm. The complex permittivity of the particle ϵ_p^* in (2) is replaced with an effective complex permittivity of *E. coli* cell ϵ_{eff}^* ; which can be calculated by using the ‘smeared-out sphere’ model [28]. Parameter values of *E. coli* are determined referring to the referenced literature [29] and listed in Table 1. Fig. 4 indicates that $\text{Re}[K(\omega)]$ or the DEP force decreases with increases in the medium conductivity σ_s at a lower field frequency. For example, when the medium conductivity increases from the

Table 1 Parameter values used in the theoretical prediction of $\text{Re}[K(\omega)]$ shown in Figs. 4 and 5

Component	Parameter	Value
cell	radius	1 μm
cell cytoplasm	relative permittivity	60
	conductivity	0.2 S m^{-1}
cell membrane	relative permittivity	6
	conductivity	0.25 $\mu\text{S m}^{-1}$
	thickness	8 nm
suspension medium	relative permittivity	80

initial value of 1 to $50 \mu\text{S cm}^{-1}$, the $\text{Re}[K(\omega)]$ value changes from a positive to a negative value, that is, DEP changes from positive-DEP to negative-DEP at the field frequency 100 kHz . This suggests that *E. coli* cells are not captured at the electrode gap by DEP under the condition of $50 \mu\text{S cm}^{-1}$. On the other hand, the DEP force is hardly dependent on σ_s at 800 kHz . The theoretical calculations agree well with the experimental results shown in Fig. 2 where DEP collection of *E. coli* is observed only for low medium conductivity ($1 \mu\text{S cm}^{-1}$) at 100 kHz but no clear differences are observed with a rise in medium conductivity until $50 \mu\text{S cm}^{-1}$ at 800 kHz .

As explained in the previous work [19], the conductance and capacitance increase rate as a DEPIM value strongly depend on the DEP force acting on the bacteria to be detected. The weaker the positive-DEP force acting on the bacteria, the fewer the bacteria captured at the electrode gap, resulting in decreased impedance change detected by DEPIM. Fig. 5 shows effects of the suspension medium conductivity on the DEPIM measurement as well as theoretical calculations of $\text{Re}[K(\omega)]$, which are normalised to the value at low medium conductivity ($1 \mu\text{S cm}^{-1}$). At a frequency of 100 kHz , the predicted positive-DEP force rapidly decreases with a rise in medium conductivity, changing to negative-DEP at $25 \mu\text{S cm}^{-1}$. On the other hand, at 800 kHz , the decrease in positive-DEP force is predicted to be suppressed. The measured DEPIM value agrees with these theoretical predictions at both 100 and 800 kHz . The normalised DEPIM values were around zero despite the normalised $\text{Re}[K(\omega)]$ value being around -4 under the condition of $50 \mu\text{S cm}^{-1}$, 100 kHz . In the light of the DEPIM theory, it can be understood that the DEPIM value will not be less than zero even if $\text{Re}[K(\omega)]$ is negative. These results indicate that frequency of 800 kHz

is more appropriate than 100 kHz for DEPIM measurement of sample with high medium electrical conductivity, σ_s .

In order to ensure applicability of DEPIM to oral bacteria inspection, bacteria were taken from oral cavity of 49 elderly persons, and these samples were measured by DEPIM equipment. The conductivities of suspended samples for DEPIM measurement (mean \pm SD among 49 subjects) were $51 \pm 37 \mu\text{S cm}^{-1}$, and the maximum value was $144 \mu\text{S cm}^{-1}$. Considering the rather high electrical conductivity as well as the experimental and calculation results obtained for *E. coli* samples, measurement was carried out at frequency of 800 kHz .

Fig. 6 shows a relationship between the bacteria concentration obtained by the culture method and the tangent slope of capacitance change measured by DEPIM apparatus. As shown in Fig. 5, the DEPIM value changes with an increase in medium conductivity even if the bacteria concentration is the same. Therefore measured DEPIM values were calibrated based on the conductivity dependence shown in Fig. 5 so that effects of sample conductivity variation, which were difficult to control for the oral samples, could be cancelled out. Pearson's correlation coefficient was $R = 0.90$, and the range of error calculated from the standard curve obtained by the measurement data was -86 – 285% . Considering the correlation between the colony counting method and ATP bioluminescence assay ($R = 0.851$) [14] and the fluorescent counting method ($R = 0.75$) [30], correlation between DEPIM measurement and the standard culture method was significantly high.

As calculated in Fig. 4, it is predicted that stronger positive-DEP will occur in the range of MHz rather than 800 kHz under higher medium conductivity. Therefore higher field frequency will be a more suitable condition. However, a rise in the field frequency will require more careful design and probably higher cost of the impedance measurement circuit. The development of an impedance measurement circuit with a higher frequency AC source for DEP, and a current detector that can sense higher-frequency AC current accurately, will be future tasks.

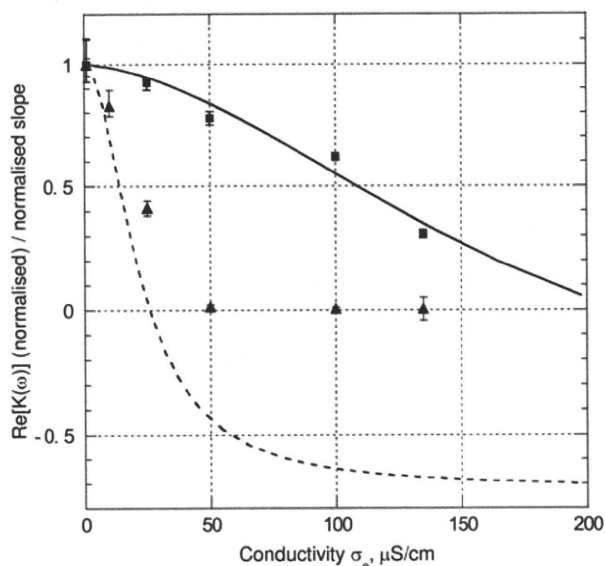


Fig. 5 Theoretical calculations of $\text{Re}[K(\omega)]$ (solid, 800 kHz ; dashed, 100 kHz) and experimental results of DEPIM at *E. coli* concentration of $5 \times 10^5 \text{ CFU/ml}$ (■, 800 kHz ; ▲, 100 kHz), these were normalised to these values at low medium conductivity ($1 \mu\text{S cm}^{-1}$)

DEPIM measurement was carried out three times under each set of conditions. The conductivity dependencies of the $\text{Re}[K(\omega)]$ and DEPIM results are almost identical, showing that the data can be employed to calibrate DEPIM results obtained for various conductivity values

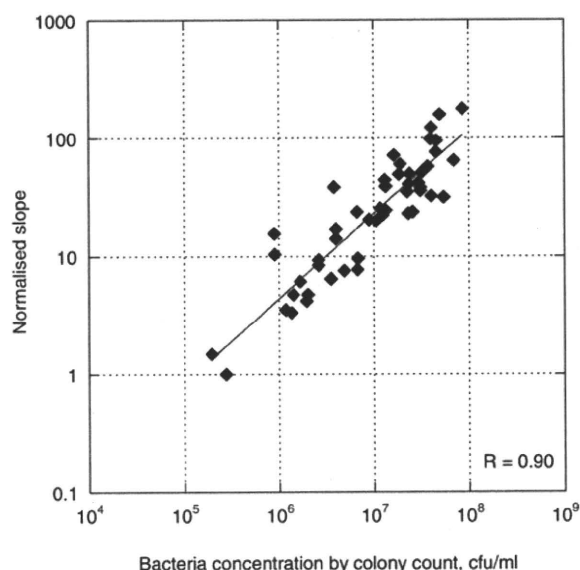


Fig. 6 Relationship between bacteria concentration determined by conventional colony counting method and tangent slope of capacitance measured by DEPIM equipment for 49 samples obtained from the human oral cavity

5 Conclusions

In this article, we have described the optimisation of AC electric field frequency in the DEPIM method to enhance the measurable range of conductivity of the sample solution to adapt the DEPIM method for the inspection of bacteria obtained from the oral cavity. The optimisation was based on the theoretical calculation of DEP force dependency on the applied field frequency in the range of the assumed medium conductivity of the sample obtained from the oral cavity. Observation of DEP-trapping process of cell was carried out to find the appropriate field frequency condition in the range of the assumed medium conductivity. Based on the investigation, the performance of the developed DEPIM apparatus was confirmed by measuring *E. coli* suspended in the medium conductivity range. By applying an AC electric field of 800 kHz, the measurable range of medium conductivity using developed apparatus was expanded up to $135 \mu\text{S cm}^{-1}$, at which point almost all samples from the oral cavity will be covered. Consequently, it was demonstrated that the apparatus is useful in the evaluation of the bacterial contamination of clinical samples from the oral cavity for quantitative evaluation of oral hygiene. In addition, the developed apparatus will be applied to other fields in which the investigation of the sample including ionic substances is necessary, for example, any clinical samples besides those taken from the oral cavity, as well as fields relating to the environment and the food industry.

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LETTER TO THE EDITOR

Effect of oral care on cognitive function in patients with dementia

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Dear Editor,

Association of oral health with cognitive performance has been reported in a previous study¹. Chewing performance might stimulate brain functions and protect against the degradation of cognitive functions. However, it has not yet been concluded if oral health degenerates cognitive activities in longitudinal studies. In the present study, we carried out oral care and prospectively observed whether oral care improved cognitive function in dementia patients

A total of 446 patients with dementia staying in 10 nursing homes were nominated for the study. The criterion for patient selection was that physical symptoms and cognitive impairment must have been stable for the preceding 3 months. During this 3-month period, no patient had acute disorders (e.g., severe infection, heart failure, or stroke requiring special treatment and intensive care). Mini mental state examination (MMSE)² was examined and 275 patients of MMSE less than 23 and more than 10 were selected. The patients fed themselves or needed help in eating, but no patient had feeding tubes. The patients were randomly selected from the same floor and nursing team in each nursing home. Randomization was made from a random-numbers table, and the list was held independently of the investigators. A total of 275 patients were randomly assigned to an oral care group or a no oral care group from September 2003 to August 2004. In all patients and/or their families, agreements of participation in the study were taken and 114 patients for the oral care group and 126 patients for the no oral care group participated to the study. However, 24 patients for the oral care group and 27 patients for the no oral care group were excluded from the analysis, because they were discharged from nursing homes, hospitalized, died or dropped out due to discontinuation of the examination. Finally, 90 patients

for the oral care group (mean age \pm standard deviation (SD) 81 ± 9 , 68 women and 22 men) and 99 patients for the no oral care group (mean age 83 ± 8 , 75 women and 24 men) prospectively completed the study during 1 year.

At baseline, each patient received a uniform evaluation, including medical history, physical and neurological examination, MMSE and Barthel Index³ for activity of daily living (ADL; maximum score was 100 and higher scores indicated better performance). The diagnosis of dementia was made according to the Diagnostic and Statistical Manual of Mental Disorders, 4th (DSM-IV) criteria⁴. Patients with dementia of either Alzheimer's disease or vascular dementia or a combination of both were involved

During follow up, nurses or caregivers cleaned the patients' teeth with a toothbrush for approximately 5 min after each meal. The brushing was carried out as usual daily tooth-brushing without dentifrice, including brushing the palatal and mandibular mucosa and tongue dorsum. In the no oral care group, several patients carried out tooth-brushing by themselves once a day or irregularly. In subjects who could not clean their mouth by themselves, oral care was carried out by caregivers if requested. A total of 81 patients used dentures. In both groups, dentures were cleaned with a denture brush every day and with denture cleanser once a week. Dentists or dental hygienists administered professional care, such as plaque and calculus control, as necessary once a week for the oral care group.

All comparisons were made between the oral care and no oral care groups using two-way analysis of variance. Statistical significance was accepted as $P < 0.05$. All data are expressed as mean \pm SD.

At baseline, MMSE of the oral care group and no oral care group were 18.2 ± 5.1 and 17.8 ± 5.7 , respectively. There was no significant difference between them.

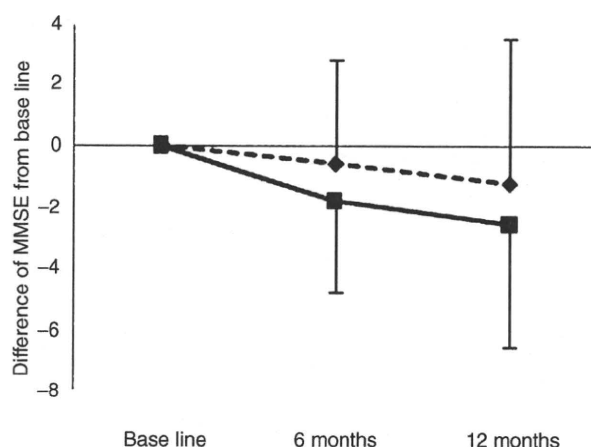


Figure 1 Differences of mini mental state examination (MMSE) from baseline are shown at 6 months and 12 months in both oral care groups (o) and no oral care group (x). There were significant differences between the two groups at 6 months ($P < 0.05$) and at 12 months ($P < 0.05$).

Differences of MMSE from baseline are shown in Figure 1, and there were significant differences between the oral care group and no oral care group at 6 months ($P < 0.05$) and 12 months ($P < 0.05$). The Barthel Indexes at baseline were 48 ± 27 and 50 ± 30 in the oral care group and no oral care group, respectively. The Barthel Index did not change during 1 year in the oral care group and no oral care group (46 ± 22 and 47 ± 27 , respectively) and there was no significant difference between them.

Until now, it has been reported that communication with others was effective for the cognitive function of the patient.⁵ The present study proved that oral care contributed to preventing degradation of cognitive function in dementia. Oral care has been proven to be a benefit for the prevention of pneumonia in self-care dependent older patients¹ and physical complaints of unknown origin,⁶ and even mortalities in older adults^{7,8}. To the best of our knowledge, this is the first report to show that oral care influences cognitive function in dementia patients.

Oral care stimulates sensory areas of the cortex related to oral function, which occupies relatively large areas of the sensory area of the cortex, and improved swallowing reflexes⁹, resulting in preventing aspiration pneumonia¹. Oral care might be a benefit to improved chewing function, which would contribute to stimulating the oral sensory area as well as nutritional improvement.¹⁰ All of these beneficial effects on brain function might contribute to cognitive function. In geriatrics, the oral organ also tightly influences other organs,

including the brain¹¹. Donepezil has been used as a unique medicine to improve cognitive function in dementia¹². Oral care would contribute to cognitive function as much as donepezil. Angiotensin-converting enzyme inhibitors have been suggested to be a benefit for Alzheimer's disease^{13,14}. Substance P would be a key material to influence Alzheimer's disease as well as pneumonia¹⁵. Stimulation of any organ would be important to improve functions in older people¹⁵. Oral care can be one of the key stimulations in cognitive function, as well as many other organ functions.

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The Effect of Tooth Loss on Body Balance Control Among Community-Dwelling Elderly Persons

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Purpose: Since tooth loss may be considered to affect postural control, the aim of this study was to compare body balance control among samples of edentulous and dentate community-dwelling elderly subjects. **Materials and Methods:** A case control study was conducted using test and control groups matched by age, gender, body fat, and muscle composition. The test group included all participants of the 2006 Kyoto Health Seminar who wore a full denture in either or both arches. The control group was blindly selected from the same population, but only included individuals who retained all of their dentition with either natural teeth or crown prostheses. The results of physical fitness examinations and stabilometer tests were compared between these two groups. **Results:** The test and control groups both included 12 male and 23 female subjects. Body balance ability, measured by time spent standing on one leg with eyes open ($P = .013$) and functional reach ($P = .037$), was significantly less in the test group when compared to the control, as shown by analysis done using the Mann-Whitney U test. The stabilometer examination also indicated that sway area (an accurate indicator of postural balance) and body sway (evidence of energy consumption for postural control) while standing with eyes closed were both significantly higher in the test group ($P = .035$ and $.048$, respectively; Wilcoxon signed ranks test) than the control. **Conclusion:** It is suggested that tooth loss is a risk factor for postural instability. This further suggests that proprioceptive sensation from the periodontal ligament receptor may play a role in body balance control. *Int J Prosthodont* 2009;22:136-139

More than one third of persons 65 years of age or older fall each year and, for half of these individuals, such falls are recurrent.^{1,2} Approximately 1 in 10 falls results in serious injury, such as hip fracture.³

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Fracturing a hip increases morbidity and mortality in this population, with death occurring within 1 year of the fracture in over 30% of elderly individuals.⁴ Also, falling and a fear of falling are risk factors for disuse syndrome, the major cause of becoming bedridden according to the Annual Report of the Aging Society published by the Cabinet Office in 2002.⁵ The prevention of falls in this rapidly increasing segment of the population is a primary concern for maintaining an adequate quality of life (QOL).

Studies have shown that demented elderly persons are twice as likely to fall as cognitively normal persons of the same age group^{1,6} and a relationship between dental occlusion and falls among the elderly with dementia has been demonstrated.^{7,8} This finding suggests that elderly individuals who lack dental occlusion are at a higher risk of falling than those whose dental occlusion has been maintained. Other investigators have suggested that occlusion and head position affect sway at the center of gravity, resulting in an increased risk suffering of a fall.^{9,10} Gangloff and Perrin¹¹ showed

Table 1 Characteristics of Test and Control Groups

	Test group	Control group	<i>P</i> value
Sex (male/female)	12/23	12/23	1.000
Mean age	75.6 ± 4.3	75.9 ± 3.9	.645
Body mass index (kg/m ²)	21.9 ± 2.9	21.8 ± 2.5	.949
Arm muscle circumference (cm)	20.8 ± 1.9	21.1 ± 2.0	.747
Hand grip (kg)	25.2 ± 6.9	25.9 ± 8.4	.941
Leg extensor power (kg)	21.0 ± 9.1	19.8 ± 8.7	.307
One-leg standing time with eyes open (s)	28.4 ± 32.6	47.6 ± 44.5	.013*
Functional reach (cm)	29.1 ± 9.8	33.7 ± 8.0	.037*

**P* < .05 (Mann-Whitney *U* test).

that proprioception of the mandibular system has a great effect on postural control. They reported that postural control significantly deteriorated in young volunteers after undergoing unilateral conduction anesthesia of the mandibular nerve. These results suggest that tooth loss may affect postural control. The aim of this study was to compare body balance control between edentulous and dentate community-dwelling elderly subjects.

Materials and Methods

This study was approved by the Ethics Committee of Kyoto Prefectural University of Medicine. All subjects were living independently in Kyoto and participated in the 2006 Kyoto Health Seminar, which is held by the Kyoto Prefectural University of Medicine each May. Individuals suffering from cerebrovascular diseases, motor neuron diseases, or otologic symptoms or those who were obese (Body Mass Index [BMI] > 30) were excluded from the study. In addition, the Geriatric Depression Scale¹² was performed and any subject considered to be depressed was excluded. Dental examinations were performed by the authors using a dental mirror and small light. A case control study was planned as follows: the test group included all seminar participants who wore a full denture in either or both arches. The control group was selected from the 149 subjects who retained all dentition with either natural teeth or crown prostheses. A blinded practitioner matched the control group to the test group by age, gender, body fat (BMI), and muscle composition, measured by arm muscle circumference (AMC).

Physical fitness examinations were performed as a part of this seminar. Hand grip and leg extensor power reflected muscle strength. Time spent standing on one leg with the eyes open and functional reach (the difference between arm length and maximal forward reach) reflected balance ability.

Body balance ability was also evaluated using a stabilometer (Stabilometer S510-U, Sakamoto). The sta-

bilometer test is a valid and reliable examination used to evaluate whirling and staggering body movements.¹³ Each subject was asked to remain as stable and relaxed as possible while standing barefoot on a vertical force platform focusing on a mark 2 m away. The parameters of the examination were sway area, serving as an accurate indicator of postural balance, and body sway, which reflected the energy consumption needed to remain steady. Measurements were recorded for 20 seconds with each subject standing with both eyes open and closed and the pressure placed at the center of the foot was displayed on a personal computer. In the test group, the same measurements were taken using the stabilometer with and without dentures.

Comparisons between test and control groups were made using the Mann-Whitney *U* test with the aid of SPSS 15.0 J for Windows (SPSS). The Wilcoxon signed ranks test was used to compare performances in the test group with and without dentures.

Results

The test and control groups each included 12 male and 23 female subjects. Mean age, BMI, and AMC for each group are shown in Table 1. Physical function, as measured by the hand grip and leg extensor tests, was not significantly different when comparing the two groups (Table 1). However, body balance ability, measured by time spent standing on one leg with the eyes open and functional reach, was significantly reduced among members of the test group (*P* < .05) (Table 1).

The results of the stabilometer test showed that sway area was significantly greater in the test group when standing with the eyes closed (*P* < .05) (Table 2). Also, body sway reflected a significantly increased energy consumption in the same group under the same condition (*P* < .05) (Table 2). In the test group, denture wearing was not shown to have any correlation to postural stabilization (Table 3). A power analysis used to analyze the beta bias of the sample demonstrated that the power of these results was 60% to 70%.

Table 2 Results of Stabilometer Test for Test and Control Groups

	Test group	Control group	P value
Sway area (mm ²)			
Eyes open	8.0 ± 5.4	9.1 ± 14.7	.716
Eyes closed	11.0 ± 7.0	7.6 ± 5.3	.035*
Body sway (cm)			
Eyes open	40.8 ± 12.9	39.5 ± 13.8	.518
Eyes closed	57.5 ± 21.5	46.9 ± 15.0	.048*

*P < .05 (Mann-Whitney U test).

Discussion

The results of this study demonstrate that tooth loss is a risk factor for postural instability among the elderly. Yamaga et al¹⁴ showed that the condition of dental occlusion is associated with reduced lower extremity dynamic strength in elderly individuals and a reduction in the amount of time they are able to stand on one leg with their eyes open. The present study agreed with this finding that tooth loss decreases body balance ability by examining the results of a series of physical examinations.

Normally, when in an upright position, frequent small oscillations are generated to maintain balance. Sensorial afferents are provided from proprioceptive, tactile, vestibular, and visual receptors. Proprioception of the mandibular system arises from the masticatory muscular system and dentoalveolar ligaments.¹⁵ It has been suggested that a more symmetric maxillo-mandibular position results in a more symmetric sternocleidomastoid muscle contraction pattern and less body sway.¹⁶ Therefore, it follows that poor or non-existent dental occlusion may decrease proprioception in this area, interfering with the stability of head posture. The removal of visual input leads to an increased difficulty in postural control and may emphasize the role of mandibular system proprioceptive sensation in body balance control. The results of the stabilometer test in this study confirmed this since the body balance ability of the test group was significantly decreased when their eyes were closed.

Proprioceptive sensation from the periodontal ligament receptor plays an important role in body balance control, shown by the fact that the results of the stabilometer test did not differ between the test groups when they were or were not wearing dentures. A study by Usumez et al¹⁷ found no significant changes in head position 30 days after new complete dentures were inserted. On the other hand, another Japanese study reported that totally edentulous patients without dentures showed a significantly higher degree of pos-

Table 3 Results of Stabilometer Test for Test Group With and Without Dentures

	With dentures	Without dentures	P value
Sway area (mm ²)			
Eyes open	8.0 ± 5.4	7.7 ± 5.5	.984
Eyes closed	11.0 ± 7.0	10.5 ± 7.4	.071
Body sway (cm)			
Eyes open	40.8 ± 12.9	41.2 ± 12.5	.829
Eyes closed	57.5 ± 21.5	53.6 ± 14.9	.072

tural swaying when compared to patients with dentures.¹⁸ In that study, 19 of 35 subjects had lost all of their teeth and another 16 subjects had some teeth in one arch or the other (mean = 5.8 teeth). The difference in the results of these two studies may be because about half of the subjects in the test group had some teeth and functional periodontal ligaments, which would influence body balance.

Furthermore, dynamic body balance-associated functions, such as quickness or recovery action of the body, appeared to have deteriorated in the absence of dental occlusion.^{19,20} In such conditions, muscle strength factors can be more important to balancing ability than static body balance, as examined in this study. An earlier report suggested that voluntary teeth clenching, in which the ankle extensors and flexors co-contract to fix the ankle joint, may contribute to the stabilization of postural stance.²¹ However, these results were obtained from young, healthy volunteers. Further research will be needed to conclude similar results for an elderly population.

Conclusion

Within the limited conditions of this study, it can be concluded that natural occlusion, which involves the presence of periodontal ligaments, may play a role in generating an adequate postural reflex through mandibular stability. A longitudinal study with a large sample will be needed to confirm that complete occlusion is linked to a reduction in the number of falls. In any case, a dental examination is recommended for inclusion in the standard health examination for elderly persons.

Acknowledgment

This study was supported by a grant-in-aid from the Ministry of Health, Labour, and Welfare (H16-kenko-021).

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Literature Abstract

Retrospective analysis of 56 edentulous dental arches restored with 344 single-stage implants using an immediate loading fixed provisional protocol: Statistical predictors of implant failure

The purpose of this retrospective study was to analyze the factors that are most likely to predict a negative outcome for the use of immediately loaded, provisional full-arch fixed prostheses supported by multiple single-stage implants. Over a period of 8 years, the author has restored 56 consecutive fully edentulous patients with same-day cross-stabilized acrylic resin-fixed provisional restorations supported by multiple single-stage implants. The cases were finally restored with metal ceramic fixed prostheses and monitored for 2 to 10 years after placement and potential risk factors were evaluated. These included smoking, grafted bone, anterior vs. posterior placement, maxilla or mandible, number of implants per arch (4 to 10), length (6, 8, 10, 12, 14, and 16 mm) and diameter of implants (3.3, 4.1, and 4.8 mm), age, gender, implant surface (SLA vs. TPS), and type of tissue retraction techniques (be it tissue punch or full-thickness flap reflection). Patients were deemed to be failures if they had at least one implant failure but no criteria was given to assess failure. Univariate tests were made using Fisher exact tests and the Cochran Armitage test was used to analyze linear trends. Logistic regression modeling was also used to determine predictive factors. The results initially showed that smoking, grafted recipient sites, and maxillary bone were predictors of high failure. However, logistic regression showed that only implant length emerged as statistically significant and short implants (ie, 6 mm), was shown to be a predictor of failure.

Kinsel R, Liss M. *Int J Oral Maxillofac Implants* 2007;22:823-830. **References:** 24. **Reprints:** Dr Richard Kinsel, Department of Restorative Dentistry, University of California, San Francisco, 1291 E Hillsdale Blvd Suite 143, Foster City, CA 94404. Fax: 650 573 8280.—Y. L. Seetoh, Singapore

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特集

歯科と医科のクロストーク



福泉 隆喜

4. 歯科医科連携が重要な疾患

3) 感染症

—口腔内細菌に起因する要介護高齢者の誤嚥性肺炎—

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●●● 誤嚥性肺炎を起こす口腔内細菌

免疫機能が低下した易感染性宿主, 例えば要介護高齢者において, しばしば重篤となる誤嚥性肺炎を起こす口腔内細菌は, 有菌顎で清掃不良な口腔内の場合, 菌種で300種以上, 総菌数で 10^{12} 個以上を超えるといわれている。肺炎患者の病巣から分離される菌の内訳(表1)は, グラム陰性桿菌が57%と最も多く, グラム陽性球菌が31%, グラム陽性桿菌が10%, グラム陰性球菌が2%となっている。嫌気性菌の分離頻度も高く, 肺炎患者の75~90%から嫌気性菌が検出されている^{1,2)}。これらの嫌気性菌の中には, *Porphyromonas gingivalis*などの黒色素産生性グラム陰性桿菌, *Fusobacterium nucleatum*をはじめとして, 歯周炎の際に増加する偏性嫌気性グラム陰性桿菌も多数含まれている。その他の多くの菌群も, 大部分は口腔内の常在細菌であり, 唾液や食物残渣とともに侵入した口腔内細菌によって, 誤嚥性肺炎が引き起こされていることが明らかになってきた。

●●● 高齢者と誤嚥性肺炎の関係

誤嚥性肺炎に関係する生理的反射は, 嚥下反射と咳反射である。嚥下反射とは, 嚥下運動の第2期, すなわち飲食物を咽頭から食道へ送り込む反射運動のことを指す。通常は, 飲食物が食道に送られるタイミングに合わせて喉頭蓋が喉頭口をふさぎ, 気管への誤嚥を防止している。万一, 気管に飲食物が侵入した場合でも, 強い咳反射が起こってこれを排出し, 下気道

表1 誤嚥性肺炎患者の病巣から検出される細菌

細菌種	検出例
グラム陰性桿菌	
黒色集落となる嫌気性桿菌 (<i>Porphyromonas gingivalis</i> , <i>Prevotella intermedia</i> など)	23
黒色集落とならないPrevotella	19
<i>Fusobacterium nucleatum</i>	18
Klebsiella属	6
緑膿菌	6
大腸菌	6
<i>Enterobacter cloacae</i>	4
その他	9
グラム陽性球菌	
Peptostreptococcus属	16
Peptococcus属	7
微好気性菌	9
黄色ブドウ球菌	8
肺炎レンサ球菌	7
腸球菌	2
化膿レンサ球菌	1
グラム陰性球菌	
Veillonella属	4
グラム陽性桿菌	
Clostridium属	5
Eubacterium属	5
Propionibacterium属	4
Bifidobacterium属	2

への落ち込みを防いでいる。誤嚥性肺炎患者では、この両方の反射が低下し、特に夜間睡眠中に低下が著しいことが明らかになった^{3,4)}。この嚥下反射と咳反射の低下は、高齢者でも健常者では認められない^{5,6)}。

嚥下運動に関する中枢は、延髄と大脳基底核にあることが知られており、これらと誤嚥性肺炎との関係が注目された。同部の脳梗塞患者では、非脳梗塞患者と比べて、有意に肺炎の発症率が高く、高率に誤嚥を起こしている⁷⁾。また、脳梗塞患者でも大脳皮質における梗塞では、誤嚥と肺炎の発生は非脳梗塞患者におけるものと変わらないことも示されている。したがって、大部分の誤嚥は、大脳基底核の脳血管障害によって引き起こされていることが明らかとなった。この反射の低下による誤嚥に、個体の免疫能の低下が重なることで、要介護高齢者に口腔内細菌による誤嚥性肺炎が多発するものと考えられるようになってきた。

要介護高齢者に誤嚥性肺炎が起ると、致死的な転帰を取ることも少なくなく、歯科医師や歯科衛生士による専門的な口腔管理が重要である。

●●●● 誤嚥性肺炎の予防における留意点

誤嚥性肺炎は、大脳基底核の脳血管障害によって嚥下反射と咳反射が低下し、口腔内細菌が下気道に落ち込むことによって生じる。誤嚥性肺炎の既往がある要介護高齢者では、肺炎が治癒した後も咳反射が持続的に低下している⁸⁾。このため、誤嚥性肺炎の再発を繰り返すことが少なくない。誤嚥性肺炎の再発に抗菌物質の投与を繰り返すと、耐性菌の出現などによって重篤化しやすい。したがって、誤嚥性肺炎の予防には、その原因に対する根本的な対処が必要となる。すなわち、摂食・嚥下機能のリハビリテーションによって誤嚥自体を防止することと、口腔ケアによって口腔の清潔を保持して、下気道に落ち込む口腔内細菌の総菌数を減少させることの両面からのアプローチが重要となる。

●●●● 脳梗塞と嚥下機能

前述のように、誤嚥性肺炎の主たる原因の1つとして、脳梗塞の後遺症による摂食・嚥下障害が挙げられる。しかし、脳梗塞後の摂食・嚥下障害は、一側性で初回の場合においては、半年後にはほとんど嚥下障害は残存しないとされている^{9,10)}。同一の病院に半年間入院できることは通常ないため、脳梗塞後の急性

期において経口摂取が不可能な場合、多くの患者が胃瘻造設などの経管栄養となって退院するものと推察されるが、経管栄養後の患者の誤嚥性肺炎の有無を調べた調査では、発症率にはかなりのばらつきがあり、経管栄養にするだけでは誤嚥性肺炎の発症を抑制できないことが示されている¹¹⁾。したがって、誤嚥性肺炎の予防のためには、専門職種による摂食・嚥下機能の適切な評価と、リハビリテーションを行うことが重要である。

●●●● 口腔ケアによる口腔内細菌のコントロール

一方、誤嚥性肺炎の原因は口腔内細菌であるため、その発症を予防するためには、口腔ケアによって清掃状態を改善し、口腔内細菌の総菌数を減少させることが有効である。誤嚥性肺炎を起こしやすい高齢者は、脳血管障害などに起因して日常生活動作(ADL)が低下した要介護高齢者であることが多いため、自発的な口腔清掃はなかなか困難である。しかし、できる限り高齢者自身による自発的な清掃を原則とし、歯科医師や歯科衛生士による定期的なチェックを行うことが勧められる。ADLがかなり低下しており、自発的な口腔清掃が困難で介助を要する場合は、歯科医師や歯科衛生士によって、歯面と粘膜面の専門的口腔ケアを行う必要がある。専門的口腔ケアは、ブラシなどによる歯や粘膜などの機械的清掃を基本とし、補助的に含嗽薬や消毒薬が用いられている。ケアが適切に行われると、口腔内で検出される総菌数が1/10程度に減少できるといわれている。

●●●● 歯科専門職の要介護高齢者への関わり

摂食・嚥下リハビリテーションや、口腔ケアにおける歯科専門職の果たす役割は、前述のように、大変大きなものであるが、現状では、要介護高齢者への提供体制は必ずしも十分ではない。例えば、要介護状態の高齢者の口腔内状態は悪く、89.4%の者が何らかの歯科治療、または専門的口腔ケアが必要であるにもかかわらず、実際に歯科治療を受診した者は26%に過ぎない¹²⁾。また、歯科診療所のうち、居宅への歯科訪問診療を行っている診療所は12.1%、訪問歯科衛生指導を行っている診療所は5.2%、歯科医師による居宅療養管理指導を行っている診療所は4.4%、歯科衛生士などに

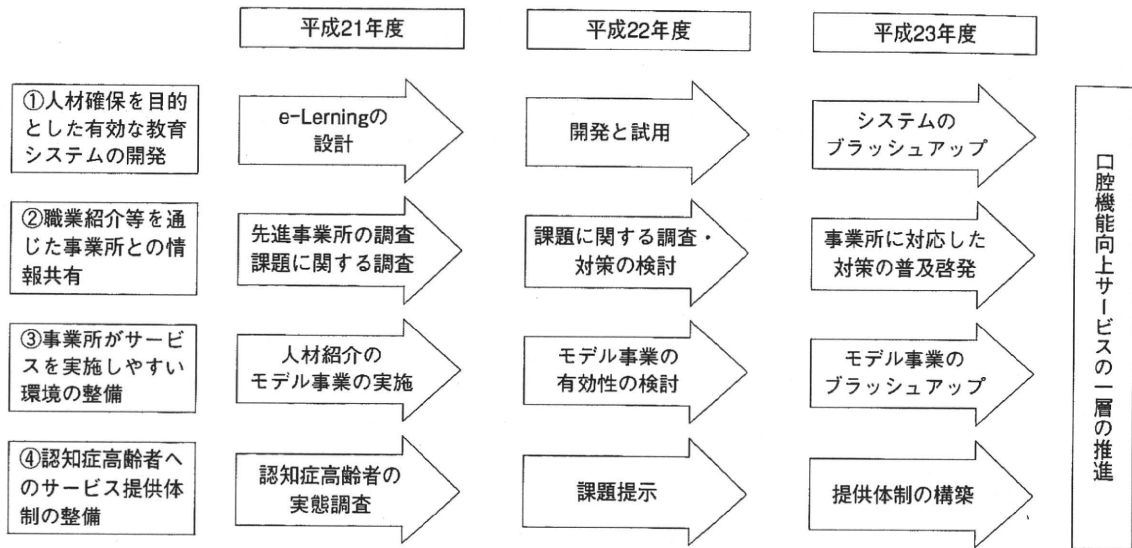


図1 口腔機能向上サービスに係る人材確保のための研究事業の概要

(文献17より引用)

よる居宅療養管理指導を行っている診療所は3.0%であった¹³⁾。居宅介護サービス利用者のうち、訪問歯科診療の制度そのものを知らない者が59%、介護保険サービスとして口腔ケアの指導を受けられることを知らない者が79%であった¹⁴⁾。

これらの現状を踏まえると、必要とされる者に必要とされるサービスを、切れ目なく提供することが、要介護高齢者の口腔機能の維持・管理にとって大変重要といえる。このため、厚生労働省の調査研究事業においても、平成21年度から在宅歯科医療と居宅介護サービスの連携を目指した取組が行われている¹⁵⁾。

●●● 介護保険における誤嚥性肺炎防止のための取組

介護保険法改正に伴い、平成18年度から予防給付の1つとして、高齢者がおいしく、楽しく、安全な食生活を営むことにより、自己実現達成の支援を行うことを目的とした通所サービスとして、口腔機能向上サービスが導入された。このサービスは、要介護高齢者の誤嚥性肺炎の防止に重要とされる、①摂食・嚥下機能のリハビリテーションによって、誤嚥自体を防止することと、②口腔ケアによって口腔の清潔を保持して、下気道に落ち込む口腔内細菌の総菌数を減少させることについて、その両者を提供するもので、要介護高齢者のQOLの維持・向上に果たす役割は大きい。

平成21年度介護報酬改定においても、介護現場のニーズ¹⁶⁾に応じた改定が行われている。

サービスの提供現場で使用される「口腔機能向上マニュアル」についても、平成21年3月の改訂版では、①今回の改定内容の反映、②課題把握のためのツール、および利用者への説明用チャートの新規収載、③記載内容の大幅な簡素化などが行われ、より使いやすい配慮が行われている。

一方で、口腔機能向上サービスを担う人材の育成確保対策については、介護報酬改定だけでは充分に対応できないため、平成21年度から平成23年度までの3カ年の厚生労働科学研究において、①人材確保を目的とした有効な教育システムの開発、②職業紹介などを通じた事業所との情報共有、③事業所がサービスを実施しやすい環境の整備、④認知症高齢者へのサービス提供体制の整備が、それぞれ行われている(図1)。また、①口腔機能向上サービスの提供状況などの把握のための、指定通所介護事業所などに対する実態調査、②同サービスの普及・啓発を図るための地域包括支援センター職員、および介護支援専門員などに対する研修もそれぞれ行われており、口腔機能向上サービスの利用も進みつつある。

さらに、介護保険施設入所者に対する口腔ケアの提供体制の向上に資するため、①歯科医師または歯科医師の指示を受けた歯科衛生士が、介護職員に対する口腔ケアに係る技術的助言、および指導を月1回以上行っている場合であって、②当該施設において歯科医師、または歯科医師の指示を受けた歯科衛生士の技術的助言および指導に基づき、入所者の口腔ケア・マネジメントに係る計画が作成する場合を評価して、平成