Loi Tonthat [ID], Shunnosuke Takahashi, Hidehiko Onodera, Kazuhiko Okita [ID], Shin Yabukami [ID], Kotone Yokota, Maiko Furuya, Hiroyasu Kanetaka, Yoshinori Miura, Hideki Takahashi, Yoshihiko Watanabe, and Ritsuko Akiyama

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A simple and rapid detection system for oral bacteria in liquid phase for point-of-care diagnostics using magnetic nanoparticles

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Loi Tonthat,[1,2,a] (iD)   Shunnosuke Takahashi,[1]   Hidehiko Onodera,[2]   Kazuhiko Okita,[2] (iD)   Shin Yabukami,[1,2] (iD)
Kotone Yokota,[1,2]   Maiko Furuya,[1]   Hiroyasu Kanetaka,[1]   Yoshinori Miura,[3]   Hideki Takahashi,[3]   Yoshihiko Watanabe,[4]
and Ritsuko Akiyama[5]

AFFILIATIONS

[1] Department of Electrical Engineering, Tohoku University, Sendai 980-8579, Japan

[2] Tohoku Gakuin University, Tagajo 985-853, Japan

[3] JNS Co., Ltd., Natori 981-1251, Japan

[4] Department of Industrial Welfare Management, Tohoku Fukushi University, Sendai 981-8522, Japan

[5] Sendan No Oka, Sendai 989-3201, Japan

Note: This paper was presented at the 64th Annual Conference on Magnetism and Magnetic Materials.
[a] Corresponding author: loi@ecei.tohoku.ac.jp

ABSTRACT

This study describes a user-friendly and rapid detection system of oral bacteria in the liquid phase for point of care testing based on magnetic immunoassay. We focused on the dependence of the strength of external magnetic field required to switch the magnetic moments of nanoparticles bound to bacteria on the bacteria concentration. The results obtained indicate that the required field strength increases linearly as a function of log concentration of *Porphyromonas gingivalis* cultured in the range of 10^3–10^9 CFU/mL. Similarly, the required field strengths for *Streptococcus mutans* and *Pseudomonas aeruginosa* increase monotonically when their concentrations increase, whereas the required field strength for *Escherichia coli* decreases monotonically when its concentration increases. We then measured the concentration of *Porphyromonas gingivalis* in the saliva collected from elderly people in a geriatric health services facility and the results using the developed system had a correlation with those using a commercial bacteria counter.

I. INTRODUCTION

Magnetic nanoparticles have been gaining great interest for use in biomedical applications as they can be easily manipulated by an external magnetic field. Recently, magnetic immunoassay using bio-functionalized magnetic nanoparticles has been widely developed for point of care testing (POCT). The nanoparticles are coated with detecting antibodies (referred to as magnetic markers) and these antibodies selectively capture biological targets such as proteins and cells (referred to as antigens) via antigen-antibody reactions.[1–3] So far, magnetic relaxation, AC susceptibility and remanence-based measurement methods have been developed for the detection of biological targets.[3] To detect magnetic fringe field produced by

the nanoparticles, magnetic sensors used, such as giant magnetoimpedance (GMI),[4] giant magnetoresistance (GMR),[5] tunneling magnetoresistance (TMR)[6] and SQUID sensors.[2,7]

Recent evidence suggests that oral microbiota is closely related to diseases including periodontal disease, caries, and pneumonia.[8] In 2018, pneumonia was the fifth leading cause of death in Japan, and most people died from the pneumonia were older than 75 years. Aspiration pneumonia, which is common in the elderly, is caused by periodontal disease bacteria (*Porphyromonas gingivalis*) which enter the lung through saliva. As such, the quantitative detection of oral bacteria has increasingly attracted attention.[9–11] To date, the standard method for quantitative detection is based on cultivation and counting of grown colonies. However, it requires time up to

several days for bacteria incubation and needs to be implemented by specialists. In this regard, as an alternative approach to the cultivation method, magnetic immunoassay can meet the requirements of POCT in rapid, highly-sensitive, low-cost and user-friendly detection.

In this study, we develop a simple and rapid detection system of oral bacteria based on magnetic immunoassay. The magnetic moments of nanoparticles without bacteria can be easily reversed under an applied switching magnetic field compared to nanoparticles bound to bacteria.[2,12] Therefore, the field strength required to reverse the magnetic moments (hereafter, referred to as required field strength) depends on the bacteria concentration. We examined the calibration curves of typical bacteria such as *Streptococcus mutans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Porphyromonas gingivalis*. We then measured the concentration of *P. gingivalis* cultured in saliva collected from the elderly people in a geriatric health services facility using the developed system and compared the obtained results to those using a commercial bacteria counter.

II. EXPERIMENTAL METHOD

A. Experimental setup

Figure 1 shows the schematic diagram of the developed system. It consists of a GMI sensor (developed by JNS Co. Ltd.), NdFeB magnet (NeoMag, 5 mm x 4 mm x 3 mm), drive coil (3000 turns), power supply (Advantest, R6243) and rotor (IAI, RCS2-RTC8HL-I-20-15(24)-360-T2-P-L). First, the nanoparticles bound to the bacteria in the sample were magnetized by the NdFeB magnet with a surface magnetic flux density of 21 mT for 600 sec, then the magnet was taken out manually. Subsequently, the sample was rotated directly above the yoke using the rotor for 300 sec. Here, the yoke was used to focus the magnetic flux density of 12 mT at the tip position of the yoke to aggerate the nanoparticles dispersed in the sample at one point (the current in the drive coil generated from the power supply was 1 A).

After the magnetization and aggregation processes above, to examine the required field strength for each bacteria concentration, the field strength was changed from 0 mT to 9.36 mT with a step interval of 0.36 mT for the upward and downward direction (i.e., the current in the drive coil was 0, 30, −30, . . ., 780, and −780 mA). Each magnetic field was applied for 30 sec, and the magnetic moments of nanoparticles tended to align themselves with the direction of the
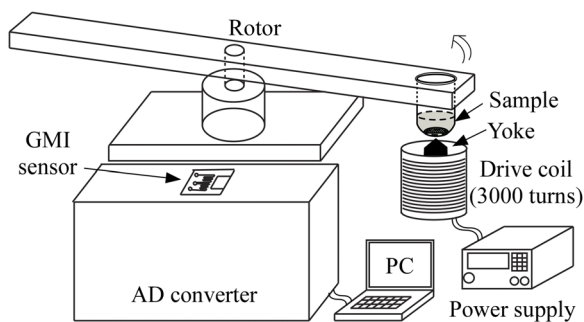

FIG. 1. Schematic of laboratory-made system for detecting bacteria in liquid phase using magnetic nanoparticles.

applied magnetic field. The sample was then rotated slowly close to the GMI sensor with a rotation speed of 100 deg/s, the magnetic fringe field from the nanoparticles was measured by the sensor with a sampling rate of 50 kHz. The distance between the bottom surface of the sample tube and the sensor was 200 μm. Here, the tube was installed as close as possible so that the tube did not touch the sensor during rotation.

An advantage of the developed system is that the bacteria concentration is measured and evaluated without any post-treatment such as washing process after the antigen-antibody reaction, which allows for rapid detection.

B. Sample preparation

Magnetic beads (Micromod Partikeltechnologie GmbH, Nanomag®-D 09-20-502) consist of magnetic nanoparticles with an average diameter of 500 nm provided with covalently bound protein A on the surface. The nanoparticles are dextran iron oxide composite particles in a highly uniform cluster-type shape with a stock concentration of 10 mg/mL. The pure magnetic beads were diluted 200-fold in phosphate-buffered saline (PBS) and the dosage of magnetic beads used for each sample was 0.01 μL/tube. The nanoparticles with antibodies (e.g. anti-*P. gingivalis*, anti-*S. mutans*, anti-*P. aeruginosa* and anti-*E. coli*) were reacted with their antigens for 30 min. The pure liquid bacteria were diluted in PBS to change its concentration. Each sample tube consisted of PBS (97 μL), antibody (1 μL), magnetic beads (2 μL), and cultured bacteria (100 μL). After the antigen-antibody reaction, the entire volume was transferred to another tube, then PBS was added to fill the tube (the maximum volume of the additional PBS was 8 mL) and the cultured sample was stored immediately at 4°C.

III. RESULTS AND DISCUSSION

Figure 2 shows the output voltage when the sample moved close to the GMI sensor and the calibration curve of *S. mutans*. The bacteria concentration was measured using an OD meter. The nanoparticle concentration was 5×10^6 in 100 μL. The nanoparticles with anti-*S. mutans* (Ab31181) were reacted with *S. mutans*. Fig. 2(a) shows that, without *S. mutans*, the peak voltage was reversed when the field direction was switched, because the magnetic moments of the nanoparticles without *S. mutans* were easily rotated. However, in Fig. 2(b) with *S. mutans* with a concentration of 1×10^6 in 100 μL, the peak voltage tended to decrease compared to the result in Fig. 2(a) because the magnetic moments of the nanoparticles with *S. mutans* became difficult to rotate. When the applied magnetic field was weak, the peak voltage of the downward magnetic field tended to be in the same direction as that of the upward magnetic field. However, when the field strength exceeded a certain value (i.e., the required field strength), the peak voltage of the downward magnetic field started in the opposite direction to that of the upward magnetic field. The field strength at that time was determined to be the required field corresponding to the bacteria concentration. In Fig. 2(c), the required field strength of *S. mutans* increased monotonically as the concentration of *S. mutans* increased.

In principle, the concentration of other bacteria can also be measured using the developed system. Fig. 3(a) shows that the required field strength in the case of *P. aeruginosa* increased monotonically when its concentration increased, whereas in
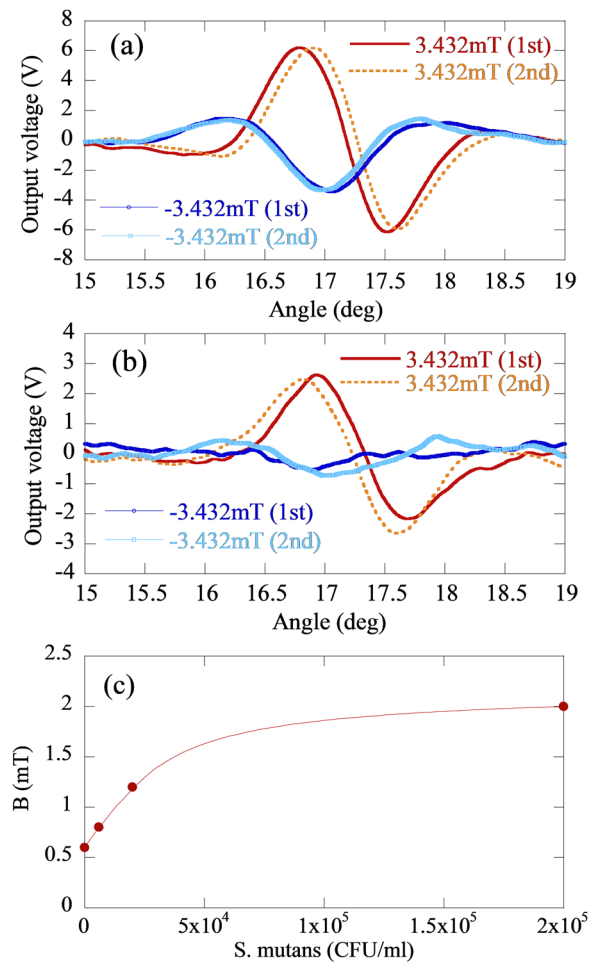
FIG. 2. The output voltage when the sample rotated close to the GMI sensor when the sample consisted of magnetic nanoparticles (a) without *S. mutans*, (b) with *S. mutans*, and (c) the calibration curve of *S. mutans*.

FIG. 3. The calibration curves of (a) *P. aeruginosa* and (b) *E. coli*.

Fig. 3(b), the required field strength in the case of *E. coli* decreased monotonically when its concentration increased. The difference in this tendency was considered that the oral bacteria originally agglutinated and lived. As such, the nanoparticles were attached to the aggregates of the bacteria as shown in Fig. 4(a). However, in Fig. 4(b) in the case of *E. coli* which normally lives in the intestines the nanoparticles were attached to a single bacterium.

Next, we collected the saliva from elderly people (n=10) in a geriatric health services facility named Sendan No Oka using Salisoft® and Salikids® purchased from Salimetrics and then measured the concentration of *P. gingivalis* in the saliva. The saliva was collected by placing the Salisoft in the mouth. The saliva collection and evaluation were approved by the ethical committee of Sendan No Oka. As reference data, we measured the total number of bacteria in the saliva using a commercial bacteria counter (Panasonic, DU-AA01). First, we obtained the calibration curve of *P. gingivalis* cultured using the developed system. Fig. 5 shows that the required field strength increased linearly as a function of log
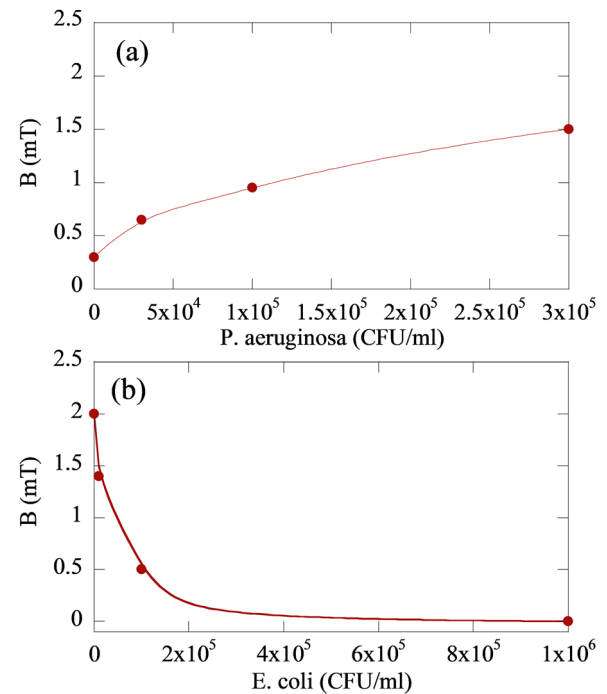
concentration of *P. gingivalis*. The result also indicates that the detection limit of *P. gingivalis* is 10^3 CFU/mL. Using the calibration curve of *P. gingivalis* above, we then measured the concentration of *P. gingivalis* in the saliva. In Fig. 6, the results using the developed system and the bacteria counter are correlated, with a correlation coefficient of 0.55. After the measurement using the developed system, the samples left in Samples 2, 7 and 10 were not enough for the bacteria counter. In addition, the people who provided Samples 4, 8 and 9 had a lot of bacteria compared to the others. These people wore full dentures and brushed their teeth themselves. According to their dental hygienist, the people may have not brushed their teeth when the hygienist had not examined them, and their oral condition was bad when the bacteria concentration was high. This suggested that useful results could be obtained at actual measurement sites.

Currently, the detection time is about 1 hour, which is significantly shorter than with the conventional cultivation method, and it can be shortened further by optimizing the field step interval, the
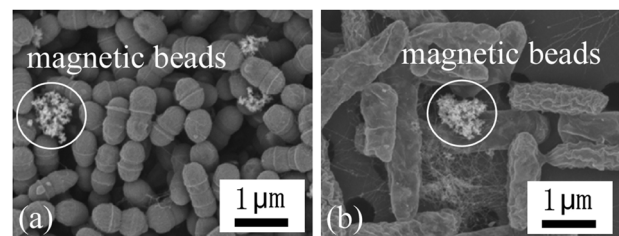

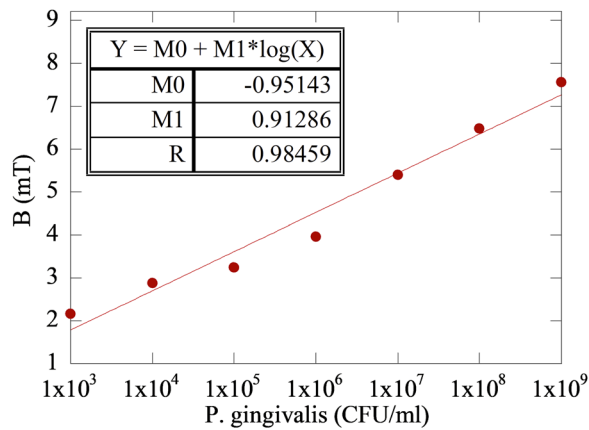FIG. 4. The microscope images of (a) *S. mutans* and (b) *E. coli*.

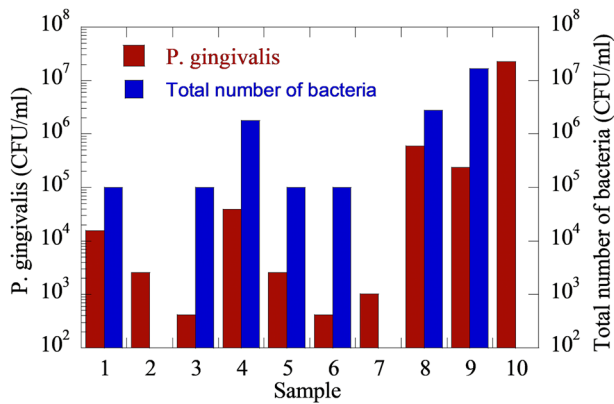**FIG. 5**. The calibration curves of *P. gingivalis*.

FIG. 6. The concentration of *P. gingivalis* cultured using our system using the collected saliva from elderly people (n=10) in a geriatric health services facility and the total number of bacteria in the saliva using a commercial bacteria counter.

magnetization time, etc. In addition, the detection limit of bacteria concentration can be improved by using more sensitive magnetic sensors and decreasing the distance between the sample and the sensor. Future works will examine in detail the relationship between health status and bacteria concentration.

IV. CONCLUSIONS

In this study, we have developed a simple and rapid detection system for oral bacteria in liquid phase for point-of-care diagnostics based on magnetic immunoassay. Using the developed system, we obtained the calibration curves of typical cultured bacteria such as *P. gingivalis*, *S. mutans*, *E. coli*, and *P. aeruginosa*. We measured the concentration of *P. gingivalis* cultured using the saliva collected from the elderly people in a geriatric health services facility and the obtained results had a correlation with the reference data using a commercial bacteria counter. The current detection limit is 10^3 CFU/mL and detection time is about 1 hour. Future work is to optimize the developed system and examine the relationship between health status and bacteria concentration.

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