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Point-of-care detection of *Tannerella forsythia* using an antigen–antibody assisted dielectrophoretic impedance measurement method

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ABSTRACT

The importance of periodontal treatment planning based on diagnosis with clinical detection of periodontal pathogens has been well recognized. However, reliable detection and quantification methods that can be conveniently used at chair-side have yet to be developed. This study aimed to evaluate the clinical use of a novel apparatus which uses an antigen–antibody reaction assisted dielectrophoretic impedance measurement (AA-DEPIM) for the detection of a prominent periodontal pathogen, *Tannerella forsythia*. A total of 15 patients with a clinical diagnosis of chronic periodontitis, three periodontally healthy volunteers and two with gingivitis were subjected to clinical and microbiological examinations. Saliva samples were analyzed for the presence of *T. forsythia* using AA-DEPIM, PCR-Invader and real-time PCR methods. The measurement values for total bacteria and *T. forsythia* using the prototype AA-DEPIM apparatus were significantly greater in periodontitis group than those in healthy/gingivitis group. Using the AA-DEPIM apparatus with tentative cut-off values, *T. forsythia* was detected for 14 (12 with periodontitis and 2 either healthy or with gingivitis) out of 20 individuals. The measurement for the detection of *T. forsythia* by the AA-DEPIM method showed a significant positive correlation with the detection by PCR-Invader ($r = 0.541$, $p = 0.01$) and the real-time PCR method ($r = 0.834$, $p = 0.01$). When the PCR-Invader method was used as a reference, the sensitivity and specificity of the AA-DEPIM method were 76.5% and 100%, respectively. The results suggested that the AA-DEPIM method has potential to be used for clinically evaluating salivary presence of *T. forsythia* at chair-side.
Trial registration: UMIN Clinical Trials Registry (UMIN-CTR) UMIN000012181.

1. Introduction

Periodontal disease is the inflammation of periodontal tissue caused by pathogenic microflora in dental plaque biofilm [1]. The presence of distinct set of subgingival bacteria within the plaque biofilm increases the risk for the pathogenesis and the level of progression of periodontitis [2,3]. Microbiological assessment of periodontal pathogens is considered to be important in selecting appropriate antimicrobial therapy, in deciding the need for periodontal surgery, and in evaluating the treatment outcome [4]. Various bacterial detection methods including polymerase chain reaction (PCR), ATP bioluminescence and a direct-count technique using epifluorescence microscopy [5] have been used. While the relevance of point-of-care detection for periodontal pathogens in clinical settings is widely recognized, a truly effective and rapid detection and quantification system is yet to be developed.

Tannerella forsythia is a non-pigmenting saccharolytic anaerobic Gram-negative rod and one of the most prominent periodontal pathogens [6–8]. *T. forsythia* is a nutritionally fastidious anaerobe which produces a wide array of activities, through the action of

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proteases, enzymes that can degrade extracellular matrix, and biomolecules that can undermine host response factors [9]. Together, *Porphyromonas gingivalis*, *Treponema denticola* and *T. forsythia* comprises the so-called "red complex", which is strongly associated with each other and with diseased periodontal sites [10].

T. forsythia grows poorly in pure culture, unless the medium is supplemented with N-acetylmuramic acid, or by growing together with other microorganisms [11]. Clinically, chair-side analysis of target species such as *T. forsythia* could be used to assess risk of periodontal breakdown, provide endpoints for therapy and as a mode to improve patient awareness of disease etiology [12]. Currently, a novel apparatus is being developed to rapidly detect and quantify this bacterium, which is based on the bacteria detection technique called dielectrophoretic impedance measurement (DEPIM) [13,14]. A production model based on the original DEPIM method that measures total bacteria in samples from the oral cavity [15] has been commercially available in Japan since 2012 and widely used at chair-side settings in health care and welfare facilities. The novel apparatus utilizes the DEPIM technology combined with an antigen–antibody reaction [16,17] (AA-DEPIM) and is being developed for the specific detection of periodontal pathogens.

This study aims to assess the ability of the prototype AA-DEPIM apparatus to detect *T. forsythia* in clinical saliva samples and to compare this with conventional detection methods based on the PCR.

2. Materials and methods

2.1. Study population

Participants were consecutively recruited from patients with mild to moderate chronic periodontitis [18,19] who visited Tokyo Dental College Chiba Hospital or Suidobashi Hospital from November 2013 to January 2014. Written informed consent was obtained from all participants, and this study was approved by the ethics committee of Tokyo Dental College (No.494).

Complete medical and dental histories were obtained from participants. Systemic exclusion criteria were the presence of cardiovascular and respiratory diseases, systemic inflammatory conditions, such as diabetes mellitus, immunodeficiency, and current pregnancy or lactation. Individuals with a history of active periodontal therapy within the past 6 months were excluded. Current smokers were also excluded. Inclusion criteria consisted of having two or more inter-proximal sites with clinical attachment level (CAL) $\geq$ 4 mm, not on the same tooth, with radiographic evidence of bone loss or two or more inter-proximal sites with probing depth (PD) $\geq$ 5 mm, not on the same tooth, and the presence of at least 16 teeth with a minimum of four molars. The participants received no medication that could affect their periodontal conditions, such as antibiotics or anti-inflammatory drugs, for at least 3 months prior to the microbiological testing. Periodontally healthy volunteers or those with minor gingivitis were also asked to participate.

This clinical study was registered in the UMIN (University Hospital Medical Information Network in Japan) Clinical Trials Registry (UMIN-CTR, No. 000012181).

2.2. Clinical examination

The following clinical parameters were recorded at six sites for each tooth: Probing depth (PD) was measured using a Williams probe (#2, YDM, Higashi Matsuyama, Japan) with an approximate force of 0.2–0.25 N and rounded to the nearest millimeter. Clinical attachment level (CAL) was measured from the cemento-enamel junction to the apical depth of periodontal probe penetration. Bleeding on probing (BOP) was recorded as the presence or absence of bleeding following measurement of PD.

The clinical examination was performed by five trained periodontists. Prior to the study, an investigator meeting was held. In the meeting, training for probing was implemented using a digital precision scale and the periodontal probe to be used in the study.

2.3. Sample collection

Study participants were asked to refrain from using antimicrobial mouthwash or toothpaste for at least 24 h before saliva sample collection. Moreover, they were asked not to eat or brush their teeth for at least one hour before sampling. From each participant, 1.5 ml of stimulated saliva was collected in a sterile calibrated plastic cup. An aliquot of 500 μl was used for the measurement with the apparatus, and another 500 μl was transferred into sample tubes supplied in a commercial kit and sent to a microbiological testing laboratory (BML, Tokyo, Japan) for the quantitative analysis of periodontal pathogens, including *T. forsythia*, using PCR-Invader method (structure-specific 5′ nuclease-based method) [20,21]. The remaining 500 μl was used for the detection of *T. forsythia* by a real-time (RT) - PCR method.

2.4. Measurement with antigen–antibody reaction assisted-DEPIM apparatus

A novel apparatus was used for the detection of *T. forsythia*. This method is based on a bacteria detection technology called dielectrophoretic impedance measurement (DEPIM) [13,14]. The DEPIM method uses positive dielectrophoretic force to capture bacteria in suspension onto an interdigitated microelectrode array. Concentration of bacteria in the suspension can be estimated by measuring impedance change of the microelectrode. The original, commercially available DEPIM apparatus requires 20 s for a measurement of total bacteria present in the sample solution [15]. The DEPIM method has been shown to be effective in measuring total bacterial numbers in a mixed oral bacterial suspension, and is unaffected by bacterial aggregates [22].

With the prototype apparatus, the total number of bacteria is estimated by conventional DEPIM method, i.e., measuring the increase rate of capacitance of the microelectrode [ΔC(fF/s)] [13,14]. Specific bacteria can be detected by antigen–antibody assisted DEPIM (AA-DEPIM) method [16,17]. Target bacteria are recognized by antibodies and attach to the microelectrode by positive dielectrophoresis (Fig. S1). Trapped bacteria can be estimated by measuring the rise in conductivity of the microelectrode immediately after the cytoplasm of trapped bacteria is eluted by electroporation [G_{TP} (μS)] [16].

For the specific detection of *T. forsythia*, murine monoclonal antibodies (mAb) raised against the whole cell antigen of *T. forsythia* ATCC43037 was prepared by Immuno Probe Co. Ltd (Saitama, Japan). The mAbs specifically recognized the epitopes located on the cell surface of *T. forsythia*. In a preliminary experiment, potential cross-reactivity of the mAbs was assessed for a panel of 34 oral bacteria (Table S1) using an enzyme-linked immunosorbent assay and they showed no cross-reactivity with any of the bacteria tested. As for the stabilities of mAbs, no significant change in reactivity was found after storing for up to 14 months at −20 °C and 4 °C. The antibodies, immobilized on magnetic beads, were used to separate and concentrate *T. forsythia* in samples as a bio-recognition element for the apparatus.

Detection of *T. forsythia* in clinical saliva samples was performed using the prototype AA-DEPIM apparatus (Fig. 1). Pre-treatment of saliva sample was performed as follows. The saliva sample was centrifuged for 10 min (14,200 × g at room temperature) and