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Detectability evaluation of novel DNA chip against periodontopathic bacteria

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ABSTRACT

[AIM]
To evaluate the detectability of novel DNA chip by using cultured bacteria and clinical samples.

[Materials]
・Cultured bacteria (P.g. , T.f. and T.d.)
・Clinical samples(Gingival crevicular fluid)

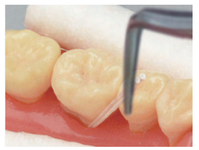
[Methods]
DNA was extracted from cultured bacteria or clinical samples. Extracted DNA was fluorescence labeled by PCR method. Fluorescence-labeled DNA was hybridized with DNA chip (Mitsubishi Chemical, Japan), and fluorescence intensity was measured. Correlation between DNA concentration and fluorescence intensity was evaluated.

[Results and Discussions]
By measuring the fluorescence intensity of the fluorescence -labeled DNA hybridized with DNA chip, positive linear relationship between fluorescence intensity and DNA concentration was observed. Coefficient of determination for each bacterium was 0.999 for P.g, 0.999 for T.f and 0.991 for T.d. From these results, it is suggested that calibration curve to determine the quantity of bacteria could be plotted by the bacterial test using novel DNA chip.

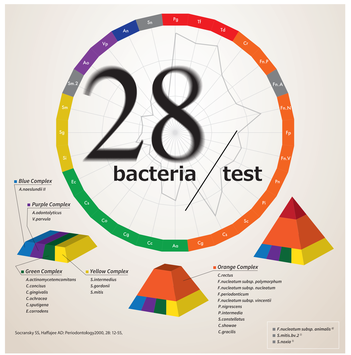
[Conclusion]
From the result of bacteria test using novel DNA chip against the DNA extracted from cultured bacteria, it is suggested that calibration curve to determine the quantity of bacteria could be plotted by fluorescence intensity. By accumulating the data for other periodontopathic bacteria, relationship between the change in bacterial number and the state of periodontal disease could be evaluated. This test method using novel DNA chip is expected to be a new way to manage the periodontal disease of the patient.

1 INTRODUCTION

Periodontal diseases are inflammatory conditions caused by bacterial infection. In previous studies, a number of periodontopathic bacteria have been reported. However, it is rare case that the periodontopathic bacteria adhere to the tooth surface directly. Adhesion of early colonizers is needed for periodontopathic bacteria (orange and red complex) to adhere and multiply on the tooth surface, which leads to the pathogenesis and progress of periodontal diseases. Therefore, it is important to evaluate the oral flora of the patient, in order to manage the periodontal disease. By the bacterial test using novel DNA chip (Mitsubishi Chemical, Japan),28 species of bacteria could be detected at once, so that information of oral flora of the patient could be obtained.



The samples for the novel bacterial test, Oral Flora DNA Inspection (GC, Japan) are GCF collected by paper point.



Aim

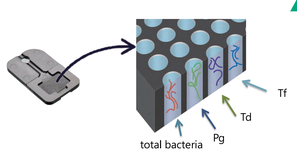
To evaluate the detectability of novel DNA chip against periodontopathic bacteria by using cultured bacteria and clinical samples.

2 MATERIALS & METHODS

Materials

DNA chip

Collection of microscopic DNA spots attached to solid surface, specifically . Numbers of DNA could be analized in single reaction.



Genopal, DNA array chip developed by Mitsubishi Chemical Corp.

Cultured bacteria

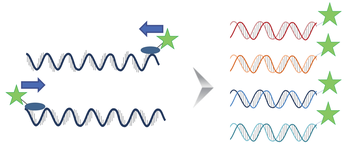
- *P. gingivalis* (P.g., JCM8525)
- *T. forsythia* (T.f., JCM10827)
- *T. denticola* (T.d., JCM8152)

Clinical samples

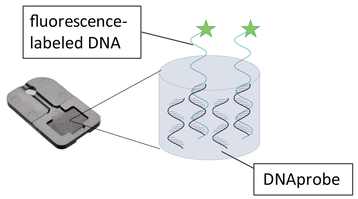
Gingival crevicular fluid (GCF) collected from 2 research sujects. This study was approved by research ethics committees in GC Corp..

Methods

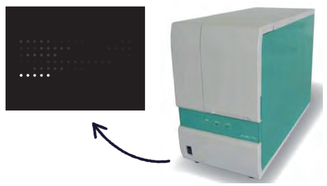
(1) DNA was extracted from cultured bacteria or GCF samples using QuickGene-800 (FUJIFILM, Japan).



(2) Extracted DNA was fluorescence-labeled by PCR method.



(3) Fluorescence-labeled DNA was hybridized with DNA chip (Mitsubishi Chemical, Japan).



(4) Fluorescence intensity was measured by Genopal Reader (Mitsubishi Chemical, Japan).

3 RESULTS

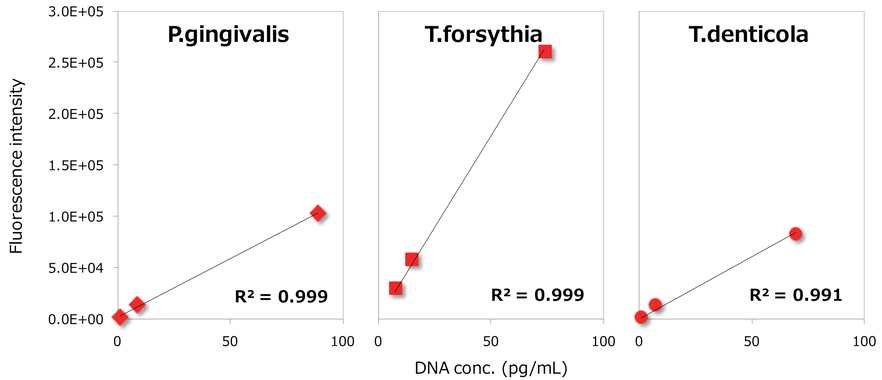
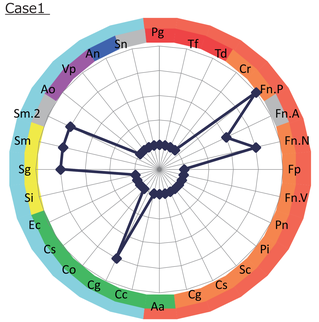


Fig.3 Calibration curve between fluorescence intensity and DNA concentration. By measuring the fluorescence intensity of the fluorescence-labeled DNA hybridized with DNA chip, positive linear relationship between fluorescence intensity and DNA concentration was observed. Coefficient of determination for each bacterium was 0.999 for P.g., 0.999 for T.f. and 0.991 for T.d. . From these results, it is suggested that calibration curve to determine the quantity of bacteria could be plotted by the bacterial test using novel DNA chip.

Case1



Case2

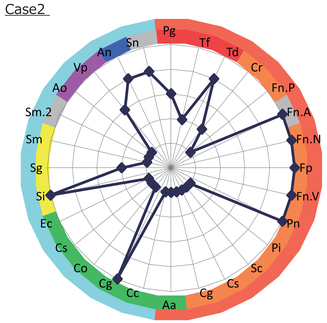


Table1 Clinical findings of each research subjects

Case	subjects	PPD	BOP	stage
1	20s female	3	-	early
2	50s male	5	+	moderate

Fig.4 Radar charts of oral bacterial flora in GCF samples measured by DNA chip. Case1 is 20s female, PPD was 3mm and BOP was negative which was diagnosed with early periodontitis. Case2 is 50s male, PPD was 5mm and BOP was positive which was diagnosed with moderate periodontitis. From clinical findings, case1 was diagnosed as early periodontitis, but from oral flora tested by DNA chip, the orange complex species, especially Fusobacterium species, which were tought to recruit the red complex species were detected. The risk unrevealed by clinical findings was visualized by DNA chip. In case2, the red complex species and more Fusobacterium species were detected, it would be concerned to cause further progression of periodontal disease.

4 CONCLUSION

From the result of bacteria test using novel DNA chip against the DNA extracted from cultured bacteria, it is suggested that calibration curve to determine the quantity of bacteria could be plotted by fluorescence intensity. By accumulating the data for other periodontopathic bacteria, relationship between the change in bacterial number and the state of periodontal disease could be evaluated. This test method using novel DNA chip is expected to be a new way to visualize the periodontal risk unrevealed by clinical findings and manage the periodontal disease of the patient.