Analysis of an Immediate Diagnostic Technique for Detecting Root Canal Bacteria
Using Light-induced Fluorescence

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Abstract

Purpose:
The decision to perform a root canal filling is influenced by many factors including subjective symptoms and clinical signs. Sterility in the root canal before filling is usually confirmed using the bacterial culture method. However, the outcomes by this method is not known until about 24 to 48-hour, and it is associated with a risk of technical contamination, which may affect the results. Therefore, we investigated the possibility of evaluating sterility inside the root canal using a blue light of wavelength approximately 405nm.

Materials/Method

A total of 31 root canals were investigated. After checking subjective symptoms and clinical signs, one drop of sterile water was introduced into the root canal. The water was absorbed 30 seconds later by inserting two sterilized paper points into the root canal. One paper point was used for bacterial culture test while the other was analyzed with high-sensitivity fluorescence-induced test using microscopic spectroscopy. The outcomes of bacteria simple culture test were evaluated after 48 hours. In the fluorescence study, the wave form of the fluorescence spectrum obtained using the microscope multi-photometry system was recorded.
Results:

Clinical signs indicated that performing a root canal filling was not appropriate in ten cases. These ten cases had positive results with both, bacterial culture and the fluorescence tests. Of the remaining 21 cases, 16 had negative results for both tests; while bacterial culture test was positive in rest five cases. Three of these five cases had negative fluorescence test results, indicating technical contamination. The remaining two cases had positive fluorescence test results, indicating bacterial infection.

Conclusion:

Light induced fluorescence can probably replace the bacteria simple culture test in the analysis of root canal bacteria.

Keyword: light-induced fluorescence, endodontic, diagnosis
Introduction

Endodontic treatment generally comprises a series of measures such as dowel preparation, irrigation, application of root canal medications, and root canal filling. According to Ingle et al, imperfect root canal filling can provide a supplementation road for bacterial growth and eventually result in failure of the root canal treatment.\(^1\)

However, Siqueira\(^2\) opined that most common reason for failure of root canal is bacterial infection, but not operational mistakes such as incomplete root canal filling, ejection of the root canal filling or ledge formation. Nevertheless, bacteria-free root canal is essential, for a successful root canal treatment and optimal healing of the periapical tissues\(^1-3\).

In most of the cases, it is not possible to know whether the canal is sterilized immediately after the treatment is initiated, and clinicians rely on signs such as loss of spontaneous pain, percussion tenderness, and apical area tenderness, along with other factors such as absence of bleeding, drainage, and reduction or absence of fistula. Bacterial culture can help evaluate the bacterial presence in the root canal. However, it takes about 48-72 hours to obtain the results, and it is difficult to cultivate some bacteria species\(^4\). Polymerase chain reaction (PCR) evaluation or meta genome analysis using high-precision sequencer can be performed to confirm the presence of uncultivable bacteria. However, these tests involve high costs and often require a longer time to obtain the results\(^5,6\).
When exposed to blue light at a wavelength of approximately 405 nanometers activates calculus\textsuperscript{7-9)} and plaque biofilm\textsuperscript{10-12)} and excites the fluorescence of the red region. Presence of Streptococcus mutans in a caries affected dentin and association with the fluorescence of the red region is also noted\textsuperscript{13)}. Other microorganisms such as Prevotella intermedia and Prevotella nigrescens emit the fluorescence of 622 nanometers and 635 nanometers; which is attributed to the presence of protoporphyrin\textsuperscript{14,15)}. The main objective of the current study was to evaluate whether light-induced fluorescence (using the blue light at a wavelength of approximately 405 nanometers) could help determine bacterial presence within the root canal, and to compare the outcomes with bacterial culture and assess its effectiveness in routine clinical use.

Materials and Methods

Subject

We evaluated 11 patients scheduled for a total of 31 root canal procedures at the Showa University dentistry hospital (Tokyo, Japan). Informed consent was obtained from all patients before the study was initiated. Ethical committee approval was obtained from the Showa University dentistry hospital clinical trials screening committee (No. 2016-019).
Sample preparation

In each case, we assessed the clinical manifestations of bacterial infection by presence or absence of percussion tenderness and apical area tenderness, and the syringeal presence. Access cavity filling was removed to check for presence (or absence) of drainage in the root canal, and putrid odor.

We filled the root canal with 17% EDTA solution (EDTA solution 17% PENTRON JAPAN INC. Tokyo, Japan) and removed calcium hydroxide preparation (Calcipex Plane II® Nippon Shika Yakuhin Co. Ltd., Yamaguchi, Japan) present within the canal, using a supersonic wave oscillation device (ENAC®OSADA INC. Tokyo, Japan). One drop of sterile water was dropped into the root canal and one sterilized paper point (JM Paperpoint®, Morita Co. Ltd., Osaka, Japan) each was placed in two root canals as aseptically possible and left it to stand for around 30 seconds. The paper points were then transferred to a sterilized glass test tube (Micro Test Tube, TGK Co.Ltd., Tokyo, Japan), and were subsequently used for fluorescence analysis and bacteria culture test.

Fluorescence analysis

We used a microscope multi-photometry system for fluorescence analysis.

The microscope multi-photometry system comprises a CCD camera (STC-TC152USB-A, OMRON SENTECH CO. Ltd., Kanagawa, Japan), 36 mW, a 406 nm laser light source (radiation diameter Φ 20 nm), fluorescence analyzer (MCPD 7700, Otsuka Electronics
Co. Ltd., Tokyo, Japan), and a commercial note PC for the analysis together in an optical microscope (VMU-LB, Mitutoyo Co. Ltd., Kanagawa, Japan) which we installed in the darkroom.

Fluorescence analysis involved excitation fluorescence to irradiate a sample with excitation light and the wave form of the obtained fluorescence spectrum was recorded. The reflectance in the region of 620 to 680 nm was considered in the fluorescence analyses. The emission peak at 620 nm, 650 nm and around 680 nm (red region) were considered positive, while that noted only with 620 nm was considered slightly positive. If the fluorescence was noted below 620 nm, then it was considered negative.

Bacteria simple culture test

We used plastic Deer (PLADIA®, Showa Yakuhin Kako Co.Ltd., Tokyo, Japan), a simple bacterial culture study agent that has conventionally been used for bacteria simple culture test. The paper point was inserted as a document in plastic Deer culture medium and cultured it with the Furan container, which was set to 37 degrees. The results were evaluated 48 hours later.

The presence of any growth at 48 hours was considered positive while the absence was considered negative.

Statistical analysis
The difference between results obtained from the two methodologies were assessed using Fisher's exact probability test and analysis of variance (ANOVA).

Results

1) By the results of the fluorescence analysis, there was the thing that a peak was not observed in 620nm, 650nm of the red region, the thing that a peak was observed in all of 680nm, the thing that a peak was observed only to 620nm, the red region except 500nm of the green region. The fluorescence analysis was considered positive in 8 cases, while it was slightly positive in 4 cases. The peak in the red region was not noted in 19 cases and were considered negative.

2) In the bacteria simple culture test, 15 cases were positive, and the rest 16 cases were negative.

3) In the clinical examination, 9 cases were noted to have a putrid odor, while drainage in root canal was noted in 2 cases. Fistula was noted in 4 cases while apical area tenderness was noted in 5 cases and 6 cases were tender on percussion. Also, Root canal filling possibility and the thing which we judged were 21 cases generally than a clinical examination.

4) Ten root canals were judged to be impossible to fill during clinical examination and all these were positive in bacteria culture test as well as in the fluorescence analysis study. A total of 21 root canals were judged to be available for root canal filling in the
clinical examination. However, 5 those had positive results with the bacteria culture test as well as fluorescence analysis while three had positive outcomes only with fluorescence analysis.

5) The positive-predictive value of the results of the fluorescence analysis and bacteria simple culture test were high with values of 90.5% and 76.2%, respectively. After performing Fischer’s exact probability test revealed a significant difference between the fluorescence analysis and bacteria culture test ($\chi^2 = 20.8842$, decayed and filled = 1, $p<0.01$).

Discussion

Assessing for the presence of bacteria in the root canal is one of the important steps to ensure successful root canal treatment\textsuperscript{1-3}. Although it was considered that number of visits in which root canal is completed impacts the outcome, reports have suggested that the prognosis is not influenced by this\textsuperscript{16-20}. In addition, the outcomes remained same when multiple visit and single visit root canal treatments were compared \textsuperscript{21}. It is believed that risk of infection increases with the number of times the root canal is worked on. The prognosis may be better if the presence of bacteria in the root canal is judged immediately and the root canal treatment is completed during a single visit. Time is necessary for the method using the established cultural method and PCR by all means, and the immediate judgment becomes difficult now. Therefore, we applied technique of
the fluorescence analysis this time and weighed it against bacteria simple culture test.

In this study, outcomes of the fluorescence analysis was almost at par with bacteria simple culture test suggesting the likelihood that it can replace bacteria testing. Additionally, bacteria simple culture test is always associated with a likelihood of contamination by technical error. This was evident in 2 cases, that showed negative results in fluorescence analysis.

In the current study, the difference between fluorescence analysis and sensitivity of the culture test for the bacterial count has not been assessed. However, comparing the intensity of fluorescence and the bacterial count seem to be necessary to evaluate the detection limit based on the difference in bacterial count. The positive predictive value of fluorescence analysis was superior to bacteria simple culture when the outcomes were compared with clinical manifestations.

Only a few of the oral bacteria can be cultured while the number of bacteria that are cultivable by bacteria simple culture is much lesser. The PCR testing and meta genome analysis on the other hand, are considered to quite expensive tests both in terms of purchasing the equipment and maintaining it. Fluorescence analysis does not incur any running costs and can hence be considered a better modality. However, the cost of the instrument used for fluorescence analysis is quite high. Nevertheless, customization of the instrument to include only the required wavelength is thought to be possible.

Further analysis to evaluate the outcomes of these cases and also to include more number
of patients is necessary. Additionally, the association between quantity of bacteria and strength of the excitation fluorescence needs to be assessed.

Conclusion

Light induced fluorescence analysis can replace bacteria simple culture test in the evaluation of root canal bacteria.

Conflicts of Interest

The authors have no conflicts of interest directly relevant to the content of this article.
Reference


9. Bittar DG, Pontes LR, Calvo AF, Novaes TF, Braga MM, Freitas PM,


蛍光発色を応用した根管内細菌診断手法の検討

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抄録

目的：根管治療を成功させるためには、細菌を残したまま根管充填を施すのではなく、根管内および根尖孔周囲の無菌化が必須であり、それは細菌培養法を用いて根管の無菌性を確認するが、この方法は24~48時間の培養期間を必要とし、さらにテクニカルエラーによる細菌混入の可能性もある。本研究では、根管内の細菌の有無を、波長約405nmの青色光を利用して根管内の無菌性を評価する方法を検討した。

材料と方法：根管治療中の31根管を対象とした。各症例において臨床症状を確認した後、仮封と前回治療時の貼薬剤を除去し、根管内に一滴程度のわずかな滅菌水を滴下した。その後、滅菌されたペーパーポイントを極力無菌的に2本根管内に挿入し、30秒程度静置した。これを資料として、それぞれ蛍光試験と細菌簡易培養試験に用いた。蛍光試験には、暗室に設置した光学顕微鏡に、CCDカメラ、36mW出力の406nmレーザー光源、分光分析器、解析用の市販ノートパソコンを組み合わせた顕微鏡マルチ測光システムを使用し、得られた蛍光スペクトルの波形を観察・記録した。細菌簡易培養検査は、37度に設定したフラントにて培養、48時間後に判定を行った。

結果：臨床所見において、根管充填を行うことが不可能と判断された根管は10根管あり、その全ての根管で、蛍光試験および細菌簡易培養検査の両方において陽性であった。残りの21根管のうち16根管でどちらの検査も陰性となった。他の5根管は細菌簡易培養検査の結果が陽性であったが、5根管のうち2根管
は分光分析の結果も陽性であり、細菌の存在が示唆されたが、3根管についてはテクニカルエラーの可能性が示唆された。

結論：励起光を用いた励起蛍光による蛍光分析を用いた検査が、細菌簡易培養検査の代わりになる可能性が示唆された。

キーワード：光励起蛍光、歯内療法、診断
付図説明

Fig1. A sterilized paper point to be sampled was inserted into the root canal and allowed to stand for 30 seconds. This was done carefully to avoid bacterial contamination.

Fig2. Microscope multi-photometry system. The microscope multi-photometry system comprises a CCD camera, 36mW, a 406nm laser light source (radiation diameter Φ 20nm) for the output, a fluorescence analyzer, a commercial note PC for the analysis along with an optical microscope.

Fig3. Output image by CCD camera of trial microscope multi-photometry system during fluorescence test.

Fig4: Correlation diagram of the results of the bacteria simple culture test and fluorescence analysis of 31 root canals.

Fig5. Fluorescence spectrum obtained by spectroscopic analysis in fluorescence test. In A, there were peaks at 620 nm, 650 nm, and 680 nm which are red regions, and it was judged as positive. In B, there was no peak in the red region and it was negative.

Table1. Cross-tabulation table of fluorescence analysis and simple culture test and the result of chi-square test. $\chi^2 = 20.884$, and $P= 4.87899801446415E-06$. 
Table 1

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<tr>
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<tr>
<td></td>
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<tr>
<td>Pearson test</td>
<td>20.884</td>
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Fig. 2

- Laser diode
- CCD Camera
- Microscope unit
- Sample
- PC
- Spectrometers (MCPD7700)
Clinical examination

Bacteria simple culture test

Fluorescence analysis

31 root canal

(+10)

(-21)

(+10)  (-0)

(+5)  (-16)

(+10)  (-0)  (+2)  (-3)  (+0)  (-16)