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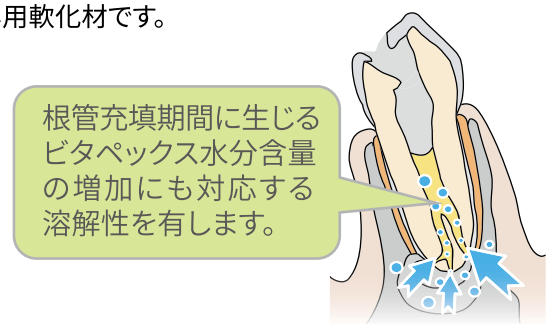

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メタルフリー材料・接着・IOSの融合で実現する次世代臨床コンセプト「オクルーザルベニア」の理論的背景と臨床ステップを提示

Dental Outlook
歯界展望 別冊

オクルーザルベニア レストレーション

進化したメタルフリー材料・接着・IOSの融合で実現する次世代臨床コンセプト

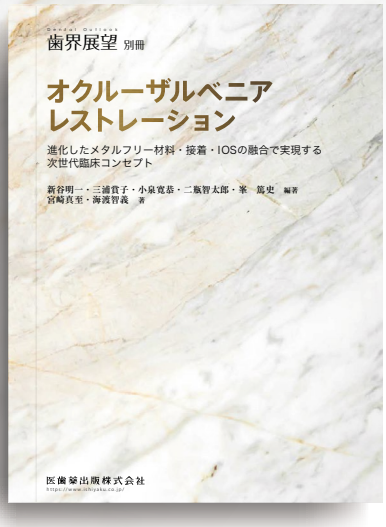
新谷明一・三浦賞子・小泉寛恭・二瓶智太郎・峯　篤史　編著
宮崎真至・海渡智義　著

オクルーザルベニアとは？

歯肉縁上へのフィニッシュライン設定による最小限の侵襲の後に，歯冠修復材料にレジン系材料やジルコニア，二ケイ酸リチウムなどのセラミックスを用いて接着により支台歯に固定．次世代の技術を活用した，新しい歯冠修復法のコンセプトです．

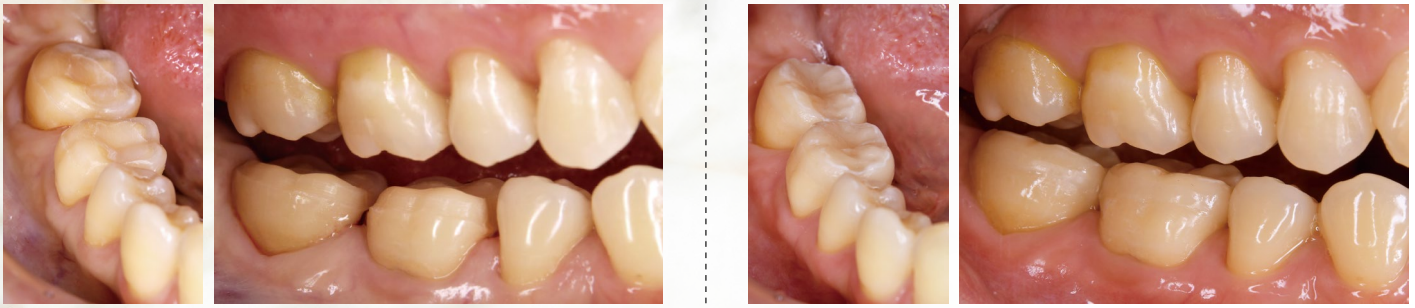
本書には，オクルーザルベニアの何が書いてある？

オクルーザルベニアのエビデンスから実際の臨床でのポイントまで網羅しました．そのなかでも，オクルーザルベニアを安全に使用するための最も重要な要素として，「支台歯形成」と「接着術式」については特に詳しく説明しています．



オクルーザルベニアの実際（76の歯冠修復）

左：修復形態（被覆範囲）の決定後，支台歯形成．フィニッシュラインを歯肉縁上に設定．
右：シリカ系セラミックスを用いてCAD/CAMにより歯冠修復物を製作．接着により支台歯に固定．



（写真提供：海渡智義先生，本書 Chapter 10 より）

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Original Articles

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Original Article

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Effect of the Thickness Ratio of Core Build-up Composite Resin to Root Dentin on the Strength of Abutment Construction

Yoh KOBAYASHI, Miki SEKIYA, Keisuke SAIGUSA,
Munehiro MAEDA and Masaru IGARASHI

Department of Endodontics, The Nippon Dental University School of Life Dentistry at Tokyo

Abstract

Purpose: After root canal treatment, the tooth is restored with an abutment, for which core build-up resins providing adhesiveness have come into use. This study examined the effect of the thickness ratio of the composite resin used for construction and the thickness of the root dentin on the strength of the construction.

Methods: This research used four core build-up resins: 1) Clearfil DC Core Automix ONE (DC), 2) BeautiCore Post Paste (BC), 3) Filtek Fill and Core Flowable Restorative Plus (FT), and 4) Estecore (EC). First, 164 experimental specimens of 2.0 mm×2.0 mm×14.0 mm, with dentin thicknesses of 0.5 (D0.5), 1.0 (D1.0), 1.5 (D1.5), and 2.0 mm (D2.0), were prepared from bovine teeth, which were subjected to a three-point bending test in a universal testing machine, and the fracture load was recorded. Second, 40 extracted human single-root teeth were used to perform compressive strength tests of dentin-resin composites. The root side of the tooth was cut to a length of 15 mm from the root apex, and root dentin with the post space of 1/3 width (1/3 P), which is a standard post formation, was used as the control group. Root dentin widths of 2/3 (2/3 P), 3/4 (3/4 P), and 4/5 (4/5 P), and human extracted teeth without core build-up (S) were used as the experimental groups. A universal testing machine was used to apply a static load in the tooth axial direction at a crosshead speed of 1.0 mm/min, and the fracture load was recorded.

Results: The bending strength of FT was significantly lower than those of BC and EC in the D0.5 group ($p<0.05$). The bending strength of FT was significantly lower than that of EC in the D1.0 and D1.5 groups ($p<0.05$).

The bending strengths of the four core build-up resin groups were significantly greater than that of the S group, which only underwent root canal shaping.

The highest force required for fracture among the four groups was 41.1±10.6 MPa in the 3/4 P group. This was followed by 39.1±8.8 MPa in the 1/3 P group, 37.5±9.5 MPa in the 4/5 P group, 33.4±4.1 MPa in the 2/3 P group, and 15.3±7.3 MPa in the S group.

Conclusion: It is suggested that construction using a core build-up resin offers similar compressive strength as conventional abutment construction based on 1/3 width, even if the remaining tooth root is thinner.

Key words: core build-up resins, bending strength, compressive strength

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Introduction

After root canal treatment, if the obturated tooth is left untreated for a long period of time, the pulp chamber becomes contaminated with bacteria and the interfacial gap between the filling material and the root canal wall causes bacterial coronal leakage[1-4], resulting in the development of periapical periodontitis. Endodontically treated teeth are constructed with the abutment construction to fill the defect and restore the functional form because the dentin of the root canal wall is removed and thinned during root canal preparation. At that time, it is important to maintain tooth strength, and metal materials have generally been used. In general, the core build-up requires a ferrule of over 1 mm and remaining tooth substance of ≥1 mm from the cervical region to the crown side as conditions[5,6]. Further, the thickness of the post is based on the standard of not exceeding 1/3 of the maximum width of the tooth root[7], and the residual amount of healthy tooth substance is important. However, metallic materials have the problem that their elastic modulus differs significantly from that of dentin, causing stress on the luting cement[8]. In addition, a removable wax pattern is required during the process of manufacturing the metal core, so a large amount of dentin needs to be removed to avoid leaving an undercut.

In recent years, core build-up resins that have adhesiveness to dentin and have similar physical characteristics to those of dentin have been developed. These are commercially available in a chemical-cured type, and dual-cured (light- and chemical-cured) type. The core built up using such adhesive materials provides excellent marginal sealing ability and can prevent coronal leakage. In particular, the direct resin core build-up can be applied with or without undercut in the tooth cavity. In cases where the metal post crown falls off, secondary caries often progresses in the dentin around the dowel post. When the softened dentin of the root canal wall is completely removed, many cases only show one layer of thin dentin remaining near the root surface. In clinical practice, most cases of endodontic re-treatment require removal of prosthetic devices, posts, and caries, resulting in less residual dentin. Under such circumstances, many cases do not match the basic morphology of conventional cavities. So far, no reports have addressed the effects on the strength of abutment construction when the ratio of the thickness of the composite resin to remnant root dentin is changed.

Therefore, this study examined the effects of the thickness of the composite resin used for construction and the thickness of the root dentin on the strength of the construction.

Materials and Methods

1. Experiment 1: Bending strength test based on differences between dentin and resin thickness

1) Experimental core build-up resin materials

This study used four core build-up resins for abutment construction: (1) Clearfil DC Core Automix ONE (DC) (Kuraray Noritake Dental, Tokyo, Japan), (2) BeautiCore Post Paste (BC) (Shofu, Kyoto, Japan), (3) Filtek Fill and Core Flowable Restorative Plus (FT) (3M, Saint Paul, MN, USA), and (4) Estecore (EC) (Tokuyama, Tokyo, Japan). The tooth surface treatment agent specified by each manufacturer was used for adhesion treatment of the tooth substance (Table 1).

2) Preparation of an adhesive model

One hundred extracted bovine single-root teeth were sliced into 2.0 mm thicknesses using a low-speed precision cutting machine (ISOMET 1000; Buehler, Lake Bluff, IL, USA), and 200 test pieces of 2.0 mm width×14.0 mm length were cut out. From these 200 samples, 164 samples were selected for the experiment.

The thickness of the test pieces was adjusted by polishing the surfaces with open dentin tubules with #600 water-resistant abrasive paper until the thickness was 0.5 mm, 1.0 mm, and 1.5 mm, respectively.

3) Preparation of resin-bonded sections

A slot of 2.0 mm width×2.0 mm depth×25.0 mm length cavity was made by two L-shaped metal plates (Fig. 1), and a dentin piece that had been treated for bonding according to the procedure specified by each manufacturer was inserted into the bottom of the slot. The four core build-up resins shown in Table 1 were embedded in the adhesive surface of the dentin piece, and while manually pressing on the glass, irradiation was performed for 10 s with a light irradiator (G-Light Prima II Plus; GC, Tokyo, Japan). The cured experimental piece was taken out from the metal frame, and

Table 1 Compositions of each core build-up resin used in this study

Materials (Maker)	Composition	Adhision
Clearfil DC Core Automix ONE (Kuraray Noritake Dental)	Paste A : Bis-GMA, hydrophobic aliphatic dimethacrylate, hydrophilic aliphatic dimethacrylate, hydrophobic aromatic dimethacrylate, filler, dl-CQ, initiators, pigments Paste B : TEGDMA, hydrophilic aliphatic dimethacrylate, hydrophobic aromatic dimethacrylate, filler, accelerators	Clearfil Universal Bond Quick ER
BeautiCore Post Paste (Shofu)	Glass powder, Bis-GMA, TEGDMA, UDMA	BeautiBond Xtreme
Filtek Fill and Core Flowable Restorative Plus (3M)	Glass powder, Bis-GMA, TEGDMA, UDMA	Scotchbond Universal Adhesive
Estecore (Tokuyama)	Paste A : Silica zirconia filler, Bis-GMA, TEGDMA, Bis-MPEPP Paste B : Silica zirconia filler, Bis-GMA, TEGDMA, Bis-MPEPP, peroxides, camphorquinone, radical amplifiers	Bondmer Lightless

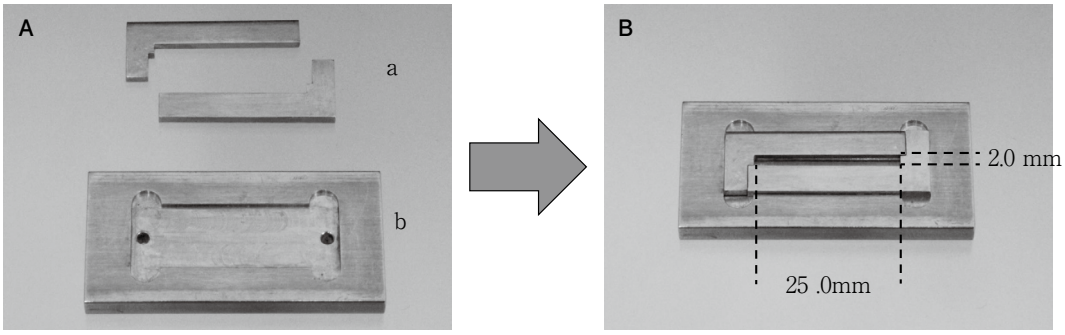

Fig. 1 The template used to prepare test pieces
A : Structure of the template. a is L-shaped plate, b is base plate. B : The combined state.

the excess resin was polished and removed with water-resistant silicon carbide paper #600 to prepare an experimental piece measuring 2.0 mm×2.0 mm×14.0 mm. A specimen made of resin alone was used as the control (2.0). Three types of samples were used as experimental groups: D0.5 group (0.5 mm dentin thickness), D1.0 group (1.0 mm dentin thickness), and D1.5 group (1.5 mm dentin thickness). Fourteen specimens of each group were used in the experiment. R2.0 was consisted of whole resin. The specimens with a dentin thickness of 2.0 mm were used as the D2.0 group for reference (Fig. 2).

4) Three-point bending test

The produced sample piece was subjected to a three-point bending test at a crosshead speed of 1.0 mm/min on a universal testing machine (Autograph AGS-X; Shimadzu, Tokyo, Japan), and the fracture load was recorded (Fig. 3).

2. Experiment 2: Compressive strength test of dentin resin complex using extracted human teeth

After obtaining approval from the Nippon Dental University Ethics Committee (approval number NDU-T2019-44), 40 extracted human single-root teeth collected from healthy subjects aged 15 to 40 years old were used in the experiment.

Mandibular premolars were used as samples because they are easily extracted for orthodontic convenience and have less caries. After access opening of the pulp chamber, the root canals were shaped using the standardized preparation technique with a size #50 K file (MANI Co., Tochigi, Japan). Size #50 master points (GC, Tokyo, Japan) and accessory points (GC), and a root canal sealer (Canals; Showa Yakuhin Kako Co., Tokyo, Japan) were used for the lateral condensation method. The crown side was then cut so that the root length was 15 mm from the apex. A diamond bar (Matsukaze Diamond Point FG-C105RX; Matsukaze, Tokyo,

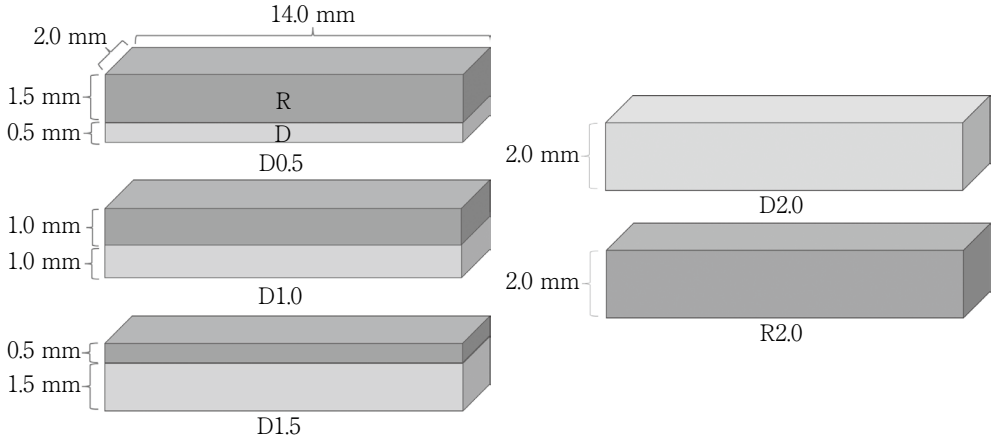

Fig. 2 Schematic diagram of each plate specimen
R : Resin, D : Dentin, D0.5 : 0.5 mm dentin thickness, D1.0 : 1.0 mm dentin thickness, D1.5 : 1.5 mm dentin thickness, D2.0 : 2.0 mm dentin thickness, R2.0 : 2.0 mm resin thickness

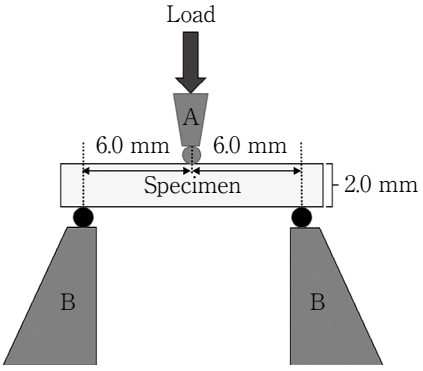

Fig. 3 Schematic diagram of the three-point bending test (head speed : 1.0 mm/min)
A : indenter, B : load bearing platform

Japan) was equipped in the micromotor of a custom-made preparing device (Nihon Mecc, Tokyo, Japan) and used for post preparation of 1/3 width of the root (1/3 P), from the apex to 5.0 mm according to the standard post formation (control group). The experimental groups (n=10) were the four groups of the 2/3 formation group (2/3 P), the 3/4 formation group (3/4 P), and the 4/5 formation group (4/5 P), and the group in which only canal shaping was performed (S) (Fig. 4). The inside of the tooth cavity was treated with an etching agent (K Etchants GEL, Kuraray Noritake Dental, Tokyo, Japan) for 40 s, washed with water and dried, then the tooth surface was treated with Clearfil Universal Bond Quick ER (Kuraray Noritake Dental) as instructed by the manufacturer. In the three-point bending test, the bending strength of EC was increased depending on its thickness. This property was excluded because it was considered to affect the results of this experiment. FT was weaker in intensity than the other groups. In this study, there was no significant difference in the three-point bending strength between DC and BC. However, DC has been reported in previous studies to have superior bond strength[9]. Therefore, DC was employed in the experiments for compressive strength.

Then, DC was filled into the tooth cavity and cured with a light irradiator. Each sample was stored in an incubator at 37℃ for 24 h. After storage, a universal tester was used to apply static load along the tooth axis direction at a crosshead speed of 1.0 mm/min, and the measured fracture load was recorded (Fig. 5).

3. Statistical analyses

Statistical analysis software (SPSS version 25; IBM, North Castle, NY, USA) was used for statistical analysis. Tukey's test was used for the three-point bending test in bovine teeth, and one-way analysis of variance and Tukey's multiple comparison test were used for the compressive strength test in extracted human teeth. The differences in the failure loads between the conditions were tested for significance at the 5% level.

Results

1. Three-point bending test

The results of three-point bending tests of the five groups in which the thicknesses of bovine dentin were

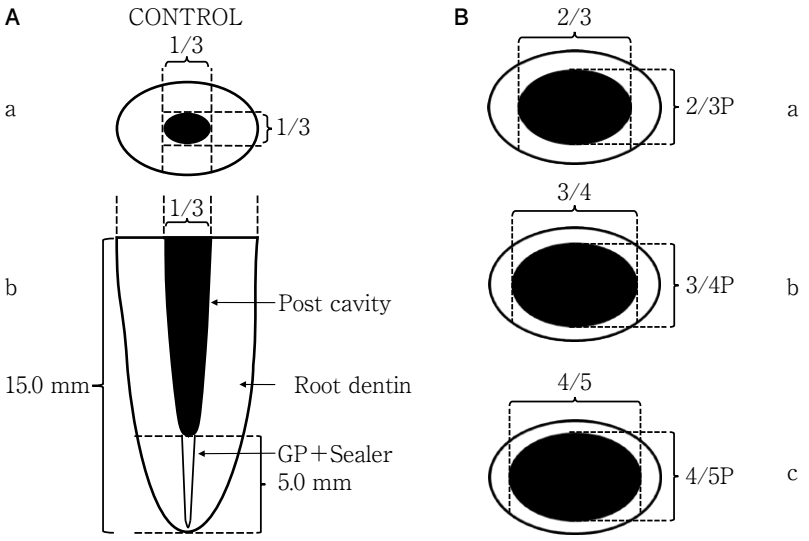

Fig. 4 Schematic diagram of the dentin and resin complex using extracted human teeth

A：Control. a：vertical view, b：horizontal view：the sample removed to 1/3 of root width

B：Horizontal views of various samples. a：the sample removed to 2/3 of root width, b：the sample removed to 3/4 of root width, c：the sample removed to 4/5 of root width

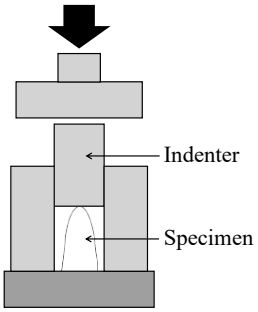

Fig. 5 Schematic diagram of static load test (head speed：1.0 mm/min)

The specimen is placed in a cylindrical jig with the root apex upward, and the indenter is used to apply pressure at a head speed of 1.0 mm/min until the specimen is destroyed.

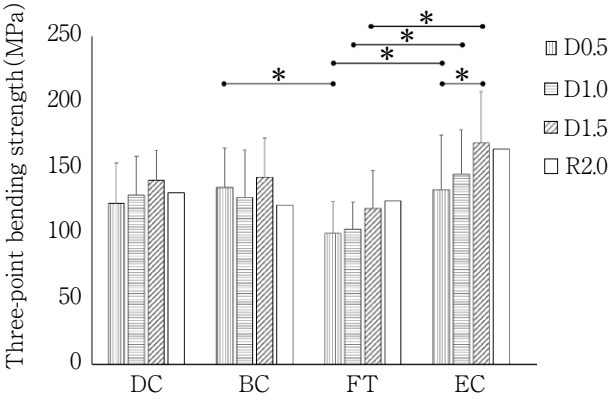

Fig. 6 Measured values of three-point bending strength for each group (＊：$p<0.05$, $n=14$)

changed using the four core build-up resins are shown in Fig. 6. Bending strength for the D0.5 group was highest in BC at 134.7±30.1 MPa, followed by 133.0±41.5 MPa in EC, 122.5±30.9 MPa in DC, and 100.0±23.9 MPa in FT. Bending strength of FT was significantly lower than that of BC or EC ($p<0.05$). Bending strength for the D1.0 group was highest in EC at 144.8 ±34.0 MPa, followed by 128.8±29.7 MPa in DC, 127.4± 36.2 MPa in BC, and 103.2±20.5 MPa in FT. Bending strength was significantly lower in FT than in EC ($p<0.05$). Bending strength for the D1.5 group was highest in EC at 168.8±38.8 MPa, followed by BC at 142.5±30.1 MPa, DC at 140.3±22.9 MPa, and FT at 118.6±29.0 MPa. Bending strength in FT was again significantly lower than that in EC ($p<0.05$). Bending strength for the R2.0 group was highest in EC at 164.16±34.2 MPa, followed by 130.73±13.7 MPa in DC, 124.68±10.6 MPa in FT, and 121.54±17.8 MPa in BC. Bending strength for the D2.0 group was 220.9±56.3 MPa.

2. Compressive strength test

The results of the load measured by applying a static load using four groups of core build-up teeth by chang-

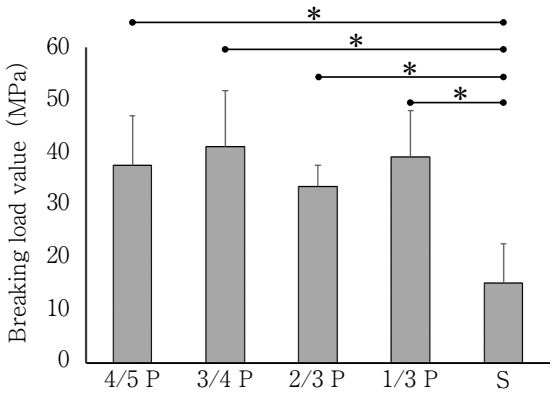

Fig. 7 Measured values of fracture load for each group (＊：$p<0.05$, $n=10$)

1/3P：post preparation of 1/3 width of the root (control group), 2/3P：2/3 formation group, 3/4P：3/4 formation group, 4/5P：4/5 formation group

S：group in which only canal shaping was performed

ing the thickness of the core build-up resins are shown in Fig. 7.

The highest force required for fracture among the four groups was 41.1±10.6 MPa in the 3/4 P group. This was followed by 39.1±8.8 MPa in the 1/3 P group, 37.5±9.5 MPa in the 4/5 P group, 33.4±4.1 MPa in the 2/3 P group, and 15.3±7.3 MPa in the S group.

However, there was a significant difference between the group of teeth in which only formation was done and the group in which each formation was done.

Discussion

For endodontically treated tooth, core build-up is required to manufacture the prosthetic device. The effect of the thickness of the core build-up resins on tooth strength needs to be determined. Therefore, a comparative study was conducted on the effect of the ratio of the thickness of the core build-up resins for construction and the thickness of the residual root dentin on the strength of the construction body.

In the experiment, extracted bovine teeth were used to allow easy creation of standard plate-shaped samples, and the physical properties of the adhesive system that is currently widely used in clinical practice and core build-up resins were compared. In this study, an adhesive sample was prepared from pieces of bovine dentin and resin, but in recent years fewer cases of human tooth extraction have been performed due to advances in dental care, making collection of samples more difficult. Bovine teeth are relatively easily available, and previous studies have shown the effectiveness of using bovine teeth as a substitute for human teeth[10-13].

A minimum of 12 mm is required for a plate-shaped sample for a three-point bending test, but cutting out 12 mm from a human dentin tooth root in an anatomical form is difficult. It was easier to prepare test pieces for the three-point bending test using bovine teeth, as they are readily prepared, can be adjusted to a length of 12 mm or more, and show similar adhesive strength to human teeth. In a comparison among the four materials, the bending strength of DC, BC, and FT increased as the thickness increased to D1.0 and D1.5 compared to D0.5, but no significant difference was observed. On the other hand, in EC, strength differed with changes in dentin thickness between the D0.5 and the D1.5 groups, indicating that strength depended on the dentin. In the present study, the strength of other experimental groups also showed a tendency to increase with increasing dentin thickness.

In addition, it was reported that EC has stronger adhesive strength than other core resins[8]. In EC, it is considered that the degree of increase in strength rose further with the increase in dentin thickness. The three-point bending strength of DC, BC, and FT tended to increase as the thickness of the dentin increased from 0.5 to 1.0 and 1.5, but there was no significant difference. The strength of FT was lower than that of the other groups. It is speculated that FT has a wide range of applications such as filling of cavities in addition to abutment construction. The D2.0 group showed higher values than the other groups. It is speculated that the structure of dentinal tubules in dentin is different from the three-dimensional structure of resin, which contributed to the dispersion of force.

With the three-point bending test, the average strength of the dentin plate-shaped test pieces with different thicknesses was 100–168 MPa, similar to the value of 106–173 MPa shown for the core build-up resins in a previous study[14]. There was a difference in bending strength between the D0.5 and D1.5 groups of EC. Since the R2.0 group of EC had the highest strength among the four groups, it is considered that even if the thickness of EC became thin, it contributed

to the bending strength of the resin.

Our results showed that the adhesion between the resin and dentin plate had a similar strength as the core build-up resins alone, regardless of the thickness. This indicates that the strength is guaranteed if an appropriate resin thickness can be achieved, even in tooth roots with a thinned tooth structure. Therefore, even a tooth in which the healthy tooth structure is thinned by caries around the metal post has sufficient strength and resin can be applied. Also, in previous research, samples of the core build-up resins alone were used for the three-point bending test, but the present study succeeded in producing a composite sample of bovine teeth and resin, and the numerical value of the bending strength was also similar to those of previous studies[15,16]. Since those results were close to the present results, reproducing the three-point bending test in more clinical situations seems feasible.

In the compressive strength testing of the dentin resin complex using extracted human teeth, no difference in strength was found regardless of the amount of dentin removed. The results showed that the mechanical properties of the composite resin used for abutment construction in recent years are very close to those of dentin, and the adhesion between dentin and resin results in an integrated composite[17,18]. In addition, the strength against vertical fracture is greatly increased when the interior is replaced with resin rather than leaving the tooth without core build-up resins. In addition, the formation comprising 1/3 of the root width, as the principle of post formation, does not necessarily represent a limitation to the use of core build-up resins, and it is not a problem even if the thickness of root dentin is thinner due to caries or excessive root canal enlargement. We clarified that the abutment can be constructed without diminishing the strength of the thinned tooth root. Even in the apexification of a tooth with incomplete root formation, where treatment is completed with a thin residual tooth substance, tooth fracture has become a problem during long-term follow-up. In such cases, even if the tooth substance is thin, it may be possible to maintain strength by appropriately treating the tooth surface and filling the cavity with core build-up resins.

Conclusions

The following conclusions were reached:

1. The results of the three-point bending test showed that there was no difference in the bending strength of each resin except EC when the thickness of dentin was changed.
2. There was no difference in the compressive strength of extracted human roots loaded with resin at different thicknesses of dentin.
3. EC had significantly greater three-point bending strength than FT in all dentin thicknesses.

These findings suggest that construction using core build-up resins offers the same strength as conventional abutment construction based on 1/3 of the tooth structure removed, even if the tooth root is thin.

Conflict of interest statement

There are no conflicts of interest to disclose regarding this paper.

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Original Article

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Effects of Sodium Copper Chlorophyllin on Human Gingival Fibroblasts *in vitro*

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Abstract

Purpose: Sodium copper chlorophyllin (SCCP) is known to have various effects such as antiallergic and tissue-activating effects, and in recent years is expected to be effectively used in the medical field. Moreover, in the field of dentistry, it is used for treating periodontal disease and trauma. Although there are many reports on the effects of SCCP, few reports have examined how it works. In this study, to elucidate the effects of SCCP on periodontal tissue, we studied physiological changes *in vitro* in human gingival fibroblasts (HGF) exposed to SCCP.

Methods: Human gingival fibroblasts were cultured in 15% FBS-containing DMEM. The cells were exposed to SCCP (5 nM, 10 nM, 100 nM, 1 μM, 10 μM), and cell proliferation and mitogens were examined.

Results: Cell proliferation increased significantly with 100 nM SCCP, and an increase in mitotic factors that promoted cell proliferation was observed.

Conclusion: The results suggested that 100 nM SCCP stimulates cell division factors of human gingival fibroblasts and promotes cell proliferation, thereby promoting wound healing.

Key words: human gingival fibroblast, sodium copper chlorophyllin, wound healing, periodontal tissue

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Introduction

Sodium copper chlorophyllin (SCCP) is an alkaline hydrolysis product of copper chlorophyll, which contains Cu in place of Mg in the center of the chlorophyll structure (Fig. 1 (A)). It is an assembly of components with similar chlorophyll skeletons, rather than a single component[1]. SCCP has various effects including antiallergic, tissue-activating, and deodorizing effects[2,3]. With expectation of these effects, it has been applied in the field of medicine. For peptic ulcer, orally administered SCCP exerts excellent healing promoting effect by reducing pepsin activity in the gastrointestinal tract and inducing the proliferation of fibroblasts in connective tissue[4]. For dermatitis and urticaria, topically applied SCCP exerts antiallergic effect and promotes healing[5]. In the field of dentistry, SCCP is used for endodontic treatment due to its antibacterial effects such as root canal application and treatment of mucosal damage in the mouth to accelerate wound healing by promoting granulation of the wound. Moreover, gargling with chlorophyll compounds is useful for improving inflammation of periodontal tissue and halitosis in patients with gingivitis and periodontal disease through the antibacterial effect of chlorophyll[2,6–10]. Periodontal disease is an infectious disease caused by bacteria in the oral cavity. The oral bacteria cause inflammation of the periodontal tissue, which is thought to trigger the destruction of the periodontal ligament and resorption of alveolar bone[11], and it has been reported that oral bacteria are implicated in systemic diseases[12–14]. The prevalence of periodontal disease has been steadily increasing in recent years. A key to the treatment of periodontal disease is to reduce dental plaque and periodontopathic bacteria that can cause inflammation. However, as the aging of the population progresses, sufficient treatment for periodontal disease cannot be performed in some cases due to association with systemic diseases. Therefore, the importance of oral care and prevention of periodontal disease is widely recognized. Furthermore, drugs that reduce the adhesion of plaque and periodontopathic bacteria and accelerate healing of periodontal tissue are being studied.

However, it remains largely unknown how SCCP acts to ameliorate inflammation and promote wound healing. In this study, to elucidate the effects of SCCP on periodontal tissue, we studied physiological changes *in vitro* in human gingival fibroblasts (HGF) exposed to SCCP.

Materials and Methods

1. Sodium copper chlorophyllin

The SCCP (Lot 60221) used in the experiment was supplied by TAMA BIOCHEMICAL Co.(Tokyo, Japan). In order to identify the primary small molecule constituents contained in the supplied SCCP, mass analysis was performed using a liquid chromatograph time-of-flight mass spectrometer equipped with a photodiode array. The measurement conditions were in accordance with the method of Simaremare et al[15].

2. Human periodontal tissue

HGF were obtained using the outgrowth method from the gingival tissue on a patient's teeth removed for therapeutic reasons at the Hospital (a female with healthy periodontal tissue without systemic disease, age 54) (Ethical Review Committee of the Nippon Dental University School of Life Dentistry at Niigata: approval number ECNG-H-336).

3. Human gingival fibroblasts

Gingival tissue of the extracted teeth was washed with phosphate buffer(phosphate buffered saline; Nissui Co., Tokyo, Japan)to remove impurities. The tissue was cut into about 1 mm^3 pieces, and the tissue pieces were placed on a 35 mm culture dish. After adding a small amount of Dulbecco's Modified Eagle's Medium (DMEM): Nutrient Mixture F-12 culture medium supplemented with 15% fetal bovine serum (FBS) (JR Scientific, Inc., CA, USA), 5 μl/ml penicillin-streptomycin (Invitrogen Corp., CA, USA) and 1 μl/ml amphotericin B(Invitrogen Corp.) (15% FBS-containing DMEM), the dish was incubated in a CO_2 incubator at 37℃ in 5% CO_2 and 95% air for the primary culture. After confirming the outgrowth from the tissue pieces, primary culturing was continued with exchanging the medium every 2 days for about 2 weeks until the migrated cells reached confluence. The cells were then cultured in 15% FBS-containing DMEM and used for the experiments. In addition, primary HGF purchased from the American Type Culture Collection (ATCC) was cultured in DMEM containing 15% FBS supplemented with 100-fold diluted penicillin streptomycin (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Cell culture was performed under the same

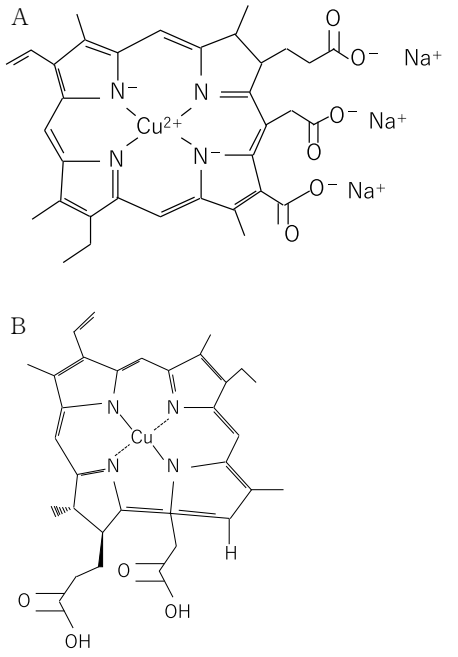

Fig. 1 Structure of sodium copper chlorophyllin (A) and Cu (II) chlorin e4 disodium salt (B)

conditions.

4. Cell culture conditions

The cells (clinically isolated cell lines) were cultured in 15% FBS-containing DMEM. Cells were exposed to SCCP (Lot 60221) added to phosphate buffer at concentrations of 5 nM, 10 nM, 100 nM, 1 μM and 10 μM. DMEM without SCCP was used as a control. Allantoin (10 nM) was used as a positive control.

5. Measurements

1) Cell proliferation

Clinically isolated cells (Passage 3) were prepared at 1.5×10^3 cells in 15% FBS-containing DMEM and seeded in a 96-well plate (Becton Dickinson Biosciences, CA, USA). Twenty-four hours after seeding, the cells were exposed to SCCP at different concentrations. After exposing for 30 s, the solution was replaced by 15% FBS-containing DMEM. The cells were exposed to SCCP every 24 h. The cells were then counted every 48 h for 8 days. Mitochondria reduction staining with Alamar Blue (Alamar Blue Cell Viability Reagent; Invitrogen Corp.) was performed to measure cell proliferation with a fluorescence plate reader (Fluoroskan Ascent FL; Thermo Fisher Scientific., MA, USA).

2) Cyclin D1 (Mitogen factor; cyclin D)

The cells purchased from ATCC were prepared at 5.0×10^3 cells in 0.5% FBS-containing DMEM and seeded in a 24-well plate (AGC TECHNO GLASS Co., Ltd., Shizuoka, Japan). Twenty-four hours after seeding, the cells were exposed to SCCP at 10 nM and 100 nM for 30 s. The medium was then replaced with 0.5% FBS-containing DMEM, and the cells were cultured for 18 h[16]. Total RNA of each cell was extracted using the RNeasy mini Kit(Qiagen, PL, NLD). cDNA was synthesized using the High-Capacity RNA-to-DNA Kit. The synthesized cDNA was then used for quantitative PCR. TaqMan Gene Expression Master Mix (Thermo Fisher Scientific Inc.) and TaqMan Gene Expression Assays (GAPDH: Hs02786624 g*l*, Cyclin D1: Hs0076553 m*l*) were used for quantitative PCR and measured by a Qubit3 Fluorometer (Thermo Fisher Scientific Inc.). From each data, relative quantitation was performed using the comparative Ct method (comparative cycle threshold; $\Delta\Delta$Ct)[17]. GAPDH was used as an internal control.

3) Statistics

The parametric test for comparing multiple unpaired groups and the ANOVA test followed by the Tukey-type test were used for the cell proliferation data and the cyclin D expression data.

Results

1. Sodium copper chlorophyllin

The HPLC chromatogram of the SCCP (Lot 60221) is shown in Fig. 2 (A). The UV-visible absorption spectrum of the largest peak (peak #10) was measured, and mass spectrum analysis was performed on peak #10. The result is shown in Fig. 2 (B) and (C).

2. Cell proliferation

On day 2 and 4 of culturing, no significant difference was observed at any concentration compared to the control (Fig. 3). On day 6 of culturing, HGF exposed to 5 nM, 10 nM, 100 nM, 1 μM SCCP and allantoin as positive control showed a significant increase in cell proliferation compared to the control (Fig. 4). On day 8 of culturing, HGF exposed to 10 nM, 100 nM, and 1 μM SCCP showed significant levels of increase in cell proliferation compared to the control. The plots of cell proliferation versus SCCP were bell-shaped with a peak at 100 nM (Fig. 5).

3. Mitogen factor: cyclin D

HGF exposed to 100 nM SCCP expressed cyclin D

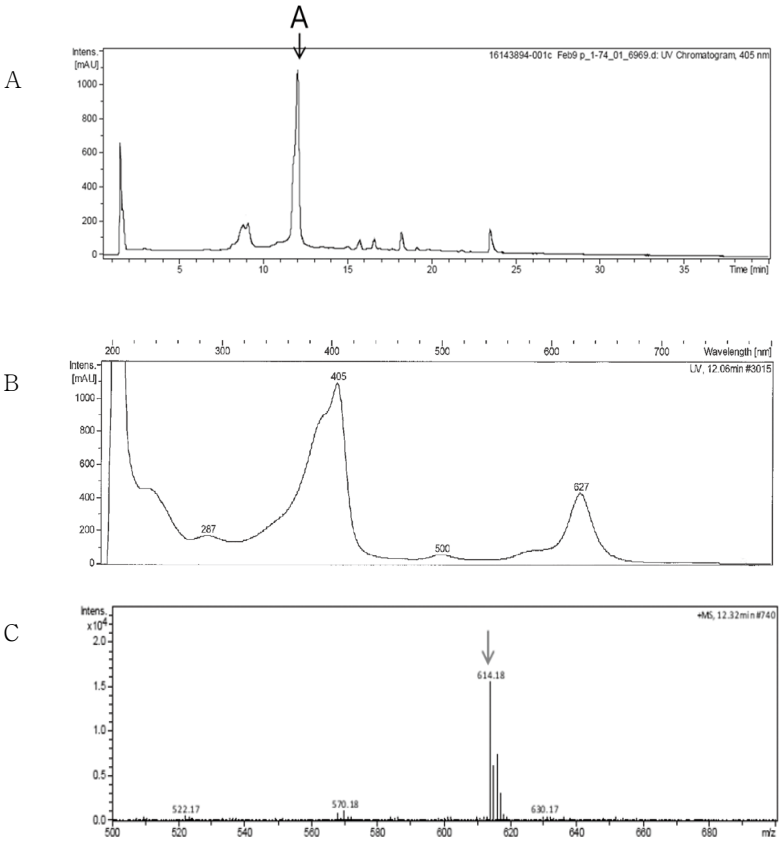

Fig. 2 Sodium copper chlorophyllin
(A) HPLC chromatogram in peak #10 of SCCP
(B) UV-visible absorption spectrum in peak #10 of SCCP
(C) Mass spectrum in peak #10 of SCCP

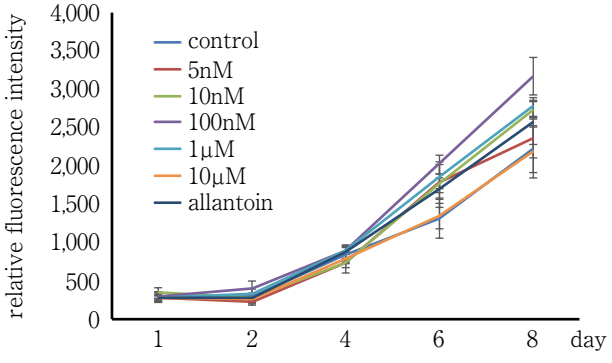

Fig. 3 HGF Proliferation

Cell suspensions containing 1.5×10^3 cells with 15% FBS-containing DMEM were implanted into a 96-well plate. Twenty-four hours after seeding, the cells were exposed to SCCP at different concentrations.

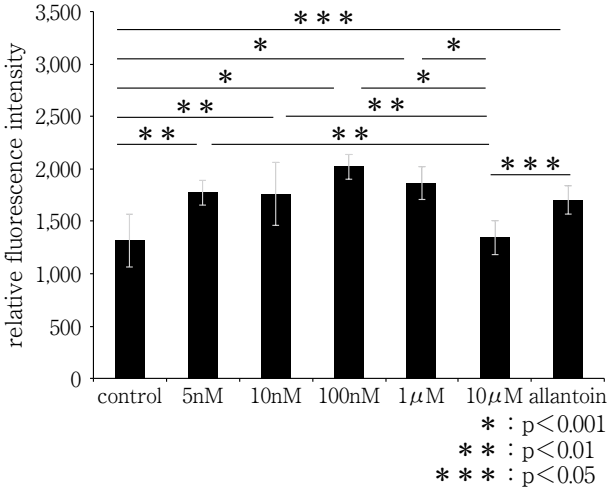

Fig. 4 HGF Proliferation day 6

Cell suspensions containing 1.5×10^3 cells with 15% FBS-containing DMEM were implanted into a 96-well plate. Twenty-four hours after seeding, the cells were exposed to SCCP at different concentrations. The cells were cultured for 6 days and measured (N=6).

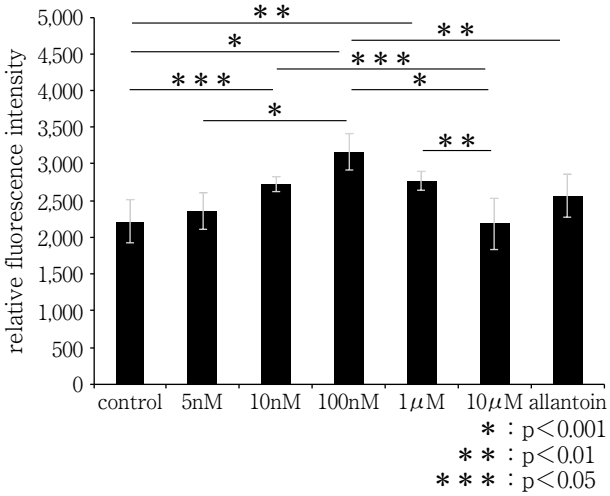

Fig. 5 HGF Proliferation day 8

Cell suspensions containing 1.5×10^3 cells with 15% FBS-containing DMEM were implanted into a 96-well plate. Twenty-four hours after seeding, the cells were exposed to SCCP at different concentrations. The cells were cultured for 8 days and measured (N=6).

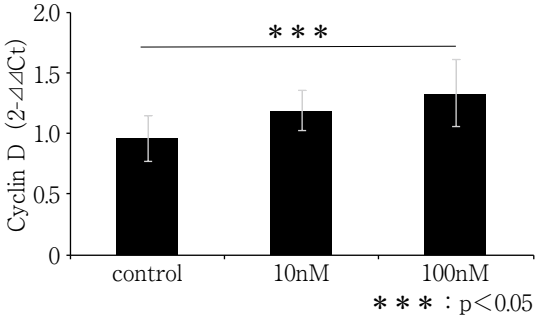

Fig. 6 Real-time PCR cyclin D1

The cells purchased from ATCC were prepared at 5.0×10^3 cells in 0.5% FBS-containing DMEM and seeded in a 24-well plate. Twenty-four hours after seeding, the cells were exposed to SCCP at 10 nM and 100 nM for 30 s. The medium was then replaced with 0.5% FBS-containing DMEM, and the cells were cultured for 18 h. Cyclin D1 was measured by real-time PCR (N=6).

mRNA at a significantly higher level compared to the control (Fig. 6).

Discussion

Periodontal disease is considered to be a chronic disease caused by periodontal pathogens. Loss of adhesion between the periodontal ligament and alveolar bone, resorption of alveolar bone, and swelling of gingiva occur in the periodontal tissue that is affected by periodontal disease and associated inflammation[11]. Because the prevalence of periodontal disease has increased, the prevention of periodontal disease and suppression of inflammation have attracted increasing attention in recent years.

SCCP has various effects, including antiallergic, tissue-activating, and deodorizing effects[2,3]. In expectation of these effects, SCCP has started to be used in the field of medicine. Particularly in the field of dentistry, SCCP has been used for periodontal disease treatment and surgical treatment in expectation of its effects on suppressing halitosis and promoting wound healing[7-10]. However, although there are many clinical reports, there are few basic research reports on the use of SCCP. In this study, we investigated the changes in the properties of HGF *in vitro* to elucidate physiological changes in periodontal tissue exposed to SCCP.

First, the ingredients of the SCCP were investigated. The UV-visible absorption spectrum of the largest peak #10 to the SCCP (Lot 60221) on the HPLC chromatogram was measured. As a result, maximum absorption was observed at 405 and 627 nm (Fig. 2 (B)). The absorption spectrum of the SCCP was very similar to that of Cu (II) chlorin e4 disodium salt and Cu (II) chlorin e6 disodium salt (Fig. 1 (B))[15]. Also, because the sodium salt dissociates in the aqueous solution, the mass spectrum of Cu (II) chlorin e4 disodium salt ($C_{33}H_{34}CuN_4Na_2O_4$) shows a mass-to-charge ratio of 614 in positive mode[15]. This is consistent with Fig. 2 (C). These results suggest that the main ingredient of the SCCP (Lot 60221) is Cu (II) chlorin e4 disodium salt.

We investigated the effect of the SCCP on cell proliferation. No significant difference was observed between days 2 and 4 of the culture as compared with the control. HGF exposed to 5 nM, 10 nM, 100 nM, and 1 μM SCCP showed significant levels of increase in cell proliferation compared to the control on day 6 of culturing. Similar results were observed on day 8 of culturing, showing a bell-shaped concentration-proliferation curve with a peak at 100 nM. This result shows the optimum concentration of SCCP for HGF in this experiment. 10 μM SCCP showed no significant difference in cell proliferation compared to untreated controls. In this study, we did not evaluate the cytotoxicity of SCCP by measuring the lactate dehydrogenase activity, mitotoxicity

assays, apoptosis assay, etc. However, 10 μM of SCCP did not show a lower value in cell proliferation compared to the untreated control. Therefore, it was considered that high concentrations of SCCP had no cytotoxic effect. This result showed the optimum concentration of SCCP for HGF in this experiment. In this study, allantoin, whose cell proliferation effect and wound healing effect by cell migration were already confirmed, was used as a positive control[18,19]. In this study, SCCP showed cell proliferation effect equal to or higher than that of allantoin. The mitogen factor, cyclin D1, is a major regulator of cell cycle and cell proliferation markers[20]. In this study of cyclin D1, 100 nM SCCP significantly promoted cell proliferation of HGF, so 100 nM SCCP and a low concentration of 10 nM SCCP were used as concentration conditions. In addition, since the primary HGF is not affected by clinical conditions, it was considered to be suitable for examining gene expression and was used. This study showed a significantly increased level of cyclin D1 expression in HGF exposed to 100 nM SCCP. Although no significant difference was observed between 10 nM SCCP as compared with the control, it is considered that continued exposure of SCCP to HGF increased the expression of cyclin D1 and promoted cell proliferation. Lee DH et al. reported that recombinant growth factor mixtures (RGFM) significantly increased cyclin D1 expression[20]. As a result, it was revealed that wound closure in mice was accelerated, re-epithelialization was promoted, and inflammatory cell infiltration was reduced[20]. Furthermore, Zhiping et al. reported increased adhesion and decreased motility of cyclin D1-deficient mouse embryonic fibroblasts compared to wild-type mouse embryonic fibroblasts[21]. These reports suggest that increased cyclin D1 expression promotes proliferation and migration of cells involved in tissue repair[20,21]. Furthermore, it has been reported that enamel matrix derivative and fibroblast growth factor, which have an action of promoting periodontal tissue repair, induce human gingival fibroblasts into the S phase of the cell cycle and promote cell proliferation[22-25]. In the present study, exposure to SCCP promoted proliferation of HGF in a concentration-dependent manner up to 100 nM. This result suggests that SCCP promotes cell activity. Recent studies have revealed that the increased cyclin D1 expression promotes the proliferation and activity of cells. This study also showed significantly increased levels of cyclin D1 expression, proliferation, and migration of HGF exposed to SCCP. In this study, the exposure time was set to 30 seconds, assuming that SCCP was used during gargling. The results suggest that SCCP is appropriate for short-term exposure such as tooth brushing and gargling. These findings suggest that exposure of periodontal tissue to SCCP induces cyclin D1 expression and promotes proliferation and migration of gingival fibroblasts. Therefore, SCCP could be effective in promoting wound healing in periodontal tissue and contribute to the prevention of periodontal disease. Future studies should clarify the mechanism by which SCCP promotes cyclin D1 expression.

Conclusions

The results of this study suggest that SCCP is appropriate for short-term exposure such as toothbrushing and gargling. The findings suggest that exposure of periodontal tissue to SCCP induces cyclin D1 expression and promotes proliferation and migration of gingival fibroblasts. Therefore, SCCP could be effective in promoting wound healing in periodontal tissue and contribute to the prevention of periodontal disease. Future studies are needed to clarify the mechanism by which SCCP promotes cyclin D1 expression.

Conflict of Interest

The authors declare no conflicts of interest associated with this manuscript.

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Original Article

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Three-year Retrospective Follow-up of Cases of Root Canal Obturation with a Bioactive Glass-based Root Canal Sealer

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Abstract

Purpose: Bioceramics-based root canal sealers are becoming essential in modern endodontics worldwide and are currently being used in Japan; however, there have been no follow-up studies on teeth treated using these sealers. The purpose of this study was to retrospectively evaluate 3-year follow-up data pertaining to teeth treated with a bioceramics-based root canal sealer.

Methods: Patients treated at the Division of Tooth Therapy (Endodontics and Restorative Dentistry) of Kyushu Dental University Hospital and a private dental clinic were recruited, and 127 teeth that were obturated with a bioactive glass-based root canal sealer were followed up for 3 years. The rates and incidence of functionally surviving and extracted teeth were analyzed. Additionally, 56 teeth with 3-year follow-up radiographs were assessed using the periapical index (PAI) score.

Results: One hundred and fifteen teeth (91%) survived functionally, and 12 teeth (9%) were extracted during the 3 years. The 12 extracted teeth were initially diagnosed as non-preservable by the dentist because of root cracks or fractures and extensive bone defects, but were endodontically treated in accordance with the patients' strong requests. There were significant associations between functional surviving and extracted teeth and tooth type, subjective symptoms, initial treatment/re-treatment, and cracks/fractures. In contrast, there was no significant difference between surviving and extracted teeth according to the root canal obturation technique, pain immediately after root canal obturation, and overfilling of the root canal. In the comparison according to PAI scores, the rates of scores 1 and 2 increased, whereas those of scores 3 and 4 decreased.

Conclusion: This 3-year retrospective follow-up study of root canal-obturated cases revealed that the bioactive glass-based root canal sealer evaluated herein, a bioceramics-based sealer, can be applied to achieve favorable outcomes in endodontic therapy.

Key words: bioactive glass, root canal sealer, root canal obturation, follow-up

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Introduction

Successful endodontic therapy depends on adequate mechanical shaping, a copious and effective irrigation protocol, and 3-dimensional obturation of the root canal system[1]. In a previous study, the association between failure of root canal treatment and poor root canal obturation was demonstrated[2,3]. The invasion of microorganisms into the interfacial region between the root canal dentinal wall and filling materials needs to be prevented to avoid re-infection[4–6]. Achieving a proper seal depends on the ability of the filling material to bind to the dentinal wall and on the root canal obturation technique. These findings highlight the importance of root canal sealers. Consequently, preventing microleakage by ensuring proper root canal obturation is essential to obtain lasting clinical success.

Bioceramics-based endodontic biomaterials have gained popularity in modern endodontics because of their physicochemical properties and biocompatibility, which is the ability of a material to achieve a stable and advantageous host response when applied[7,8]. In particular, bioceramics-based root canal sealers have been incorporated into endodontic procedures to improve the outcome of root canal treatment[9]. Several studies also indicated that bioceramics-based root canal sealers used with a single-cone technique are useful for root canal obturation and that teeth obturated using these sealers confer a favorable outcome[10,11].

Though bioceramics-based root canal sealers are used in Japan, there have been no reports on the follow-up of teeth obturated using these sealers. The purpose of this study was to retrospectively evaluate follow-up data pertaining to non-surgical root canal treatment using Nishika Canal Sealer BG (CS-BG; Nippon Shika Yakuhin Co., Ltd., Yamaguchi, Japan), an easily available and widely used bioceramics-based root canal sealer.

Materials and Methods

1. Patient selection

Patients treated at the Division of Tooth Therapy (Endodontics and Restorative Dentistry) of Kyushu Dental University Hospital and a private practice clinic (Miura Dental Clinic, Fukuoka, Japan) were recruited to this study. Ethical approval for the study was obtained from the ethics committee of Kyushu Dental University (approval no: 17-39; Kyushu Fukuoka, Japan). One hundred and twenty-seven teeth that were endodontically treated by six dentists (two endodontists and four dentists with at least 5 years of experience) and then followed up for 3 years were selected for this study.

2. Endodontic treatment procedures

Individual dentists examined and diagnosed each patient, and the same dentist was assigned for endodontic treatment. Local anesthesia was administered for pulpectomy. The anesthetic effect was verified based on subjective symptoms (tingling and numbness). In case of severe loss of tooth structure, the pre-endodontic build-up of the crown was prepared to facilitate rubber dam placement and temporization of the tooth between visits.

After isolation of the tooth by a rubber dam, the access cavity was prepared using sterile diamond points and carbide burs. The canal patency was checked using a #10 K-file (MANI Inc., Tochigi, Japan), and a glide path was established using a #15 K-file (MANI Inc.). The working length was determined using an electronic apex locator (Root ZX II; Morita, Kyoto, Japan). In cases where a reliable electronic apex locator reading could not be obtained, a radiograph was taken to confirm the working length. The canals were irrigated with 3% sodium hypochlorite (NaClO; Nippon Shika Yakuhin Co., Ltd.) using an endodontic syringe with a closed-ended side-vented needle after each filing. The master apical file size was case-specific and determined based on the initial canal size. In re-treatment cases, the previously applied root canal filling materials were removed using a combination of ultrasonics, a gutta percha solvent (GP-Solvent; Nippon Shika Yakuhin Co., Ltd.), rotary instruments, and K-files.

After root canal preparation, all the canals were irrigated by applying ultrasonic activation (P-Max plus; Acteon Inc., France) at two sets of 30 s each with 3% NaClO and 3% ethylenediaminetetraacetic acid (EDTA) solution (Nippon Shika Yakuhin Co., Ltd.). Subsequently, the canals were washed with sterilized saline (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). After the completion of irrigation, the canals were dried using an endo aspirator and paper points and filled with calcium hydroxide paste (Calcipex II; Nippon

Shika Yakuhin Co., Ltd.). The coronal cavity was sealed with an intermediate restorative material.

After improvement of the patient's symptoms, the root canals were obturated with CS-BG and gutta percha points (GP; Morita). CS-BG is a two-phase paste that includes Paste A, which consists of fatty acid, bismuth subcarbonate, and silicon dioxide, and Paste B, which consists of magnesium oxide, calcium silicate glass (a type of bioactive glass), and silicon dioxide. CS-BG was prepared according to the manufacturer's instructions. After irrigation and drying of the canals with root canal vacuum and paper points, a master GP was adapted, and the canals were obturated using the single-cone technique (Single), multi-cone technique without condensation (Multi-Non-press), or lateral condensation technique (Multi-Lateral), as follows:

- Single: A dentist used a single master GP and root canal sealer.
- Multi-Non-press: A dentist used a single master GP, several accessory GPs, and a root canal sealer, without lateral condensation.
- Multi-Lateral: A dentist used a single master GP, several accessory GPs, and a root canal sealer, with condensation.

Excess sealer and GPs were removed using a heated plugger. The coronal cavity was sealed with an intermediate restorative material. The obturation was verified using radiography. Permanent restorations of the teeth were performed as soon as possible.

3. Follow-up and outcome assessment

Patient attributes including gender, age, arch, type of tooth, subjective and objective symptoms, initial treatment or re-treatment, endodontic treatments (pulpectomy or infected root canal treatment), detection of root crack (Crack) or root fracture (Fracture), root canal obturation techniques (Single, Multi-Non-press, or Multi-Lateral), presence or absence of pain immediately after root canal obturation, presence or absence of overfilling of the root canal at the time of filling, and functionally surviving teeth (Survived) or extracted teeth (Extracted) at 3 years were examined. Information on the extracted teeth was sought from the patient or the referring dentist, or was derived from the patient's records. In addition, the incidence of Survived or Extracted was examined. Clinical follow-up was performed at 3 years for each tooth.

Periodical analysis of radiographic images during the 3-year follow-up period was archived for 56 of the 115 surviving teeth, and the periapical region status at each time-point—pre-operative (Pre-op), root canal obturation (RCF), and 3-year follow-up—were assessed according to a periapical index (PAI) score[12,13] by two blinded, independent, and trained examiners, as follows (Figure 1):

PAI 1: Normal periapical structure

PAI 2: Bone structural changes indicating, but not pathognomonic for, apical periodontitis

PAI 3: Bone structural changes with some mineral loss characteristic of apical periodontitis

PAI 4: Well-defined apical radiolucency

PAI 5: Radiolucency with radiating expansion of bone structural changes

Multi-rooted teeth were assigned the highest score for any of the roots.

4. Statistical analysis

The data were analyzed using the chi-squared test; SPSS Statistics 25.0 software (IBM Corp., Armonk, NY, USA) was used. The statistical significance was set at $p<0.05$.

Results

The patient demographic data are shown in Figure 2. A total of 127 teeth were included in this study. Thirty-eight (30%) teeth belonged to male patients, and 89 (70%) to female patients (Figure 2-a). The patient ages ranged between 10 and 89 years as follows (age range, number of teeth): 10–19, 1 (1%); 20–29, 2 (2%); 30–39, 5 (4%); 40–49, 15 (12%); 50–59, 25 (20%); 60–69, 31 (24%); 70–79, 39 (31%); and 80–89, 9 (7%) teeth (Figure 2-b). Figure 2-c shows the arch and tooth types. Seventy-five (59%) teeth were in the maxilla, and 52 (41%) in the mandible. Forty-eight (38%) of the treated teeth were located in the anterior segment, 29 (23%) were premolars, and 50 (39%) were molars. Thirty-four (27%) teeth underwent initial treatment and 93 (73%) underwent re-treatment (Figure 2-d). Figure 2-e shows the incidence rates of "Survived" and "Extracted" at 3 years. One hundred and fifteen (91%) teeth survived functionally, and 12 (9%) teeth were extracted within three years. Of the 115 teeth that survived, 108 were diagnosed as preservable and seven as non-preservable at the time of the initial examination; however, the teeth were treated endodontically in accordance with

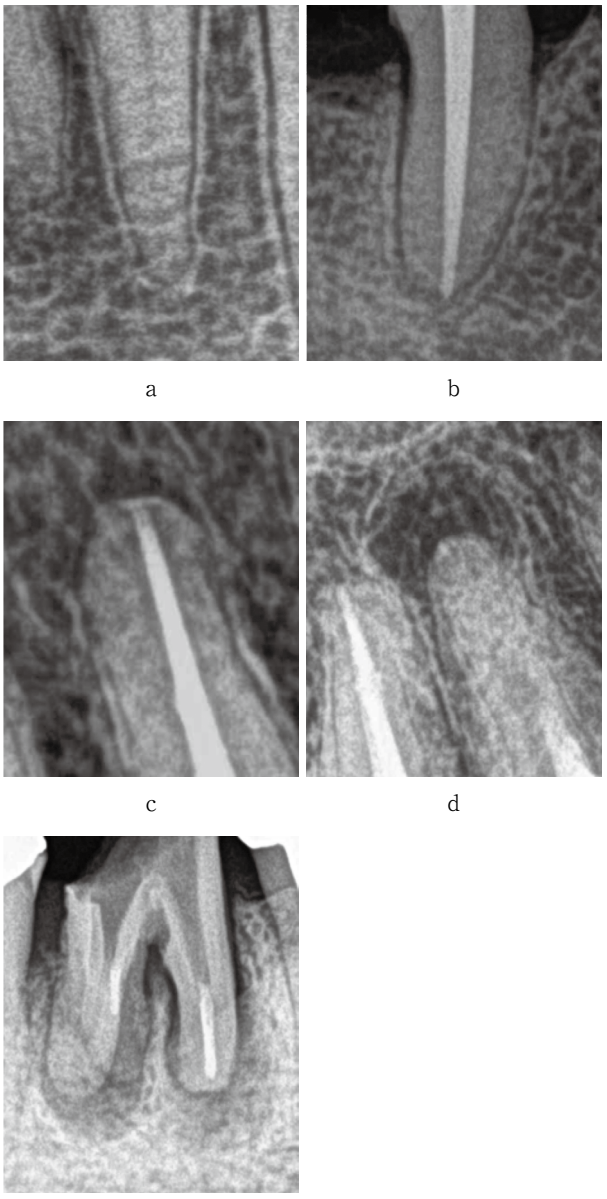


Fig. 1 Examples of teeth with periapical index (PAI) score of 1 (a), 2 (b), 3 (c), 4 (d), and 5 (e)

the patients' strong requests (data not shown). All the 12 extracted teeth were diagnosed as non-preservable at the time of the initial examination, but were endodontically treated in accordance with the patients' strong requests (data not shown).

Table 1 shows the incidence of the various patient characteristics and operative characteristics in the Survived and Extracted groups. There were significant associations between Survived/Extracted and type of tooth, subjective symptoms, initial treatment/re-treatment, and Crack/Fracture.

Table 2 shows the incidence of root canal obturation in the Survived and Extracted groups. There were no significant associations between Survived/Extracted and the root canal obturation technique, pain immediately after root canal obturation, and overfilling of the root canal immediately after the obturation.

Figure 3 shows the PAI score of the 56 surviving teeth with 3-year follow-up radiographs, and the mean/median/mode at Pre-op, RCF, and 3-year follow-up. During the 3-year follow-up period, the rates of PAI scores 1 and 2 increased in a time-dependent manner, whereas those of scores 3 and 4 decreased.

Discussion

In this study, we retrospectively evaluated follow-up data pertaining to non-surgical root canal treatment using CS-BG, an easily available and widely used bioactive glass-based root canal sealer. CS-BG was developed in 2017 and is now being applied as a bioactive glass-based root canal sealer and pulp-capping material, Nishika Canal Sealer BG multi (Nippon Shika Yakuhin Co., Ltd.). CS-BG is delivered as two pastes, which can be mixed as needed; this promotes consistency and efficiency in formulating and using this biomaterial in a clinical setting. The superior flowability and the ability to slightly expand upon setting allow this sealer to be used with the single-cone technique[14–17]. Several *in vitro* and *in vivo* studies have also reported that CS-BG has excellent biocompatibility with periapical tissues[15–17]. Additionally, a clinical study reported that CS-BG can reduce the distress of patients during root canal obturation[18]. However, the clinical significance of these characteristics remains unclear.

In the present study, 12 extracted teeth that had a cracked or fractured root were endodontically treated in accordance with the patients' preferences, even though they were diagnosed as non-preservable at the time of the initial examination. Additionally, many of the extracted teeth had thinning of root dentin pre-operatively or post-operatively (data not shown). It has been reported that thinning of root dentin by not only endodontic treatment but also the length and thickness of the post hole affects root cracking and fracture[19]. A previous study including Kaplan-Meier analysis of 5-year follow-up data reported that most lost teeth are extracted within 2 years after treatment[20,21]. Consistent

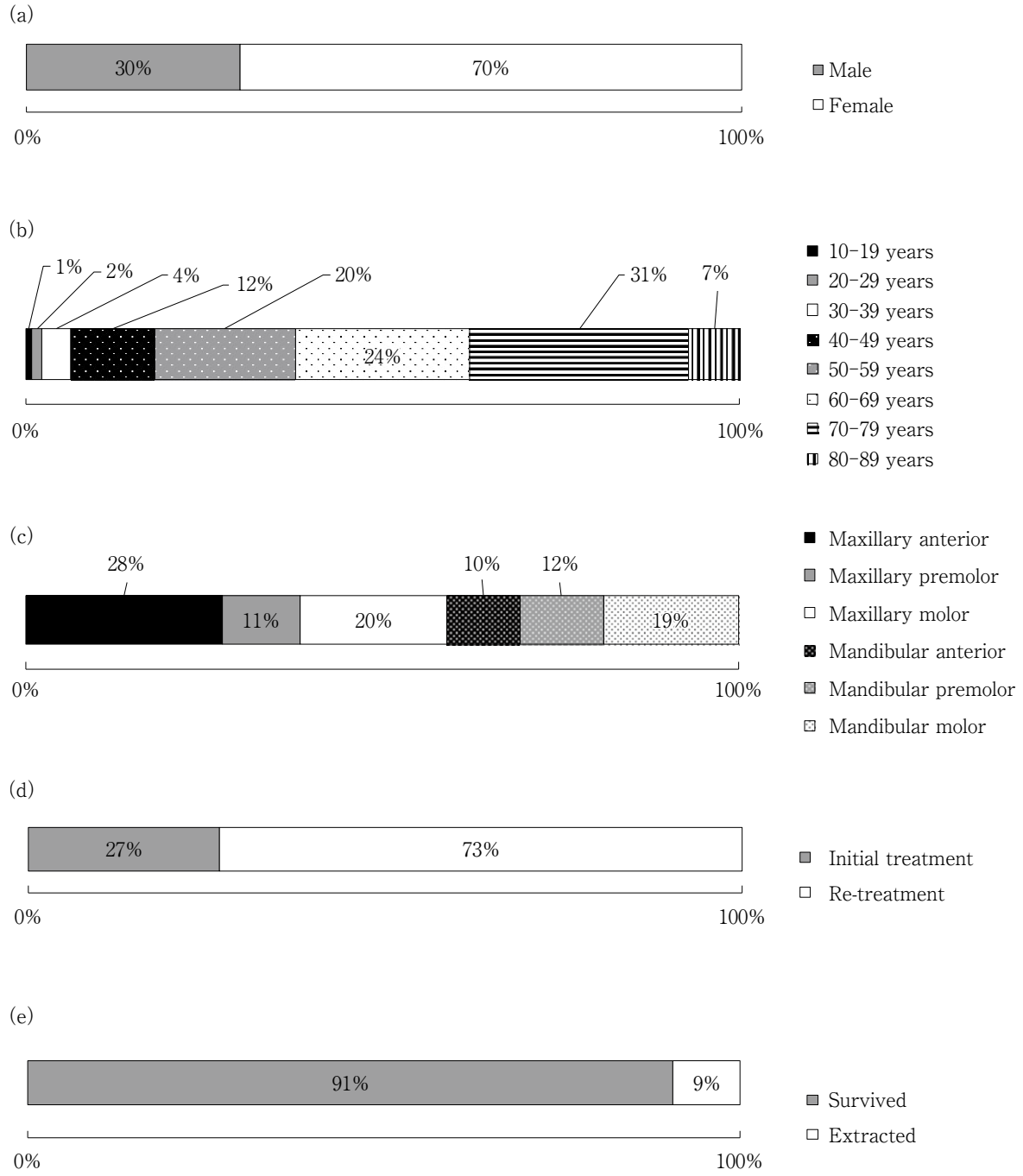


Fig. 2 Demographic data (a) gender, (b) age, (c) arch and type of tooth, (d) initial treatment or re-treatment, (e) teeth that survived or were extracted

with the literature, in this study, 11 of the 12 teeth were extracted within 2 years of treatment (data not shown). Interestingly, seven functionally surviving teeth with root cracks or fractures were diagnosed as non-preservable at the initial examination but survived and functioned after endodontic treatment. Based on the evaluation of the PAI score, the median score of the 12 extracted teeth and 7 functionally surviving teeth with root cracks or fractures was 5 and 3, respectively (data not shown). Therefore, teeth with root cracks or fractures can survive and retain function in the absence of extensive bone defects.

Previous studies have examined the incidence of survival or extraction in root canal-obturated teeth and reported that the survival of such teeth was significantly related to patient age[22]. Also, it has been

Table 1 Patient characteristics and pre-operative variables of teeth that survived (Survived) or were extracted (Extracted)

Total, n=127		Survived (n=115)	Extracted (n=12)	p value
Gender	Male	35	3	.696
	Female	80	9	
Age	<50 years	21	2	.891
	≥50 years	94	10	
Arch	Maxillary	69	6	.503
	Mandibular	46	6	
Type of tooth	Anterior	47	1	.0004*
	Premolar	29	0	
	Molar	39	11	
Subjective symptoms	No symptoms	70	0	.0001*
	Symptoms	45	12	
Objective symptoms	No symptoms	37	2	.268
	Symptoms	78	10	
Initial-/Re-treatment	Initial treatment	34	0	.028*
	Re-treatment	81	12	
Endodontic treatments	Pulpectomy	22	0	.096
	Infected root canal treatment	93	12	
Crack/Fracture	Absence	108	7	.0001*
	Presence	7	5	

* : χ^2-test ($p<.05$).

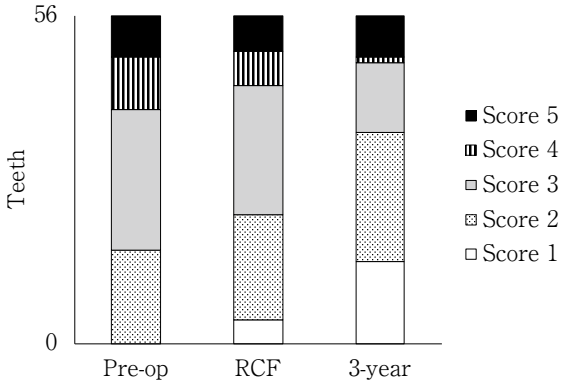
Table 2 Root canal obturation-related variables of teeth that survived (Survived) and were extracted (Extracted)

Total, n=127		Survived (n=115)	Extracted (n=12)	p value
Root canal obturation technique	Single	34	4	.772
	Multi-Non-press	76	7	
	Multi-Lateral	5	1	
Pain immediately after root canal obturation	No pain	113	11	.152
	Pain	2	1	
Overfilling of root canal	Absence	77	10	.245
	Presence	38	2	

reported that the pulp chamber is often constricted in patients over 50 years of age, making the procedure more complicated and affecting the prognosis[23-25]. However, the present study showed that age did not affect the survival of root-obturated teeth; the reason for this difference in results remains unclear.

The most common reason for tooth extraction after endodontic treatment is tooth fracture[26,27], and molars are more likely to be extracted after endodontic treatment than other teeth[28]. In addition, re-treatment has been reported to have a lower success rate than initial treatment[29,30]. However, the relationship between the presence or absence of subjective symptoms and follow-up has rarely been reported previously, although the presence of subjective symptoms is believed to be important in the need for root canal treatment. The results of the present study suggest that a molar tooth with subjective symptoms and crack or fracture is likely to be extracted within 3 years if the tooth is re-treated.

Root canal obturation techniques have been reported to be related to the extrusion of the root canal sealer



	Pre-op	RCF	3-year
Mean	3.2	3.0	2.5
Median	3	3	2
Mode	3	3	2

Fig. 3 Periapical index (PAI) score distribution, mean, median, and mode at the pre-operative stage (Pre-op), root canal obturation (RCF), and 3-year follow-up

with post-obturated pain, but these factors do not affect the outcomes of endodontically treated teeth[31,32]. The results of the present study are in agreement with those of these previous studies. An ideal root canal obturation should ensure that the filling materials reach the cement-dentinal junction[33]. Studies have shown that the highest success rate of endodontic treatment is observed in teeth with root canal fillings ending 0–2 mm short of the radiographic apex, and cases with underfillings or overfillings are associated with significantly lower success rates[33–35]. In contrast, unintentional extrusion of the root canal sealer is a common event that, in low amounts, is usually well tolerated by the periradicular tissues[36,37]. Evidence indicates that the unintentional apical extrusion of root canal fillings seems to have no direct correlation with treatment failure, provided that infection is absent[38–41]. The unintentional extrusion of filling materials can occur owing to the lack of apical constriction as a result of inflammatory apical root resorption or an incompletely formed root apex, or over-instrumentation due to errors in working length assessment[42]. In other words, the incidence of sealer extrusion seems to be related more closely with operator parameters and root canal preparation and filling techniques than with the root canal sealer selected for use. However, to avoid accidents and complications caused by the extrusion of root canal sealers, it is important to select materials with satisfactory physicochemical properties and low toxicity[43]. In the present study, there were no significant associations between the 3-year follow-up data and apically extruded sealers in the periradicular tissues at RCF. The apically extruded sealers of several teeth were absent in the periradicular tissues on 3-year follow-up radiographs, and a collateral (or original canal) seen in the root canal at pretreatment appeared to have been sealed by the obturation material (data not shown). CS-BG has been reported to be biocompatible with periapical tissues[38,39], and a case report showed that the presence of CS-BG in periapical tissues did not affect the healing of periapical lesions[44]. Therefore, the single-cone technique using CS-BG is expected to be useful. However, the intentional and excessive extrusion of the sealer into the periapical region should be avoided.

The PAI score[12,13] was employed in the current analysis. This scoring system has been used in many longitudinal studies, and its significant prognostic value has been proven, particularly in repeated radiological evaluations. Many authors have suggested that the time period for evaluating healing parameters in teeth with periapical lesions is 4 to 5 years[45,46]. However, it was demonstrated that a 1-year period may be ideal to control for periapical changes in bone density when using PAI scores[47]. The PAI scores were divided into healed (PAI$\leq$2, no signs or symptoms) and non-healed (PAI $>$2, with/without signs or symptoms)[7,48]. The PAI scores for 56 of the 115 surviving teeth included in this study suggest that root canal obturation using a bioactive glass-based root canal sealer is acceptable for achieving favorable endodontic outcomes. However, the present data are insufficient because of the small sample size; follow-up studies for longer periods are currently underway.

Conclusions

The findings of this retrospective study suggest that bioactive glass-based root canal sealers are useful for cases diagnosed as preservable at the start of endodontic therapy, and that tooth obturation by the single-cone technique using this sealer is a viable option. Additionally, the results show that in the present study, the reason for teeth extraction in most cases was not underlying periapical disease but root cracks or fractures with

extensive bone defects detected at the time of the initial examination.

Conflict of Interest

The authors declare no conflicts of interest associated with this manuscript.

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Original Article

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Influence of Light Irradiation Modes on the Polymerization of Light-curing Resins in a Root Canal Model

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Abstract

Purpose: Fiber posts with resin cores have been widely used as direct build-up materials for the restoration of endodontically treated teeth. However, the influence of light irradiation mode, including the irradiation intensity and time, on resin polymerization in the apical root canal remains an open research problem. This *in vitro* study investigated the influence of light irradiation mode on the polymerization of different light-curing resins with and without a fiber post.

Methods: A translucent fiber post, namely i-TFC Luminous fiber, was used in combination with five commercial light-curing resins: i-TFC System Universal (UN), i-TFC System Blue (BL), i-TFC Luminous core LC flow (LC), Filtek U (FU), and MI core LC flow (MI). Each resin was filled into an artificial root canal model (diameter: 3 mm, depth: 15 mm) with or without the fiber and light-cured using a handy light-curing unit in two irradiation modes: mode-F3: 2,000 mW/cm^2 for 3 s, and mode-10: 1,200 mW/cm^2 for 10 s×3. The cured resins were characterized by their polymerization ratios, depths, and optical transmittances.

Results: The polymerization ratio of each resin was significantly higher in mode-10 than in mode-F3 with and without the fiber post. In the fiber-post groups with mode-10, the polymerization ratio was over 96% for each resin. In the fiber-post groups with mode-F3, the polymerization ratio of each resin was relatively low: 90.5±3.6 for UN, 92.8±4.8 for BL, 85.2±3.7 for LC, 92.7±2.3 for FU, and 75.1±5.2 for MI. The polymerization depth of each resin with the fiber post reached 15 mm for each mode. The LC resin produced the highest optical transmittance, followed by the UN, BL, MI, and FU resins.

Conclusion: Longer light irradiation and the use of a fiber post were found to be essential to achieve sufficient resin polymerization in the apical root canal.

Key words: fiber, polymerization, root canal

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Introduction

Fiber-post/resin-core construction systems have become more popular for preparing tooth abutments than conventional metal post systems because their mechanical properties, such as the elastic modulus, are similar to those of dentin[1-3]. In the direct restoration of the tooth in a system, the resin is filled into the tooth root canal with a fiber, followed by polymerization via light irradiation. The degree of polymerization of the resin is one of the most important factors influencing the mechanical and physicochemical properties of the material[4-6], and it affects the long-term success of tooth restoration[7, 8]. To maximize the performance of the fiber-post/resin-core construction system, it is preferable to polymerize the resin as much as possible. However, complete polymerization of the resin in an apical root canal is difficult because of high humidity and light shielding in the narrow cavity[9, 10].

To overcome this issue, much effort has been made on optimizing the polymerization of resin in the root canal. For instance, a study reported that the transparency of the fiber post effectively influences the polymerization of the resin[11]. The polymerization of the resin in an apical root canal was higher with translucent fiber posts than with an opaque fiber post[12]. The fiber-post type and dowel space levels influence resin polymerization[13]. Light polymerization of the resin was insufficient in areas deeper than 4-6 mm and further away from the fiber post[14]. The degree of conversion of the resin decreased as the post length increased[15, 16]. Insufficient polymerization of the resins was observed in deep areas of the root canal using dual-curing resins[17, 18]. In addition, our previous study demonstrated that the combination of a highly translucent fiber post and light-curing resin facilitates resin polymerization in deeper areas of the root canal[19]. However, there is room for further investigation on the influence of light irradiation conditions (intensity and exposure time) on resin polymerization in the root canal.

This study investigated the influence of light irradiation mode on the polymerization of different light-curing resins in a fiber-post/resin-core construction system. An *in vitro* experiment was performed using an artificial root canal model with five commercial light-curing resin products and one commercial fiber product.

Materials and Methods

1 Materials

Table 1 lists the fibers and resins used in the fiber-post/resin-core system. The fiber was used at the as-received length (18 mm) without cutting to minimize light scattering at the cutting interface.

2 Polymerization ratio for the fiber-post/resin-core system

The polymerization weight of the resin core was evaluated by weight changes before and after light irradiation according to a previous study[19]. A Teflon block with a cylindrical cavity (diameter: 3 mm, depth: 15 mm) was fabricated and used as the artificial root canal model (Fig. 1-a). Figure 1-b shows the light polymerization process of the resin. Prior to the test, the Teflon mold, shielding silicone, and acrylic plate were weighed (A). The fiber post was also weighed (B). Resin was injected into the cavity, and the fiber was inserted into the cavity through an acrylic plate. Subsequently, the surrounding mold, except for the top surface, was covered with silicone to shield it from ambient light. This setup imitates a dentin root canal, in which irradiation light can enter the root canal cavity from the coronal direction. Light irradiation was carried out at a distance of 3 mm from the top surface of the root canal using a handheld light-curing unit (G-Light Prima, GC, Tokyo, Japan) in two different modes: mode-F3, 2,000 mW/cm^2 for 3 s; and mode-10, 1,200 mW/cm^2 for 10 s×3. After light irradiation, the total weights of the mold, silicone, plate, resin, and fiber were measured (C). The resultant resin was removed from the mold and washed by immersing in acetone for 30 s to eliminate the uncured resin. The washed resin was kept under ambient conditions at 25℃ for 24 h to dry, and then weighed (D). The polymerization ratio of the resin was determined using the equation:

$$\text{Polymerization ratio (\%)} = (D-B)/(C-B-A)\times 100 \quad \cdots\cdots(1)$$

Using this procedure, the polymerization ratio of each resin was measured with and without the fiber (n=5).

3 Polymerization depth for the fiber-post/resin-core system

After the polymerization ratio test, the resultant res-

Table 1 Materials used in this study

	Product name	Manufacturer	Code
Fiber post (∅1.0 mm/tapered)	i-TFC Luminous fiber	Sun Medical Corp., Moriyama, Japan	
Light curing resin	i-TFC Luminous core LC flow	Sun Medical Corp., Moriyama, Japan	LC
	i-TFC System Universal	Sun Medical Corp., Moriyama, Japan	UN
	i-TFC System Blue	Sun Medical Corp., Moriyama, Japan	BL
	Filtek U	3M Corp., MN, USA	FU
	MI core LC flow	GC Corp., Tokyo, Japan	MI

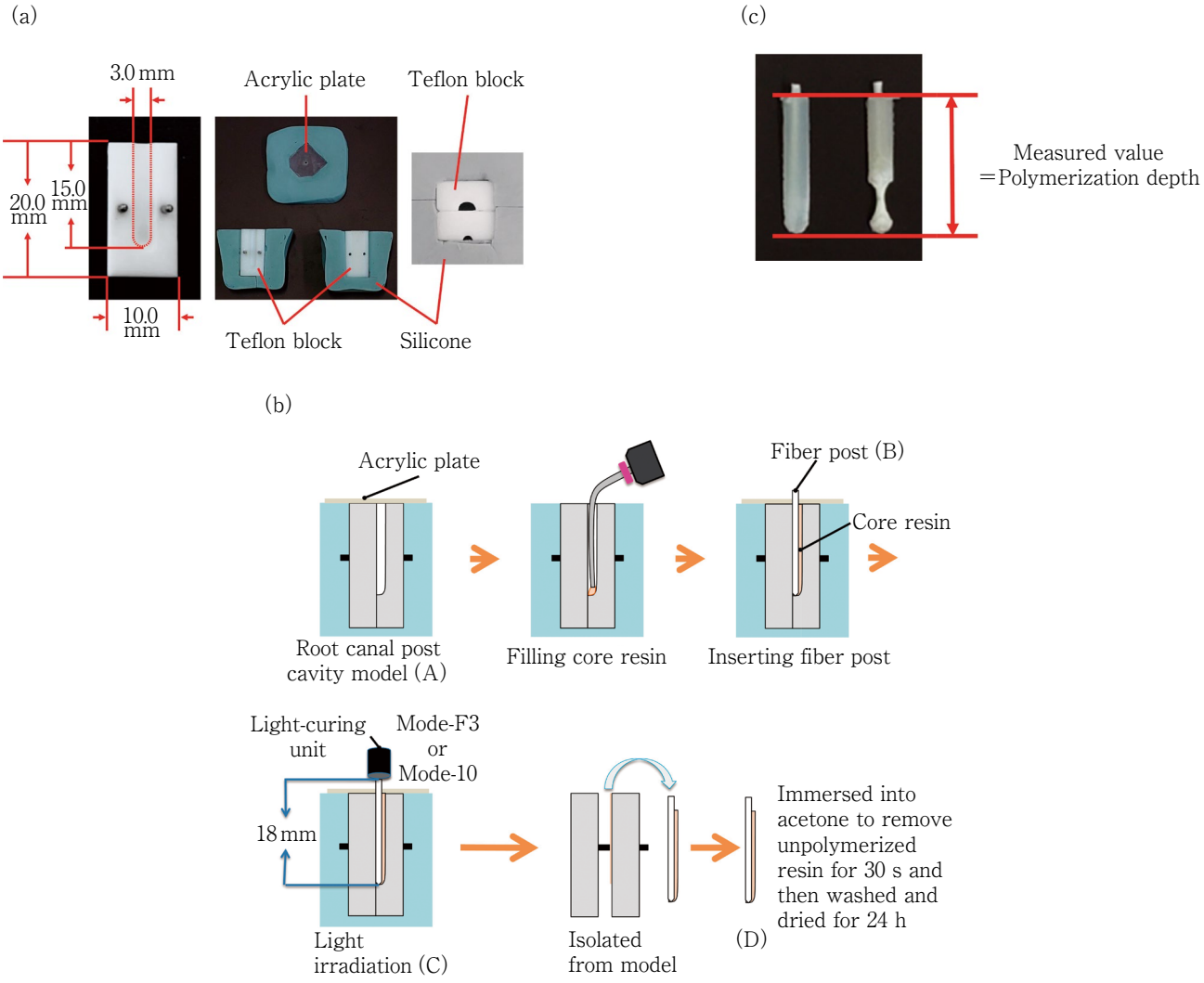

Fig. 1 Experimental system for polymerization ratio and depth measurement

(a) Image of a Teflon mold with shielding silicone for the root canal model. (b) Procedure for measuring polymerization ratio and depth of the resin in the root canal. (c) Image of cured resin after acetone washing.

ins were used for polymerization depth measurements, as shown in Fig. 1-c. The length of the resin was measured using a digital scale to determine the polymerization depth. Depth measurements were performed for each resin with and without the fiber ($n=5$).

4 Transmittance of resin

The transmission spectra of the resin were measured in the wavelength range of 200–800 nm using an ultra-

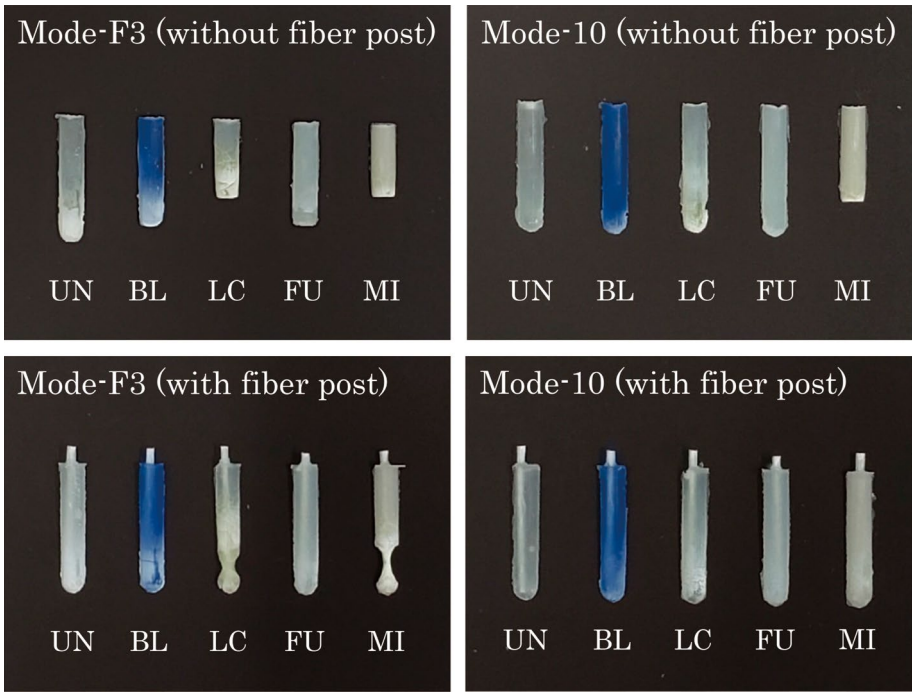

Fig. 2 Representative images of polymerization resins after acetone washing

violet-visible (UV-Vis) spectrophotometer (V-650S, JASCO Corp., Tokyo, Japan). Prior to the measurement, each resin was stacked with glass plates and light-cured to 1 mm thickness. A cured resin plate was used for these measurements.

5 Statistical analysis

Statistical differences in the polymerization ratio and depth among the resins were confirmed using the R software (Version R 3.6.3, The R Function, Vienna, Austria). Data were analyzed by performing a Student's *t*-test or one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test.

Results

Figure 2 shows representative images of the polymerized resin with and without the fiber post in the two light irradiation modes. For the samples with no fiber post, in each irradiation mode, the apical area of each resin was not polymerized. In particular, polymerization of the apical area was lower in the mode-F3 groups than in the mode-10 groups. In comparison, the fiber post improved resin polymerization in both the irradiation modes. However, for the mode-F3 groups, the resin in the apical area was not partially polymerized, even when the fiber post was used. In particular, the resin further away from the fiber was not polymerized.

Table 2 shows the polymerization ratio of the resins with and without the fiber post in the two light irradiation modes. The polymerization ratio of each resin was significantly higher in mode-10 than in mode-F3 with and without the fiber post. In the fiber-post groups with mode-10, the polymerization ratio was over 96% for each resin. Among these groups, there was no statistically significant difference between the polymerization ratios of each resin. However, in the fiber-post groups with mode-F3, the polymerization ratio of each resin was relatively low: 90.5±3.6 for UN, 92.8±4.8 for BL, 85.2±3.7 for LC, 92.7±2.3 for FU, and 75.1±5.2 for MI.

Table 3 shows the polymerization depth of the resins with and without the fiber post in the two light irradiation modes. The polymerization depth showed the same trend as the polymerization ratio. The polymerization depth was greater in mode-10 than in mode-F3 for each resin. For the no-fiber-post groups with mode-F3, the polymerization depth significantly depended on the resin type; the UN resin produced the highest polymerization depth, followed by the BL, FU, LC, and MI resins. This result indicates that the polymerization depth depends on the resin type. The polymerization depth of the samples with fiber posts reached 15 mm regardless of the light irradiation mode.

Table 2 Polymerization ratio (%) of the resin with/without fiber post in two irradiation modes

		Mode-F3			Mode-10			Significant difference**
	Resin	Mean	SD	Category*	Mean	SD	Category*	
Without fiber post	UN	93.1	3.6	A	98.3	1.5	F	Significant
	BL	81.4	2.6	B	97.6	1.3	F	Significant
	LC	57.4	2.8	C	96.7	1.0	F	Significant
	FU	80.1	0.7	B	98.3	0.6	F	Significant
	MI	55.7	2.0	C	74.7	1.8	G	Significant
With fiber post	UN	90.5	3.6	D	96.6	3.6	H	Significant
	BL	92.8	4.8	D	97.8	0.6	H	Significant
	LC	85.2	3.7	D	97.7	0.5	H	Significant
	FU	92.7	2.3	D	97.0	1.6	H	Significant
	MI	75.1	5.2	E	96.2	1.2	H	Significant

n=5, SD : standard deviation ; *Category : Same letters indicate that the values are not significantly different as confirmed by Tukey's test ($p<0.05$) in each group. **Significant difference : statistical difference between the polymerization ratio of each resin by mode-F3 and mode-10, analyzed using Student's *t*-test ($p<0.05$).

Table 3 Polymerization depth (mm) of the resin with/without fiber post in two irradiation modes

		Mode-F3			Mode-10			Significant difference**
	Resin	Mean	SD	Category*	Mean	SD	Category*	
Without fiber post	UN	15.5	0.3	A	16.4	3.6	I	Significant
	BL	14.1	0.5	B	16.4	0.1	I	Significant
	LC	10.2	0.5	C	16.2	0.2	I	Significant
	FU	12.9	0.2	D	16.5	0.1	I	Significant
	MI	8.9	0.3	E	12.1	0.3	J	Significant
With fiber post	UN	15.6	0.1	F	15.6	3.6	K	No significant
	BL	15.5	0.2	F	15.5	0.2	K	No significant
	LC	15.4	0.3	F	15.6	0.1	K	No significant
	FU	16.5	0.2	G	17.1	0.4	L	Significant
	MI	15.1	0.1	H	15.7	0.2	K	Significant

n=5, SD : standard deviation ; *Category : Same letters indicate that the values are not significantly different as confirmed by Tukey's test ($p<0.05$) in each group. **Significant difference : statistical difference between the polymerization depths of each resin by mode-F3 and mode-10, analyzed using Student *t*-test ($p<0.05$).

Figure 3 shows the transmission spectra of each resin. The LC resin produced the highest transmittance at 465 nm, where the maximum intensity of the pen light was located, followed by the UN, BL, MI, and FU resins.

Discussion

This *in vitro* study investigated the effect of irradiation mode on the polymerization of different light-curing resins in a root canal model with and without a fiber post. To maximize the effectiveness of the fiber posts, i-TFC Luminous fibers, which have a high optical

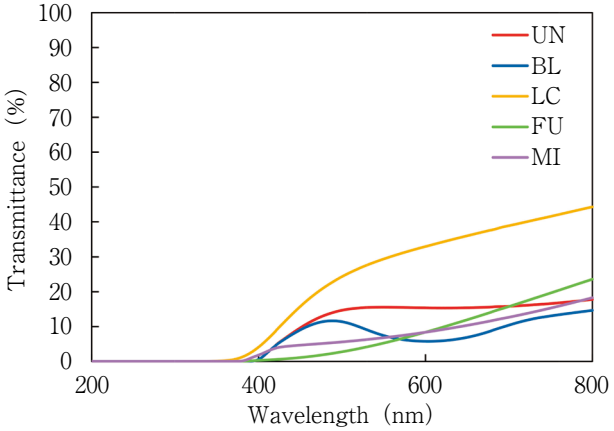

Fig. 3 Transmission spectra of each polymerization resin

transparency[19], were used as the post material. Our previous study revealed that the polymerization of the core resin with i-TFC Luminous fiber was significantly higher than that with other types of fiber post[19]. Therefore, i-TFC Luminous fibers could effectively facilitate resin polymerization in the apical root canal.

Conventionally, resin polymerization is estimated by the degree of conversion, which can be obtained by means of Fourier transform infrared (FT-IR)[20-22] or Raman[23-25] spectroscopy. These methods help measure the spectrum of the resin and derive the degree of conversion for the resin using the measured characteristic peaks. This method quantitatively determines the polymerization degree of the resin. The drawback of this method is that the degree of conversion is determined only for some spots on the sample, that is, a small size with a thin layer on the sample surface. It is difficult to estimate the polymerization of the entire bulk resin core using FT-IR and Raman spectroscopy. To evaluate the polymerization of the bulk sample, the present study adopted the polymerization ratio and depth, which were obtained by washing the light-cured resin with acetone, followed by measuring its weight change and polymerization length. The present method was demonstrated in a previous study on the polymerization of resins in a fiber-post/resin-core construction system[19]. The method allows easy estimation of the polymerized/unpolymerized locations in a bulky resin core, and hence is suitable for evaluating resin polymerization in an apical root canal.

The present study used a root canal model with a cylindrical cavity 3 mm in diameter. This cavity size was chosen considering the maximum diameter of a human root canal; for example, the largest diameter of the mandibular first premolar is reportedly 2.78 mm[26].

The present results clearly indicate that the fiber post facilitated the polymerization of the core resin in the root canal in each light irradiation mode. In particular, the use of the fiber post helped improve the polymerization of the apical area. These results are in line with those of previous studies on the use of fiber posts for resin polymerization[27, 28]. Highly translucent fiber posts can help better irradiate light into the apical root canal. As a result, free radical polymerization occurs via excitation of the photoinitiator. Based on our present results, a remarkable improvement in the degree of polymerization provided by the fiber post could be observed under short-time light irradiation, mode-F3.

Light irradiation using a handheld light-curing unit was used in two modes[29]: high intensity with a short irradiation time and low intensity with a long irradiation time. Therefore, this study examined light curing at mode-F3 and mode-10; the irradiation mode affected the polymerization of the resin. These irradiation modes of the light-curing unit are similar to those of other commercial light-curing units[29, 30]. In many fundamental studies on the polymerization of resins using light-curing units, experiments were performed under similar irradiation intensity and time as in the present irradiation modes[29, 30]. Hence, the irradiation modes used in this study were considered acceptable.

Every resin achieved a relatively higher polymerization ratio and depth in mode-10 than in mode-F3 because the total excitation energy for mode-10 was much higher than that for mode-F3. The resins polymerized by light irradiation with mode-F3 showed insufficient polymerization in the apical area of the root canal. This result implies that even high-intensity light irradiation cannot achieve sufficient polymerization under such short-duration irradiation. A similar result was reported in a previous study in which short-time light irradiation of less than 10 s did not fully polymerize the resin, and so the partially polymerized resin did not exhibit optimal properties[31]. Therefore, prolonged irradiation is mandatory for complete polymerization of the resin in an apical root canal.

Resin polymerization depended on the transparency of the resin; the highly translucent resin tended to exhibit a relatively higher polymerization ratio and depth. For instance, the UN resin had a relatively higher transparency, polymerization ratio, and depth than the MI resin. Hence, resin transparency is one of the most important factors for ensuring polymerization in the root canal. In contrast, the FU resin exhibited a relatively higher polymerization ratio and depth, despite having a low optical transparency. This suggests that there are other factors influencing the polymerization. Examples include the color, filler amount, and chemical compositions of the initiator and resin monomer[32, 33]. Further study on the fiber-post/resin-core construction system is required to clarify the correlation between the polymerization of the resins and their properties.

Within the limitations of this *in vitro* study, we

revealed that a highly translucent fiber post and long irradiation mode can help improve the polymerization of the apical area of light-curing resins. However, the present study was performed *in vitro* using an artificial root canal model instead of human teeth, and the experimental conditions differed from clinical situations in terms of humidity, the complex shape of the root canal, and salivary contamination. These environmental factors may adversely affect resin polymerization. In clinical practice, sufficient polymerization may not be achieved even with long-time light irradiation using highly translucent fibers. Both highly translucent fiber posts and long-time light irradiation are essential factors for the long-term success of tooth reconstruction. Furthermore, resin polymerization is related to its mechanical and physicochemical properties. Fundamental research is required to reveal the correlation between polymerization and the mechanical and physicochemical properties of resins.

Conclusions

Within the limitations of this study, we concluded that the resin in an apical area cannot be fully polymerized by short-time irradiation, even with high intensity and a highly translucent fiber post. Resin polymerization in a root canal requires both a long irradiation time and a highly translucent fiber post.

Conflict of Interest

The authors have no conflicts of interest to declare regarding the publication of this paper.

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Original Article

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In vivo Assessment of Tissue Compatibility of Root Canal Sealer Containing Surface Pre-reacted Glass-ionomer Fillers

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Abstract

Purpose: Surface pre-reacted glass-ionomer (S-PRG) fillers possess properties of releasing ions such as fluoride, strontium, and borate ions, and exhibit bioactive effects. In this study, we assessed the *in vivo* pre-clinical effect of a sealer containing S-PRG filler (S-PRG sealer). Specifically, we evaluated the inflammatory response of periapical tissues to the use of S-PRG sealer in root filling, compared to that of sealers with other main components (mineral trioxide aggregate (MTA), resin, and noneugenol-zinc oxide (ZO)).

Materials and Methods: Under general and local anesthesia, premolar pulpectomies were performed in 10-month-old beagle dogs. After root canal preparation, cleaning, and drying, root filling was performed by applying various sealers with gutta-percha master points. Periapical lucency was analyzed radiographically, and the area of inflammatory cell infiltration with bone resorption was analyzed histologically. The inflammatory response of periapical tissues to S-PRG sealer was compared to those of clinically used sealers including MTA (Pro Root MTA), resin (AH Plus), and ZO (Canals N). Tissue specimens were prepared at 1 month and 6 months after obturation.

Results: Periapical lucency was observed at 1 month in all groups. However, at 6 months, periapical lucency persisted with Canals N, but was infrequent with the other three fillers. In histological sections at 1 month, all groups showed similar levels of infiltration of inflammatory cells into the periodontal tissues adjacent to the root apical foramen. After 6 months, inflammation of the periodontal tissues was significantly improved ($p<0.05$) for all sealers except for Canals N.

Conclusion: The inflammatory response to S-PRG sealer at periapical tissues was milder than that to Canals N. The S-PRG sealer may provide biocompatibility comparable with that of commonly and clinically used Pro Root MTA and AH Plus in the healing of the periapical region.

Key words: surface pre-reacted glass-ionomer (S-PRG) fillers, root canal sealer, biocompatibility, dog

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Introduction

Endodontic root canal sealers have been developed for root canal obturation in combination with a master gutta-percha point. Many types of endodontic sealers, variously composed of zinc oxide (ZO), calcium hydroxide, epoxy resin, and mineral trioxide aggregate (MTA), have been used clinically[1-5]. In addition to the sealing ability and physical properties of the sealer, its biocompatibility is an important factor, given that endodontic sealers directly contact periapical tissue at the apical foramen after root canal filling[6,7].

Recently, a root canal sealer containing surface pre-reacted glass-ionomer (S-PRG) filler (S-PRG sealer) has been developed for endodontic use[8,9]. The bioactivity of the S-PRG filler has previously been reported to promote tooth remineralization, while additionally providing acid buffering capacity, antibacterial activities, and caries prevention, via the releasing and recharging abilities of six ions, including fluoride, sodium, strontium, aluminum, silicate, and borate[10-15]. In particular, bioactive ions, including those of strontium, fluoride, and borate, have the potential to provide the root canal sealer with bioactive effects. *In vitro* experiments have shown that strontium ions promote osteoblast proliferation and inhibit bone resorption[16-18]. Fluoride has been shown to stimulate osteoprogenitor cell growth *in vitro*[19], and to induce the expression of genes involved in bone formation and mineral density, reflecting an improved bone remodeling activity *in vivo*[20]. In addition, fluoride has been shown to exhibit antibacterial effects on gram-negative organisms[21], including bactericidal activity against *Porphyromonas gingivalis in vitro*[22]. Furthermore, like fluoride, borate has been shown to exhibit antibacterial and osteogenic effects[23,24]. Clinically, borate exposure has been used previously for the treatment of eye irritation and dryness of the mouth, nose, and throat.

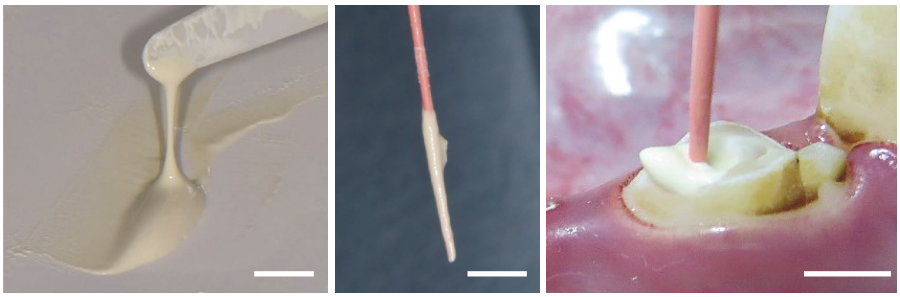
Endodontic sealer incorporating S-PRG fillers has been shown to release these ions for at least 90 days[8]. Therefore, S-PRG filler-containing root canal sealer (S-PRG sealer) is expected to facilitate the healing of periapical tissue, including alveolar bone. In rat subcutaneous implantation tests evaluating sealer cyto-compatibility, S-PRG filler sealers demonstrated lower inflammatory reactions than those seen with silica filler-containing sealer[25]. In the present study, we sought to assess the clinical effects of S-PRG filler sealers in root filling. Specifically, we employed radiographic and histologic endpoints to evaluate the healing of periapical tissues when S-PRG sealer was used in root filling with a master gutta-percha point, following the pulpectomy of dog premolars; comparisons were made with three other types of sealers: Pro Root MTA, AH Plus, and Canals N.

Materials and Methods

1. Pulpectomy and root canal filling with root canal sealers

Four healthy female beagle dogs (age 10 months; weight, approximately 10 kg) were used as experimental animals (approved by the Animal Committee of Hokkaido University, Approval No. 13-122). The experimental sites were mandibular second, third and fourth premolar teeth, for a total of 48 root canals. For endodontic treatments, animals were administered general anesthesia with medetomidine hydrochloride (0.04 mg/kg Domitor, Nippon Zenyaku Kogyo, Koriyama, Japan), butorphanol tartrate (0.15 mg/kg Vetorphale, Meiji Seika, Tokyo, Japan), and midazolam (0.15 mg/kg Dormicum injection 10 mg, Astellas Pharma, Tokyo, Japan), and local anesthesia with 2% lidocaine hydrochloride with 1 : 80,000 epinephrine (Xylocaine Cartridge for Dental Use, Dentsply Sirona, Tokyo, Japan).

After preparation of the access cavity and removal of pulp tissue, the root canal was spread with #40 K-files (Shofu, Kyoto, Japan), while measuring with an apex locator (Root ZX, J Morita, Tokyo, Japan) until the periapical tissue was reached. Cleaning with 10% sodium hypochlorite (Neocleaner "Sekine," Neo Dental Chemical Products, Tokyo, Japan) and 3% ethylenediaminetetraacetic acid (Smear Clean, Nippon Shika Yakuhin, Shimonoseki, Japan) was followed by drying using sterilized paper points. The root canal was then filled with one of the four types of sealers by insertion of a gutta-percha master point (Shofu), as shown in Figure 1. The choice of sealer for a given tooth was assigned randomly. The following sealers were used in this experiment: S-PRG sealer (Shofu; S-PRG group), Pro Root MTA (Dentsply Sirona; MTA group), Canals N (GC Showayakuhin, Tokyo, Japan; Canals N group), and AH Plus (Dentsply Sirona; AH Plus group) (Table 1).



A B C

Fig. 1 Application of S-PRG sealer to root canal of premolar
(A) S-PRG sealer after mixing. Scale bar : 2 mm.
(B) The gutta-percha was coated with sealer. Scale bar : 5 mm.
(C) Root canal filling with gutta-percha master point with adequate application of S-PRG sealer. Scale bar=5 mm.
S-PRG : surface pre-reacted glass-ionomer.

Table 1 Root canal sealers used in this study

Product	Composition
S-PRG sealer	Powder : zinc oxide-based inorganic compound filler, S-PRG filler, additive Liquid : poly carboxylic acid derived, water, others
Pro Root MTA	Powder : calcium oxide, silicon dioxide, bismuth oxide, aluminum oxide, others Liquid : purified water
Canals N	Powder : zinc oxide, rosin, bismuth subcarbonate, barium sulphate, yellow pigment Liquid : fatty acid, propylene glycol
AH Plus	Paste A : epoxy resin, calcium tungstate, zirconium oxide, silica Paste B : diphenyldiamine, aminoamadantane, tricyclodecanediamine, calcium tungstate, zirconium oxide, silica, silicon oil

S-PRG : surface pre-reacted glass-ionomer ; MTA : mineral trioxide aggregate

The cavities were temporarily sealed with glass ionomer cement (Base Cement, Shofu). Dental X-ray images of the periapical tissues were obtained at 1 month and 6 months after obturation. In four groups, 6 roots each at 1 month and 6 months postoperatively (48 roots in total) were used.

2. Histologic assessments

After the imaging at 1 month and 6 months, the dogs were euthanized with an overdose of sodium pentobarbital (Somnopentyl, Kyoritsu, Tokyo, Japan) under general anesthesia. After perfusion fixation in 10% neutral phosphate-buffered formalin solution, tissue blocks including each tooth were removed, demineralized in 10% formic acid, and paraffin-embedded. Serial sections were prepared parallel to the tooth axis, subjected to hematoxylin and eosin (HE) staining, and observed under a light microscope. To evaluate the degree of inflammatory response, the area of inflammatory cell infiltration with bone resorption around the root apex (inflammatory cell infiltration area) was measured using image analysis software (Image J 1.14, National Institutes of Health, Bethesda, MD, USA).

3. Statistical analysis

The means and standard deviations of inflammatory cell infiltration area were calculated for each of the four groups. Differences among the groups were analyzed using two-tailed one-way ANOVA with Scheffé's post hoc test. Statistical analysis was performed using the SPSS software package (version 11.0; IBM Corporation, Armonk, NY, USA). p-values of <0.05 were considered statistically significant.

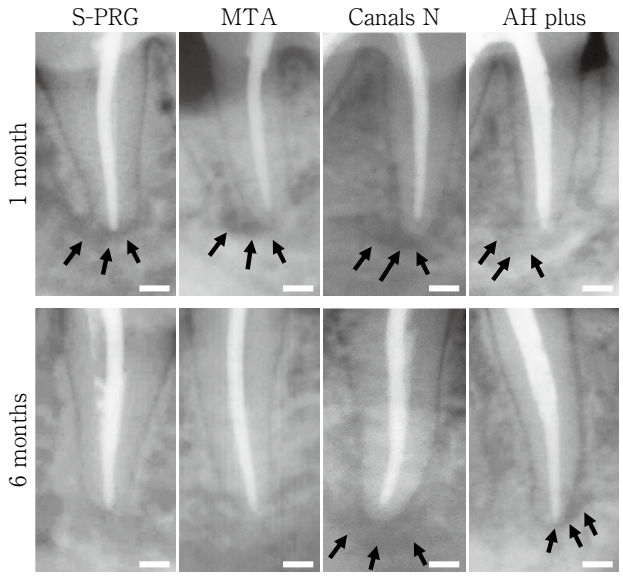


Fig. 2 Dental X-rays at 1 month and 6 months after obturation with root canal sealers

Black arrows show apical lesion. Scale bar : 1 mm. S-PRG : surface pre-reacted glass-ionomer ; MTA : mineral trioxide aggregate.

Results

1. Radiographical observation

Representative radiographical images and periapical lucency frequency at 1 month and 6 months postoperatively are shown in Figure 2 and Table 2, respectively. In all groups at 1 month, periapical lucency (X-ray transparency adjacent to the root apical foramen) was frequently observed. At 6 months, periapical lucency persisted in the Canals N group, but was observed only infrequently in the other three groups.

2. Histological observation

Representative histological images at 1 month and 6 months postoperatively are shown in Figure 3. At 1 month in all groups, infiltration of inflammatory cells that were strongly stained with hematoxylin (i.e., lymphocytes and mononuclear cells) was observed in the periodontal tissues adjacent to the root apical foramen. At 6 months, the S-PRG, MTA, and AH Plus groups showed lower levels of inflammatory cell infiltration. In contrast, the Canals N group continued to show extensive inflammatory cell infiltration at the later time point.

3. Measurement of inflammatory cell infiltration area

To evaluate tissue response at periapical tissues, the area of inflammatory cell infiltration was measured at 1 month and 6 months postoperatively. The measuring area of a representative sample in each group is shown in Figure 3, and the results are shown in Figure 4.

Inflammatory cell infiltration areas in the S-PRG, MTA, Canals N, and AH Plus groups were 1.59 ± 1.33 mm^2, 1.39 ± 0.89 mm^2, 1.84 ± 0.87 mm^2 and 1.16 ± 0.97 mm^2 at 1 month, and 1.17 ± 0.75 mm^2, 0.80 ± 0.42 mm^2, 2.49 ± 0.31 mm^2 and 1.08 ± 0.47 mm^2 at 6 months, respectively. No significant differences were observed among the groups at 1 month. The inflammatory cell infiltration area in the S-PRG and MTA was improved at 6 months compared to 1 month, but not significantly. Values in the AH Plus group were similar at 1 month and 6 months. However, in the Canals N group, the inflammatory cell infiltration area was increased at 6 months compared to that at 1 month. The 6-month values in the Canals N group were significantly larger ($p<0.05$) than the 6-month values in the other three groups.

Discussion

In this study, root canals of premolars in dog were filled using an S-PRG sealer after extraction of the pulp; S-PRG sealer was compared to three types of commercial root canal sealers that are used clinically. At 1 month, apparent inflammatory cell infiltration in the periapical tissue was observed in all four groups. There was no significant difference in inflammatory cell infiltration area among the four groups at this time point. This observation may reflect irritation induced by the sealers where the sealers came in contact with periapical tissue, or the effects of root filing and root canal spread. However, at 6 months, the Canals N group exhibited a significantly larger inflammatory cell area than that seen in the other three groups. These results suggested that the S-PRG sealer, as well as Pro Root MTA and AH Plus, ameliorate inflammation in periapical tissues compared to Canals N. Similar results were obtained by X-ray observation. Several previous reports have shown the anti-inflammatory effects of strontium ions. S-PRG filler has been proven to release strontium, which may be effective in suppressing inflammation[8,9,16-18,23,24]. Therefore, we postulate that, in the pres-

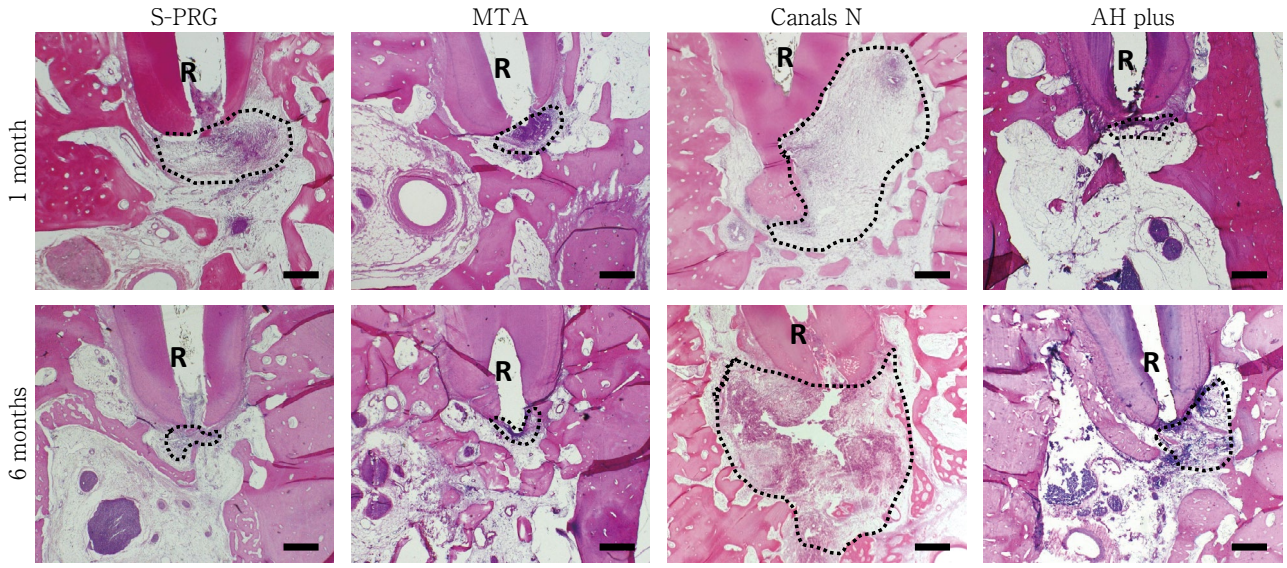


Fig. 3 Histological findings in periapical tissues at 1 month and 6 months after obturation

Dashed lines indicate the extent of inflammatory cells infiltration. Scale bar : 500 μm. Staining : hematoxylin-eosin staining.

S-PRG : surface pre-reacted glass-ionomer ; MTA : mineral trioxide aggregate ; R : root.

Table 2 Frequency of periapical lucency (lucency cases/n =6)

	S-PRG	MTA	AH Plus	Canals N
1-month	3	3	2	4
6-month	1	1	1	4

S-PRG : surface pre-reacted glass-ionomer ; MTA : mineral trioxide aggregate

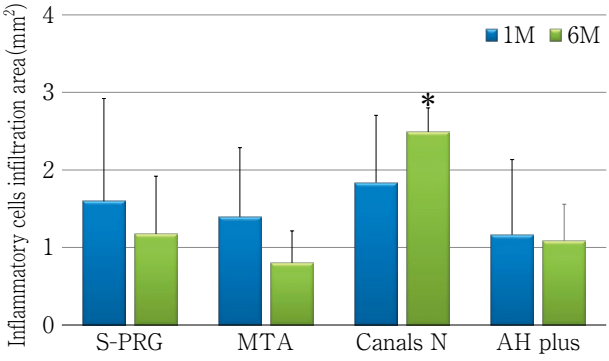


Fig. 4 Histological measurements of inflammatory cell infiltration area (mm²) at 1 month and 6 months after obturation with root canal sealers

n=6 per group, mean+standard deviation.

* : $p<0.05$ vs. other groups.

S-PRG : surface pre-reacted glass-ionomer ; MTA : mineral trioxide aggregate.

ent study, the S-PRG sealer released strontium ions at the root apex, decreasing inflammation of the periapical tissue. Previous work has shown that S-PRG fillers significantly promote cell viability of MC3T3-E1 cells (an osteoblast-like cell line) compared to silica filler[25], facilitating bone healing. On the other hand, it has been reported that the solution eluted from S-PRG filler and S-PRG sealer has antibacterial activity[8,9,26–29]. This property may maintain antibacterial effects in the root canal. The results of the present study may reflect the biosafety and antibacterial properties of the S-PRG sealer associated with the ion release effects of this filler.

MTA has been reported to have excellent hard tissue-forming abilities[29] and persistent strong alkalinity, as well as antibacterial properties and strong biocompatibility[30]. In the present study, the inflammatory cell infiltration areas in the MTA group at 6 months were less than those in the Canals N group at the same time point. We infer that MTA has superior biocompatibility compared to Canals N. The other comparator, AH Plus, is an epoxy resin-based sealer that has been reported to have high sealing properties and to reduce coronal leakage. However, a moderate inflammatory reaction has been observed in implantation when AH Plus is used

experimentally for direct filling in the jaw bone[31]. In the early stages of polymerization of AH Plus, a slight cytotoxicity (thought to result from elution of a small amount of formaldehyde) has been reported, suggesting that the irritation caused by formaldehyde in AH Plus is the source of this inflammation[32]. However, while many previous reports have shown the cytotoxicity of AH Plus, the present study observed only slight cellular infiltration at 1 month and 6 months.

Canals N is well-known as a biocompatible sealer, as it does not contain eugenol, in contrast to Grossman's sealer. In the present study, teeth filled with Canals N had significantly larger inflammatory cell infiltration areas (compared to the other three sealers), even at 6 months after obturation. Previously, a histological study of Canals N implanted subcutaneously in rat tissue detected an accumulation of inflammatory cells around the Canals N, even after 35 days[33]. It has also been reported that disintegrated Canals N in the body stimulates the expression of interleukin (IL)-1β[34], a cytokine that contributes to bone resorption activity[35]. Thus, Canals N may have an adverse effect on apical healing even at 6 months after use.

Conclusion

The inflammatory response to S-PRG sealer was compared, both radiographically and histologically, with those of Pro Root MTA, Canals N, and AH Plus after the root obturation of premolars in beagle dogs. The results showed that X-ray transparency adjacent to the root apical foramen (periapical lucency) was observed at 1 month in all groups. At 6 months, periapical lucency persisted with Canals N, but was observed only infrequently in the other three groups. In addition, when assessed at 6 months, teeth filled with the S-PRG sealer, as well as those filled with Pro Root MTA and AH Plus, exhibited significantly decreased inflammatory cell infiltration and bone resorption compared to those filled with Canals N. These results suggested that the S-PRG sealer may provide good biocompatibility, comparable to those of the commonly and clinically used Pro Root MTA and AH Plus, in healing of the periapical region.

Conflict of Interest

The authors confirm that there is no conflict of interest related to this article.

Ethics Approval

Animal experiments were conducted in accordance with the regulations on animal experiments of Hokkaido University, with the approval of the Animal Committee of Hokkaido University (Approval No. 13-122). Experiments on animals were carried out in accordance with relevant guidelines and regulations.

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Original Article

ODEP 2 (1) : 40～48, 2022

Maillard Reaction Product of Rare Sugar Allulose Decreases Bacteria-derived and Chemically-prepared Hydrogen Sulfide

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Abstract

Purpose: Oral malodor, which concerns many people, is associated with volatile sulfur compounds (VSCs) produced by periodontopathic bacteria such as *Porphyromonas gingivalis*. Maillard products are used in the food industry to decrease hydrogen sulfide (H_2S), which disturbs the flavor and taste of food. In the present study, we planned to apply Maillard reaction products for reducing VSCs to meet the needs demand of patients who want to rapidly decrease oral malodor.

Methods: The effect of maple sugar solution (a Maillard reaction product) on VSC decrease was examined by adding it to sonicated extract of *P. gingivalis*. Next, the rare sugar allulose was used for the Maillard reaction (to generate Maillard-allulose), because allulose has no calories and does not cause dental caries. The effect of Maillard-allulose on VSC decrease was examined by adding it to *P. gingivalis*-derived and chemically-prepared H_2S. After 1 min, headspace air was collected and the concentration of H_2S was measured using the Oral Chroma portable gas chromatography system. Separately, headspace air from a mixture of sodium mono-hydrogen sulfide and Maillard-allulose was analyzed by a solid phase micro extraction method with gas chromatography-mass spectrometry.

Results: Both maple sugar and Maillard-allulose decreased bacteria-derived and chemically-prepared H_2S. New material seemed to be generated after mixing sodium mono-hydrogen sulfide and Maillard-allulose, indicating that H_2S may have bound to a component of Maillard-allulose, resulting in loss of volatility of H_2S.

Conclusion: Both maple sugar and Maillard-allulose had H_2S-decreasing activity. Maillard-allulose is not associated with dental caries and obesity, so it may be a good candidate for use in foods for decreasing oral malodor.

Key words: oral malodor, Maillard reaction, rare sugar, allulose

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Introduction

Many people worry about oral malodor[1,2], and the number of such patients seems to be increasing because of the wearing of masks to prevent infection[3]. Oral malodor is associated with volatile sulfur compounds (VSCs) produced by periodontopathic bacteria such as *Porphyromonas gingivalis* and *Tannerella forsythia*[4]. These bacteria, which are included among the red complex bacteria[5], have high proteinase activity and degrade proteins in the oral cavity, resulting in VSC production[6].

To decrease oral malodor, maintenance of oral health by proper mouth cleaning is important. Mechanical plaque control, such as by using a toothbrush, interdental brush, dental floss, and tongue scraper, is the first line of prevention. Chemical plaque control, such as mouth rinse and dentifrice, used in addition to mechanical oral hygiene procedures, is helpful in decreasing oral infectious diseases. Many studies have supported significant plaque decrease by use of chemical plaque control measures[7]. However, the side effects and safety of these measures are often of concern. For example, chlorhexidine, the bactericidal agent that has been most studied and is recognized as the most effective for inhibiting plaque and preventing gingivitis, periodontitis, and oral malodor, has several adverse effects, including extrinsic tooth staining, calculus build-up, transient taste disturbance, and effects on the oral mucosa[8].

Thus, we have been examining safer methods to decrease oral malodor, one of which is the use of probiotics. Probiotics have traditionally been used to treat diseases related to the gastrointestinal tract. However, recently, many studies have investigated the effects of probiotic bacteria on oral health[9]. Several clinical trials have reported that regular consumption of *Lactobacillus salivarius* WB21 decreases periodontitis and oral malodor[10–13]. The use of oil drops containing *L. salivarius* WB21 resulted in an improvement in periodontal condition and a decrease in oral malodor in a randomized clinical trial[12]. A 14-day, double-blind, placebo-controlled, randomized crossover trial of tablets containing *L. salivarius* WB21 in patients with oral malodor resulted in a significant decrease in the concentration of VSCs and the average probing pocket depth in the probiotic period compared with the placebo period[13].

However, there is a weakness associated with probiotic treatment: it aims to improve the oral microbiota, so it takes time to obtain good results and does not help patients who want to rapidly decrease oral malodor. Thus, we added green tea catechins to the probiotic tablet[14]. The probiotic tablet containing green tea catechins was effective in controlling cariogenic and periodontopathic bacteria. It also lowered the concentration of methyl mercaptan, a VSC. However, although the VSC-decreasing effect was statistically significant, the change was not so drastic and patients may not notice the effect. Therefore, we tried another strategy of decreasing the amount of hydrogen sulfide (H_2S), which is another major VSC in oral malodor.

The Maillard reaction is an amino-carbonyl reaction. It is a non-enzymatic browning reaction, which plays an essential role in food processing to improve the appearance and functional properties of food[15]. The Maillard reaction product is also known to decrease H_2S produced in the food industry; H_2S disturbs the taste and flavor of food[15]. We considered using a Maillard reaction product, maple sugar, to decrease H_2S in oral malodor. However, maple sugar may cause dental caries, so instead we considered using a rare sugar to control oral malodor."Rare sugar" is a comprehensive term for monosaccharides and sugar alcohols that are rare in nature. Strategies to produce rare sugars using epimerases have recently been reported by Kagawa University[16]. Rare sugars cannot be used by organisms for their metabolism; indeed, they are known to inhibit cariogenic bacteria[17]. Allulose is a rare sugar, and as an additional advantage, it is non-calorific, which is important for people who are concerned about their visceral fat or blood sugar level[18]. Allulose can be chemically produced by epimerization of D-fructose, and its price has decreased in recent years[19]. Therefore, we used allulose for the Maillard reaction to generate Maillard-allulose, aiming to decrease levels of H_2S.

Materials and Methods

1. Bacterial strain and culture conditions

P. gingivalis ATCC 33277 was maintained on CDC anaerobic blood agar (Becton Dickinson, Cockeysville, MD, USA) in an anaerobic atmosphere (80% N_2, 10% H_2, 10% CO_2), and inoculated into tryptic soy broth

(Difco Laboratories, Detroit, MI, USA) supplemented with hemin (5 μg/m*l*, FUJIFILM Wako Pure Chemical Co., Osaka, Japan) and menadione (1 μg/m*l*, FUJIFILM Wako Pure Chemical Co.).

2. Preparation of sonicated extracts of *P. gingivalis* cells

Sonicated extracts (SE) of *P. gingivalis* cells were prepared as reported previously[20]. Briefly, *P. gingivalis* cells in the late logarithmic growth stage in tryptic soy broth were harvested by centrifugation and washed with phosphate-buffered saline (PBS). Five hundred micrograms of bacterial cells were suspended in 5 m*l* of PBS, and the cells were disrupted by sonication on ice. Intact cells were removed by centrifugation.

3. Measurement of *P. gingivalis*-derived VSCs

One milliliter of distilled water or maple sugar was added to 1 m*l* of *P. gingivalis* SE in a plastic tube with a soft cap, and vortexed at room temperature. After 1 min, the cap of the tube was penetrated with a needle attached to a plastic syringe, and the headspace air was collected. The level of H_2S was measured using a portable gas chromatography system, Oral Chroma CHM-2 (Nissha FIS, Osaka, Japan)[21].

4. Maillard reaction of allulose

Allulose (final conc. 0.1 M; FitLane Nutrition, Conroe, TX, USA) was mixed with various amino acids (final conc. 0.1 M; FUJIFILM Wako Pure Chemical Co.) and the pH was adjusted to 9.0 with sodium hydroxide if the pH of the solution was $<$9.0. The solution was heated in a water bath at 95℃ for 90 min[22]. Completion of the Maillard reaction was confirmed by the browning of the allulose solution. The reaction product is referred to hereafter as Maillard-allulose.

5. Decrease of *P. gingivalis*-derived H_2S by maple sugar suspension and Maillard-allulose

Maple sugar solution (0.3 g/m*l*; Maple-Farms Japan Co., Osaka, Japan) or Maillard-allulose solution was added to *P. gingivalis* SE suspension. After 1 min, headspace air was collected with a needle and the concentration of H_2S was measured using the Oral Chroma CHM-2 apparatus.

6. Treatment of chemically-prepared H_2S by Maillard-allulose

Chemically-prepared H_2S was generated by dissolving $NaHS \cdot nH_2O$ (FUJIFILM Wako Pure Chemical Co.) in distilled water. It was diluted to 10^{-5}%.

7. Heat-concentration and dialysis of Maillard-allulose

Maillard-allulose was concentrated by heating until the volume became half of the original. The concentrated Maillard-allulose solution was then dialyzed against distilled water using a Spectra/Por membrane (molecular weight cutoff 6–8,000; The Spectrum Companies, Charlotte, NC, USA), and reconcentrated to the original volume by heating.

8. Experiment to assess the possible mechanism of H_2S reduction by Maillard-allulose

Headspace air from solution containing sodium mono-hydrogen sulfide, Maillard-allulose, or a mixture of sodium mono-hydrogen sulfide and Maillard-allulose was collected and adsorbed to Stableflex Fiber Carb/PDMS 85 μm (Merck KGaA, Darmstadt, Germany) by a solid phase micro extraction (SPME) method[23]. The air component of each sample was analyzed by gas chromatography-mass spectrometry (7890A GC system, Agilent Technologies Japan, Ltd., Tokyo, Japan) connected to a VF-WAXms (Agilent CP9205) column (Agilent Technologies Japan, Ltd.).

9. Statistical analysis

To analyze the decrease of H_2S by maple sugar, Student's *t*-test was applied. To compare the decrease of H_2S by various materials and test the homogeneity of variance, the Levene test was applied. Analysis of variance was also performed. For multiple comparison, Tukey's test was applied.

Results

1. Decrease of *P. gingivalis*-derived VSCs by maple sugar

Three milliliters each of distilled water and either maple sugar suspension (final conc. 0.3 g/m*l*) or glucose suspension (final conc. 0.3 g/m*l*) were added to *P. gingivalis* SE suspension. After 1 min, the headspace air was collected and the H_2S concentration was measured using the Oral Chroma system (Figure 1). Maple sugar suspension significantly decreased the H_2S concentration in the *P. gingivalis* SE suspension tube ($p<0.05$).

Maple sugar decreased the concentration of H_2S from just after addition of the maple sugar suspension. The H_2S concentration then increased slightly over 40 min. The amount of H_2S in the control started to decline after 30 min (Figure 2).

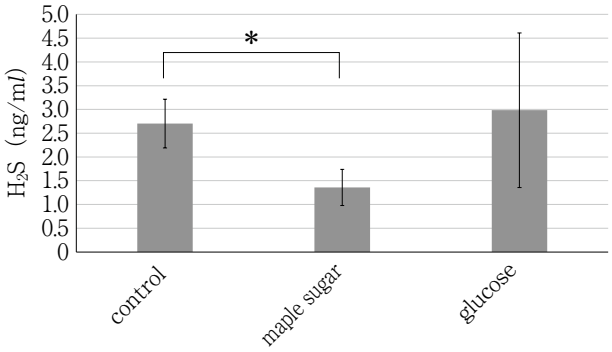

Fig. 1 Effect of maple sugar solution on H_2S concentration

An equal amount of maple sugar solution or glucose solution (0.3 g/m*l*) was added to *Porphyromonas gingivalis* sonicated extract suspension. After 1 min, headspace air was collected and the H_2S concentration was measured using the Oral Chroma system. Maple sugar suspension significantly decreased the H_2S concentration ($p<0.05$). Data show the means and standard deviations for three samples.

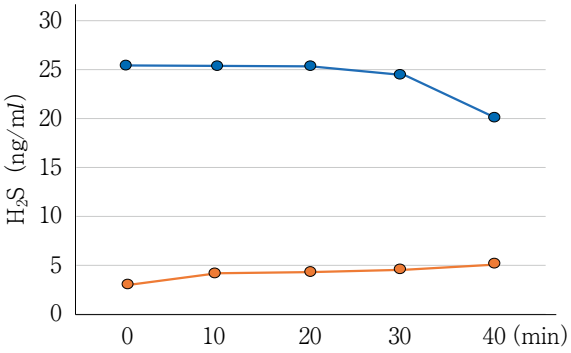

Fig. 2 Time course of H_2S concentration

An equal amount of maple sugar solution (0.3 g/m*l*) or distilled water was added to *P. gingivalis* sonicated extract suspension. At each time point, headspace air was collected and the H_2S concentration was measured using the Oral Chroma system. The H_2S concentration decreased just after addition of maple sugar suspension. The control H_2S concentration (blue line) began to decrease after 30 min (no statistical analysis). The H_2S concentration with maple sugar solution (orange line) was low but increased slightly over 40 min. The results shown are representative data for four similar sets of experimental data.

2. Maillard reaction of allulose

An illustration of Maillard-allulose is shown in Figure 3. A mixture of allulose and L-arginine was almost colorless. It became brown after heating at 95℃ for 90 min, and the Maillard reaction was considered to be completed.

Fig. 3 Color change after Maillard reaction

Allulose (final conc. 0.1 M) was mixed with L-arginine (final conc. 0.1 M) and the pH was adjusted to 9.0 with sodium hydroxide. The solution was heated in a water bath at 95℃ for 90 min. The mixture of allulose and L-arginine was initially colorless (right), but after heating, it became brown (left), which indicates the completion of the Maillard reaction. The results shown are representative data for six similar sets of experimental data.

3. Combination with various amino acids

Various amino acids were mixed with allulose for the Maillard reaction. Among the 11 tested amino acids, the Maillard reaction product obtained using arginine had the greatest effect in decreasing the concentration of H_2S, and the amount of H_2S became 0 in this experimental condition. L-serine, L-alanine, and L-cysteine had no H_2S-decreasing effect (Figure 4).

4. Confirmation of necessity of Maillard reaction for H_2S reduction

Maillard-allulose produced with L-arginine showed a strong decreasing effect on H_2S, but an unheated mixture of allulose and L-arginine had no effect ($p<0.01$), indicating that the heating process (i.e., the Maillard reaction) is necessary for H_2S decrease (Figure 5).

5. Effect of heat-concentration and dialysis on the effect of Maillard-allulose

Maillard-allulose was concentrated by heating. The concentrated Maillard-allulose showed a strong decreasing effect on the level of H_2S, indicating that the reagent was heat-stable. Even after dialysis against distilled water, it retained a strong effect (Figure 6).

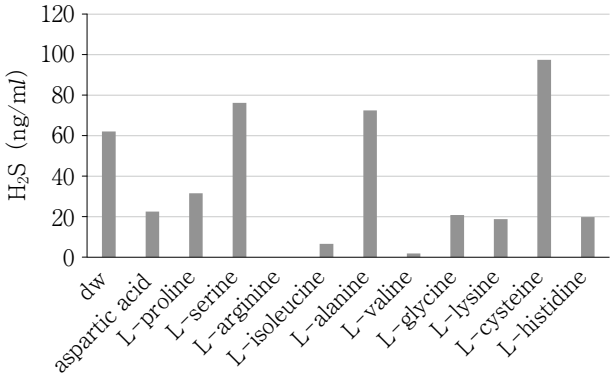

Fig. 4 Effect of various amino acids on reduction of H_2S

Allulose (final conc. 0.1 M) was mixed with various amino acids (final conc. 0.1 M) and the pH was adjusted to 9.0 with sodium hydroxide if the pH of the solution was <9.0. The resulting solution was heated in a water bath at 95℃ for 90 min. Among the 11 amino acids tested, the product with L-arginine showed the strongest reducing effect on H_2S (no statistical analysis). The results shown are representative data for three similar sets of experimental data.

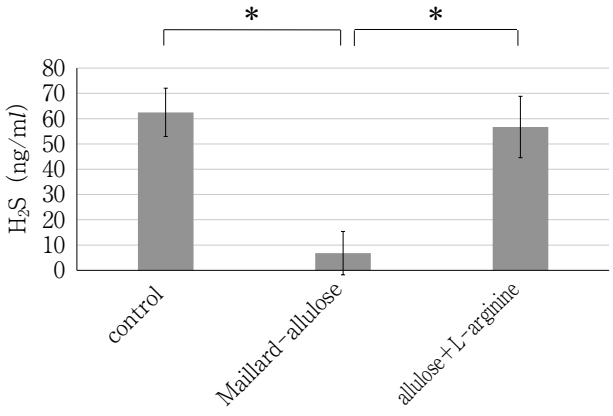

Fig. 5 Effect of Maillard reaction on reduction of H_2S

Allulose (final conc. 0.1 M) was mixed with L-arginine (final conc. 0.1 M) and the pH was adjusted to 9.0 with sodium hydroxide. The solution was heated in a water bath at 95℃ for 90 min. Control solution was not heated. Maillard-allulose significantly decreased the H_2S concentration, but the unheated mixture of allulose and L-arginine had no effect, indicating that Maillard-allulose is necessary for the H_2S reduction ($p<0.01$). The data show the means and standard deviations for eight samples.

6. SPME-gas chromatography analysis

The results of SPME-gas chromatography-mass spectrometry analysis are shown in Figure 7. The peak patterns of H_2S alone and Maillard-allulose were different. Then, H_2S and Maillard-allulose were mixed and the headspace air was subjected to gas chromatography. The peak pattern of this mixture mostly looked like a mixture of the two individual peak patterns. However, a new big peak, which was above the scale limit, was generated at a retention time of around 21.6 min. An analysis of the retention time and a database search by Kumamoto Industrial Research Institute indicated that there was a 97% probability that the new peak was caffeine.

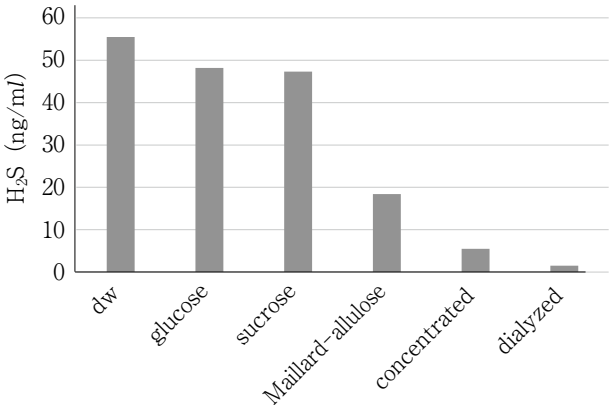

Fig. 6 Effect of heat-concentration and dialysis of Maillard-allulose on decrease of H_2S

Maillard-allulose was concentrated by heating until the volume became half of the original. The concentrated Maillard-allulose was then dialyzed against distilled water, and reconcentrated to the original volume by heating. Heated Maillard-allulose decreased the concentration of H_2S, indicating that the effect was heat-stable. Dialysis had no effect on the activity, indicating that large molecules in the Maillard-allulose reaction mixture are responsible for H_2S reduction (no statistical analysis). The results shown are representative data for four similar sets of experimental data.

Discussion

The Maillard reaction is necessary in the food industry because it can decrease H_2S, which worsens the flavor of various foods. This fact prompted us to think of using a Maillard reaction product to decrease oral malodor. Maple sugar, which is a Maillard reaction product, showed a strong decreasing effect on H_2S, as we had expected. The effect was observed just after addition to sonicated bacterial extract. However, continuously taking maple sugar to decrease oral malodor could lead to

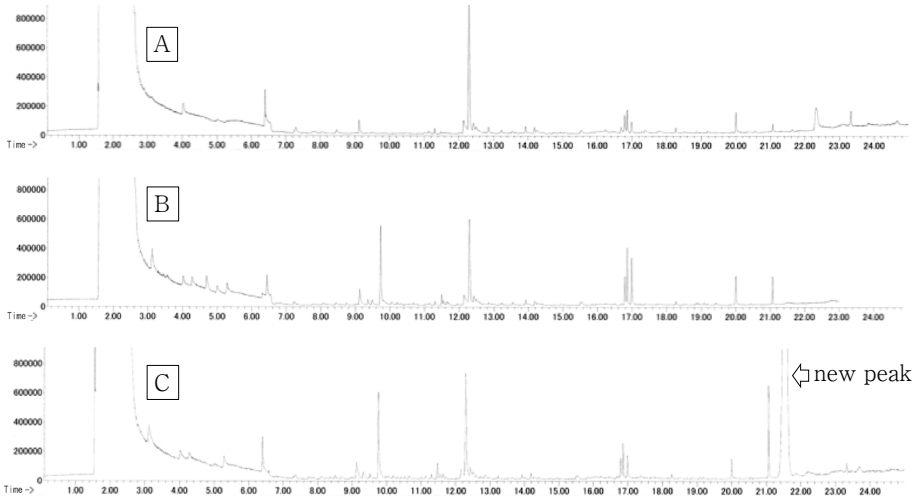

Fig. 7 Solid phase micro extraction-gas chromatography-mass spectrometry analysis

Headspace air from solution containing sodium mono-hydrogen sulfide, Maillard-allulose, or a mixture of sodium mono-hydrogen sulfide and Maillard-allulose was collected and adsorbed to Stableflex Fiber Carb/PDMS 85 μm by a solid phase micro extraction method. The air component of each sample was analyzed by gas chromatography-mass spectrometry using a VF-WAXms column. A : Peak pattern of headspace air of solution of sodium mono-hydrogen sulfide alone. B : Peak pattern of headspace air of solution of Maillard-allulose alone. C : Peak pattern of headspace air of mixture of sodium mono-hydrogen sulfide and Maillard-allulose. The arrow indicates the new peak (above scale limit) that was generated on mixing sodium mono-hydrogen sulfide and Maillard-allulose.

dental caries and obesity, so it is not appropriate to use maple sugar for oral malodor control.

Next, we considered using a rare sugar instead of maple sugar to control oral malodor. Rare sugars are non-calorific[18] and do not cause dental caries[17]. Therefore, we used allulose for the Maillard reaction, to generate Maillard-allulose to decrease levels of H_2S.

The Maillard reaction is completed by mixing sugars and amino acids under alkaline conditions, followed by heating. The proportion of sugar and amino acid affects the viscosity of the reaction product, so we used conditions that did not become too sticky. Various kinds of amino acids may be used for the Maillard reaction, so we first determined the most suitable combination for decreasing H_2S. We tested inexpensive amino acids with a view to applying our approach clinically in the future. Among the 11 amino acids used in this experiment, the Maillard reaction product of L-arginine was found to be the most effective in decreasing the level of H_2S, so we used L-arginine for further experiments. The Maillard reaction was shown to be necessary, because simply mixing allulose and L-arginine without heating did not decrease H_2S. The H_2S concentration of the *P. gingivalis* SE-maple sugar mixture was low, but it slightly increased over 40 min. The reason is not clear yet, but the binding between H_2S and maple sugar may have been reversible; further study is required.

To clarify the mechanism of H_2S reduction, we performed SPME-gas chromatography-mass spectrometry experiments[23]. Headspace air from samples containing H_2S alone and Maillard-allulose alone showed different peak patterns. The peak pattern of the mixture of H_2S and Maillard-allulose mostly looked like a mixture of those two peak patterns, but an intense new big peak was generated when H_2S and Maillard-allulose were mixed. Analysis of the retention time and a database search by Kumamoto Industrial Research Institute indicated that there was a 97% probability that the new peak was caffeine. These results indicate that some molecule in Maillard-allulose combined with H_2S to form a new product. By combining with this molecule, H_2S is considered to have lost its volatility. To confirm this

hypothesis, identification of the putative caffeine-like reaction product is under way.

This work has some limitations. Even in the controls, the level of H_2S began to decrease during the assay, due to evaporation. The Oral Chroma measurement took >8 min for each assay. Thus, we could not repeat assays many times and could not present standard deviations in some experiments. To overcome this problem, we repeated each experiment under the same conditions and confirmed reproducibility. We need to find new assay methods that allow quicker measurement.

Oral malodor sometimes affects patients' quality of life and disturbs communication with others[24,25]. Some patients use antibiotics and disinfectants to decrease oral malodor[26], and this can be effective for a time. Periodontitis is caused by infection with periodontal pathogens, but periodontal tissue damage is thought to be caused by various factors[27]. Continuous use of disinfectants may give rise to side effects such as bacterial resistance and microbial substitution[28]. Thus, we are focusing on non-disinfectant control of oral malodor. In our previous experiments, the use of probiotics was found to improve the oral microbiota and decrease oral malodor, because oral malodor is strongly associated with the oral microbiota[29,30]. Gut microbiota are also related to oral conditions, including oral malodor[31,32]. However, improvement of the oral microbiota by using probiotics is slow. Zinc ions are also known to effectively decrease oral malodor[33], but care is required to avoid excessive intake.

Maillard-allulose has a good taste and little effect on obesity[34] or caries formation. If the new method developed here using Maillard-allulose to decrease levels of H_2S is successful in clinical application, oral malodor could be decreased instantly. We are also considering combining Maillard-allulose and probiotics, which would contribute to both improvement of the oral microbiota and rapid decrease of oral malodor.

Conclusions

Maple sugar solution decreased *P. gingivalis*-derived H_2S. The Maillard reaction product with rare sugar allulose decreased *P. gingivalis*-derived H_2S and chemically-prepared H_2S. Among various amino acids, the Maillard reaction product with L-arginine was most effective in decreasing the concentration of H_2S. The Maillard reaction was required to achieve this effect because an unheated mixture of allulose and L-arginine did not decrease H_2S. New material may have been generated after mixing sodium mono-hydrogen sulfide and Maillard-allulose, indicating that some molecule in Maillard-allulose combined with H_2S to form a new product, which reduced the volatility of H_2S. From these results, Maillard-allulose may be a good candidate for use in foods for decreasing oral malodor.

Conflict of Interest

The authors declare that there is no conflict of interest in this study.

Ethical Statement

Not applicable.

Acknowledgments

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Original Article

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Study on Sealability of Dentinal Tubules Irradiated by Nd: YAG Laser with TiO_2 Using a Model of Hypersensitive Dentin

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Abstract

Purpose: In recent years, there have been reports on the application of Nd: YAG laser (the laser) combined with titanium dioxide (TiO_2), a photoreactive agent, to dental treatment, but there have been no reports on its use for dentin hypersensitivity for the purpose of high reactive level laser therapy. In this study, to investigate the transmittance of the laser combined with TiO_2 through dentin, the transmitted energy of the laser was measured. Furthermore, to investigate the sealing ability of the laser on dentinal tubules, Nd: YAG laser combined with TiO_2 was irradiated to a model dentin affected with hypersensitivity, and the dentin permeability inhibition ratio (permeability inhibition ratio) was measured.

Methods: STREAK-1 (ALTECH) was used as the laser system. Human extracted teeth were used as test teeth, and dentin disc specimens of 1.0 mm in thickness were prepared.

Experiment 1: Examination of laser penetration through dentin

The irradiation conditions were 50 μs-50 mJ-10 pps, 100 μs-100 mJ-5 pps, and 200 μs-300 mJ-5 pps, and the irradiation time 5 seconds. The laser was irradiated through one to five dentin discs, and the transmittance was calculated. In the same way, the laser was irradiated together with TiO_2 solution, and the transmittance was calculated for a single dentin disc.

Experiment 2: Examination of dentinal tubule sealing ability of laser combined with TiO_2 solution

The irradiation conditions were 50 μs-50 mJ-50 pps, 100 μs-100 mJ-20 pps, 100 μs-100 mJ-99 pps, and 200 μs-300 mJ-20 pps, and the irradiation time was set to 10 s, 20 s, and 30 s. The specimens were connected to the measuring device and laser was irradiated with TiO_2 solution. The permeability inhibition ratio was measured, and the dentin disc surface was observed by SEM.

Results: Laser transmittance into dentin significantly decreased with increasing dentin thickness under all the conditions. In addition, the transmittance of the laser significantly decreased under all the conditions when the TiO_2 solution was combined.

Regarding the sealing ability of the laser combined with TiO_2 solution on dentinal tubules, the permeability inhibition ratio increased significantly with increasing irradiation time at 50 μs-50 mJ-50 pps and 100 μs-100 mJ-20 pps. No significant difference was observed in the permeability inhibition ratio at 100 μs-100 mJ-99 pps and 200 μs-300 mJ-20 pps. SEM observation of the surface of the specimens showed that the dentinal tubules were sealed by cohesive structures under all the conditions.

Conclusion: The investigation indicates that Irradiation with Nd: YAG laser combined with TiO_2, a photoreactive agent, attenuates the penetration of the laser into dentin and it is effective for sealing dentinal tubules.

Key words: Nd: YAG laser, TiO_2, dentin hypersensitivity

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Introduction

In various branches of dentistry, many fundamental studies and clinical reports on various types of lasers have been conducted, and lasers are now used in various treatments such as conservative restorative procedures, endodontic procedures, periodontal procedures, and surgical procedures[1-7]. It has been reported that Nd: YAG laser is effective for removing enamel and dentin[8-10], imparting acid resistance to dentin[11], and sterilizing gingiva and periodontal pockets[11,12] by using titanium dioxide (TiO_2), a type of semiconductor, as a photoreactive agent during irradiation.

Recent years have seen an increase in the number of surviving permanent teeth per person in Japan owing to dental care based on minimal intervention and the promotion of oral health care[13]. At the same time, however, increasing numbers of patients are suffering from dentin hypersensitivity, which is characterized by transient pain due to cold or rubbing pain[14].

The primary theory behind the onset of pain due to dentin hypersensitivity is the hydrodynamic theory, which posits that pain is caused by stimulation of free nerve endings in the pulp by the movement of fluid in the dentinal tubules caused by external stimuli transmitted through the dentinal tubules opening on the surface of the dentin[15]. Treatments for dentin hypersensitivity include conservative therapies such as application of medicament, iontophoresis, laser treatment, and coverrage with adhesive materials. However, the cure rate of conservative therapy is reported to be about 60–70%[16], and symptoms may recur. Laser treatment is a less invasive method that involves the use of photochemical and photothermal effects in biological interactions[17,18]. Pain relief and accelerated healing can be expected with low reactive level laser therapy (LLLT) for alleviating hypersensitivity and high reactive level laser therapy (HLLT) for sealing dentinal tubules by protein denaturation and coagulation using a surface-absorbing laser[19-24].

In this study, the transmitted energy of Nd: YAG laser was measured to investigate the effects of dentin thickness and TiO_2, a photoreactive agent, on the transmittance of Nd: YAG laser through dentin. Moreover, with the aim of sealing the dentinal tubules on the dentin surface, Nd: YAG laser irradiation combined with TiO_2 was performed on a hypersensitivity-affected dentin model, and the dentin permeability inhibition ratio (permeability inhibition ratio) was measured.

Materials and Methods

STREAK-1 (400 μm diameter quartz fiber, ALTECH Co., Ltd., Yamanashi, Japan, Fig. 1) was used as the Nd: YAG laser (the laser) unit. 10% TiO_2 solution was prepared by adjusting 40% TiO_2 solution (Avant tooth liquid XBS, Miyako Chemical Co., Ltd., Kyoto, Japan) with distilled water. The density of TiO_2 solution was set based on the preliminary experiment and the method of Waga, etc.[25,26] TiO_2 solution was dripped at the rate of about 0.3 m*l*/s at the time of laser irradiation.

1. Dentin disc specimens

After extraction for therapeutic reasons at the Department of Oral and Maxillofacial Surgery of our university hospital, caries-free healthy human molars frozen at −40℃ in saline solution were thawed immediately before the experiment and used as test teeth.

Flat dentin surfaces were prepared by planing from the occlusal surface with a model trimmer. The root was then cut near the cervical area and the pulp was removed. We polished it up to #600 with waterproof abrasive paper, and prepared a cylinder-shaped dentin disc with an exposed dentin surface of 8.0 mm in diameter and 1.0 mm in thickness. A 10% phosphoric acid solution (Kishida Chemical Co., Ltd., Osaka, Japan) was applied to the occlusal side of the dentin disc for 30 seconds, and the disc was rinsed under running water for 5 seconds. Then, a 5% sodium hypochlorite solution (Kishida Chemical Co., Ltd.) was applied to the pulp side for 10 seconds, followed by a 5-second rinse under running water and a 5-minute ultrasonic rinse with distilled water to open the dentinal tubules for use as dentin disc specimens.

This study was approved by the Ethics Committee of Osaka Dental University (Approval No.: Osaka Dental University Ethics Committee No. 110767).

2. Experiment 1: Review of laser penetration through dentin

Table 1 shows the laser irradiation conditions. The irradiation conditions were set based on the preliminary experiment. A laser power meter (FieldMax II, Coherent Inc., California, USA) was used to measure the

Fig. 1 Nd: YAG laser unit used in this study

irradiation energy.

1) Effect of dentin thickness

Laser was irradiated from a distance of 1.0 cm from the measured surface, and the energy was measured. Measurements without the dentin disc were used as the control. Next, dentin specimens of 1.0 to 5.0 mm were prepared and placed on the measured surface using dentin discs, and the laser energy after transmission through dentin specimens of different thicknesses was measured. Based on the dentin thickness, the specimens were classified into groups DT0, DT1, DT2, DT3, DT4, and DT5, respectively. From the obtained values, the transmittance of the laser was calculated according to the thickness of the dentin specimen.

2) Effects of TiO_2 solution

The thickness of the dentin specimen was set to 1.0 mm. Dentin specimens were placed on the measured surface and laser was irradiated from a distance of 1.0 cm from the surface. Then, laser energy transmitted through the dentin specimens with and without TiO_2 solution was measured. The group without solution was designated as the E (−) group, and the group with solution was designated as the E (+) group. The value without solution was designated as the control, and the transmittance of the laser was calculated.

Five measurement points were used. The calculated transmittance was statistically processed by a one-way analysis of variance and Tukey's test ($p<0.05$).

3. Experiment 2: Review of dentinal tubule sealing abilities

Table 2 shows the laser irradiation conditions. The irradiation conditions were set based on the preliminary experiment.

As the experimental system, a model dentin affected with hypersensitivity was prepared based on the method of Zennyu, etc.[27] (Fig. 2). A rubber ring with an inner diameter of 6 mm was placed on the pulp side of the dentin disc specimen and sandwiched between the upper and lower stainless-steel holders to be used as the specimen stage. The center of the specimen stage was opened into a square with a diameter of 5 mm (approximately 0.25 cm^2 in area) to expose the surface to be irradiated by the laser. Dentinal tubular fluid (DF) was prepared by diluting parent bovine serum (Invitrogen) fourfold with distilled water to reproduce the protein content of DF in a clinical setting[28-30]. After filling the specimen stage with DF to prevent air from entering, the DF was connected to a device that can specify the internal pressure, made according to the method reported by Pashley et al.[31]. The internal pressure of the specimen stage was adjusted to 25 mmHg, which is the same as the internal pressure in human dental pulp, to reproduce pulp pressure in a clinical setting[32-35]. DF from the direction of the syringe was shut off with the three-way cock B, and the glass capillary tube connected to the specimen stage and the experimental device was connected. In this state, air was blown onto the exposed enamel surface of the central opening of the specimen stage for 60 seconds and allowed to stand for another 30 seconds, after which the DF movement in the glass capillary tube was measured[36-38]. The air pressure was set to 0.3 MPa. Next, the three-way cock B was adjusted to shut off the DF flow to the glass capillary tube, and DF was adjusted so that DF could flow only from the syringe to the specimen stage. Next, the three-way cock A was adjusted to inject DF from the syringe to the pressure gauge and specimen stage, and the internal pressure was adjusted to 25 mmHg. Laser was irradiated together with TiO_2 solution. As before laser irradiation, the specimen was air-blown for 60 seconds and allowed to stand for another 30 seconds, then the amount of DF movement was re-measured. The respective permeability inhibition ratios were measured from the amount of DF movement before and after laser irradiation. Using the amount of DF movement before treatment (x) and the amount of DF movement after treatment (y), the ratios were calculated as follows:

Table 1 Laser irradiation conditions in Experiment 1

	code	Irradiation output			Iradiation time (s)
		Pulse duration (μs)	Pulse energy (mJ)	Pulse rate (pps)	
Condition 1	50-10	50	50	10	5
Condition 2	100-5	100	100	5	5
Condition 3	300-5	200	300	5	5

Table 2 Laser irradiation conditions in Experiment 2

	code	Irradiation output			Fluence (J/cm^2)		
		Pulse duration (μs)	Pulse energy (mJ)	Pulse rate (pps)	Iradiation time (s)		
					10	20	30
Condition 1	50-50	50	50	50	100	200	300
Condition 2	100-20	100	100	20	80	160	240
Condition 3	100-99	100	100	99	396	792	1,188
Condition 4	300-20	200	300	20	240	480	720

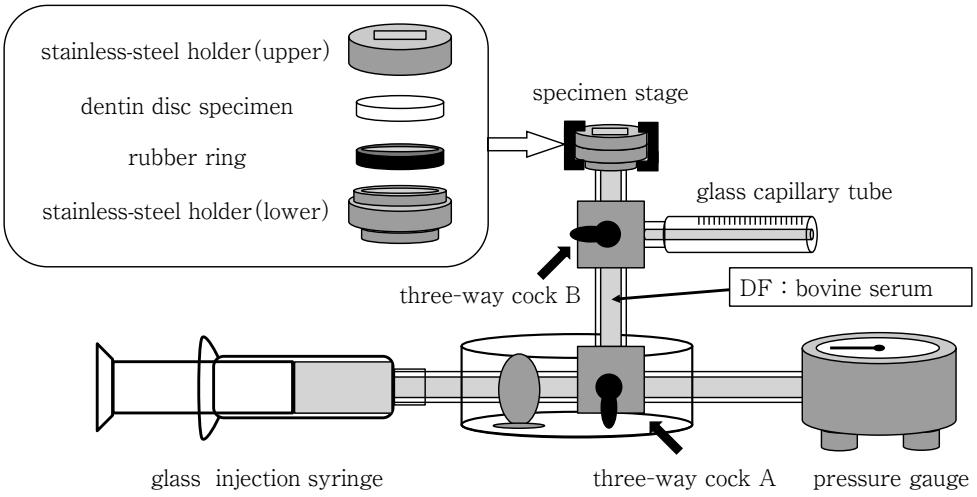

Fig. 2 Schematic diagram of a dentin hypersensitivity model
A rubber ring with an inner diameter of 6 mm was placed on the pulp side of the dentin disc specimen, inserted between the upper and lower stainless-steel holders, and used as a specimen stage. The pressure in the specimen stage was adjusted to 25 mmHg, equal to human dental pulp, to reproduce the pressure in clinical practice.

$$\text{Permeability inhibition ratio } (\%) = \{(x-y)/x\} \times 100 \quad \cdots (1)$$

The number of specimens was 5. The calculated permeability inhibition ratios were statistically processed by one-way analysis of variance and Tukey's test ($p < 0.05$) for the difference in irradiation time at each irradiation power and for the difference in irradiation power at each irradiation time.

Each specimen was deposited with gold in accordance with the conventional method and observed with a scanning electron microscope (JSM-5610LV, JEOL Ltd., Tokyo, Japan).

Results

1. Experiment 1: Review of laser penetration through dentin

Figure 3 shows the results of laser transmittance for different dentin thicknesses.

The inhibition ratio under irradiation condition 50-10

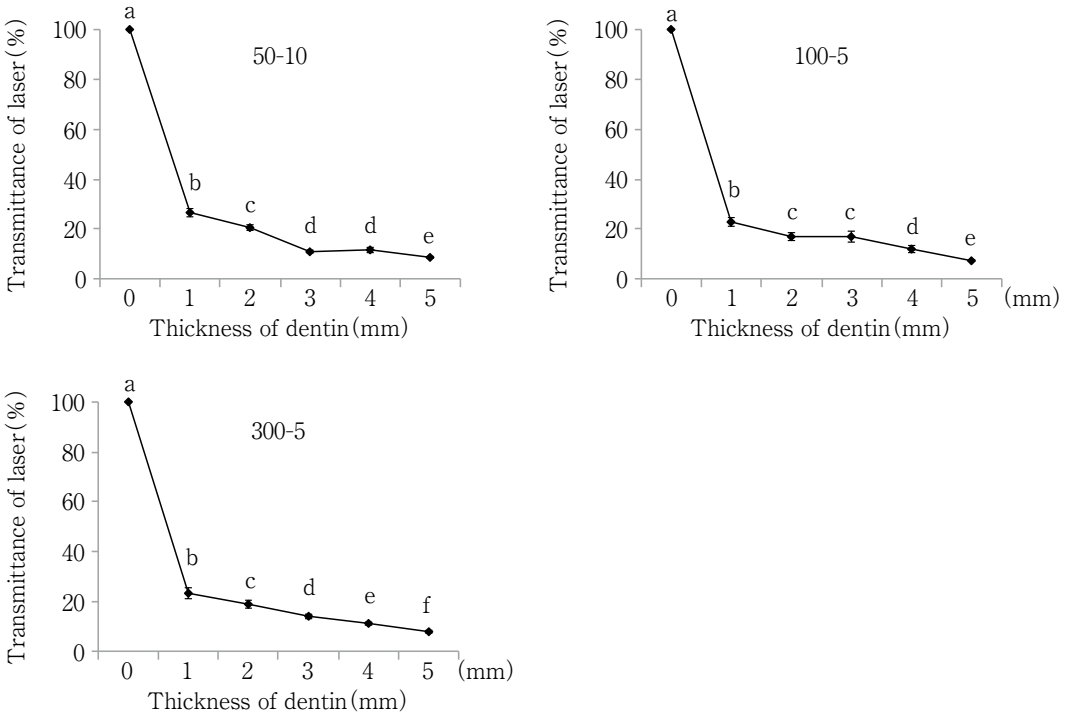

Fig. 3 Transmittance of Nd: YAG laser for different dentin thicknesses
Different letters indicate statistically significant differences at $p<0.05$.

was 26.5±1.7% for the DT1 group, 20.5±1.2% for the DT2 group, 10.9±0.6% for the DT3 group, 11.5±1.1% for the DT4 group, and 8.6±0.4% for the DT5 group. Transmittance was significantly attenuated with increasing dentin disc thickness except for the DT3 and DT4 groups.

The inhibition ratio under irradiation condition 100-5 was 22.8±1.8% for the DT1 group, 16.9±0.5% for the DT2 group, 16.9±2.3% for the DT3 group, 11.9±1.4% for the DT4 group, and 7.3±0.4% for the DT5 group. Transmittance was significantly attenuated with increasing dentin disc specimen thickness except for the DT2 and DT3 groups.

The inhibition ratio under irradiation condition 300-5 was 23.2±2.2% for the DT1 group, 18.8±1.6% for the DT2 group, 13.9±0.9% for the DT3 group, 11.1±0.7% for the DT4 group, and 7.8±0.5% for the DT5 group. In all groups, transmittance was significantly attenuated with increasing dentin disc specimen thickness.

Under all the irradiation conditions, the transmittance of the DT1 group was significantly lower than that of the DT0 group, with greater attenuation.

Figure 4 shows the results of transmittance of the laser in combination with TiO_2 solution.

Under all the irradiation conditions, the E (+) group showed a significant attenuation of the transmittance compared to the E (−) group.

2. Experiment 2: Review of dentinal tubule sealing abilities

Table 3 shows the results of measuring the permeability inhibition ratio after laser irradiation under each condition.

The permeability inhibition ratio under irradiation condition 50-50 was 24.4±3.2% in the 10 s group, 44.0 ±6.3% in the 20 s group, and 55.4±3.6% in the 30 s group. The permeability inhibition ratio of the 30 s group was significantly higher than that of the 10 s and 20 s groups, while that of the 20 s group was significantly higher than that of the 10 s group.

The permeability inhibition ratio under irradiation condition 100-20 was 26.8±10.0% in the 10 s group, 43.1±7.3% in the 20 s group, and 53.2±10.1% in the 30 s group. The permeability inhibition ratio of the 20 s and 30 s groups was significantly higher than that of the 10 s group, and no significant difference was found in the permeability inhibition ratios of the 20 s and 30 s groups.

The permeability inhibition ratio under irradiation

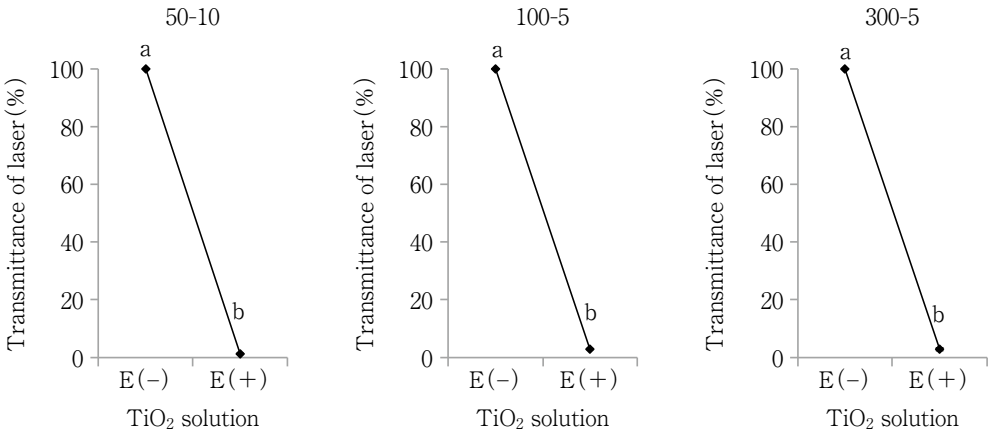

Fig. 4 Transmittance of Nd: YAG laser in combination with TiO_2 solution for the dentin
Different letters indicate statistically significant differences at $p<0.05$.

Table 3 Measurement of the dentin permeability inhibition ratio in Experiment 2

Irradiation time		Dentin permeability inhibition ratio (%) 10 s	20 s	30 s
Irradiation condition	50-50	$24.4\pm3.2^{A,a}$	$44.0\pm6.3^{B,a}$	$55.4\pm3.6^{C,a}$
	100-20	$26.8\pm10.0^{A,a}$	$43.1\pm7.3^{B,a}$	$53.2\pm10.1^{B,a}$
	100-99	$49.3\pm6.9^{A,b}$	$43.1\pm15.2^{A,a}$	$40.5\pm9.5^{A,a}$
	300-20	$27.5\pm12.7^{A,a}$	$43.2\pm10.9^{A,a}$	$41.2\pm8.4^{A,a}$

The results are shown as the mean±SD. Different lowercase letters indicate statistically significant differences at $p<0.05$ for the same irradiation time. Different uppercase letters indicate statistically significant differences at $p<0.05$ for the same irradiation conditions.

condition 100-99 was 49.3±6.9% in the 10 s group, 43.1 ±15.2% in the 20 s group, and 40.5±9.5% in the 30 s group. There was no significant difference in the permeability inhibition ratio among the 10 s, 20 s, and 30 s groups.

The permeability inhibition ratio under irradiation condition 300-20 was 27.5±12.7% in the 10 s group, 43.2±10.9% in the 20 s group, and 41.2±8.4% in the 30 s group. There was no significant difference in the permeability inhibition ratio among the 10 s, 20 s, and 30 s groups.

The permeability inhibition ratio of the 100-99 group was significantly higher than that of the 50-50, 100-20, and 300-20 groups during the 10 s irradiation time. The 50-50, 100-20, and 300-20 groups showed similar permeability inhibition ratios with no significant difference. The permeability inhibition ratios of all the groups were not significantly different and were similar at the irradiation times of 20 s and 30 s, respectively.

Figure 5 shows the results of SEM observations of the dentin disc specimen surfaces under each irradiation condition. The SEM images of each group indicated that dentinal tubules were sealed by cohesive structures. Under each irradiation condition, the longer the irradiation time, the more the dentinal tubules were sealed by the cohesive structures and the layers formed by the reactants of TiO_2 solution and laser (white arrow). In the 100-99 group (20 s and 30 s) and the 300-20 group (20 s and 30 s), the dentin surface was found to be melted in some areas (black arrow).

Discussion

Dentin hypersensitivity is treated by a method (HLLT) using a surface-absorbing laser (Er: YAG laser[21,22] or CO_2 laser[20,24]) to seal the dentinal tubules by denaturing the proteins in the tubules and to close the exposed dentinal tubule openings, thereby suppressing

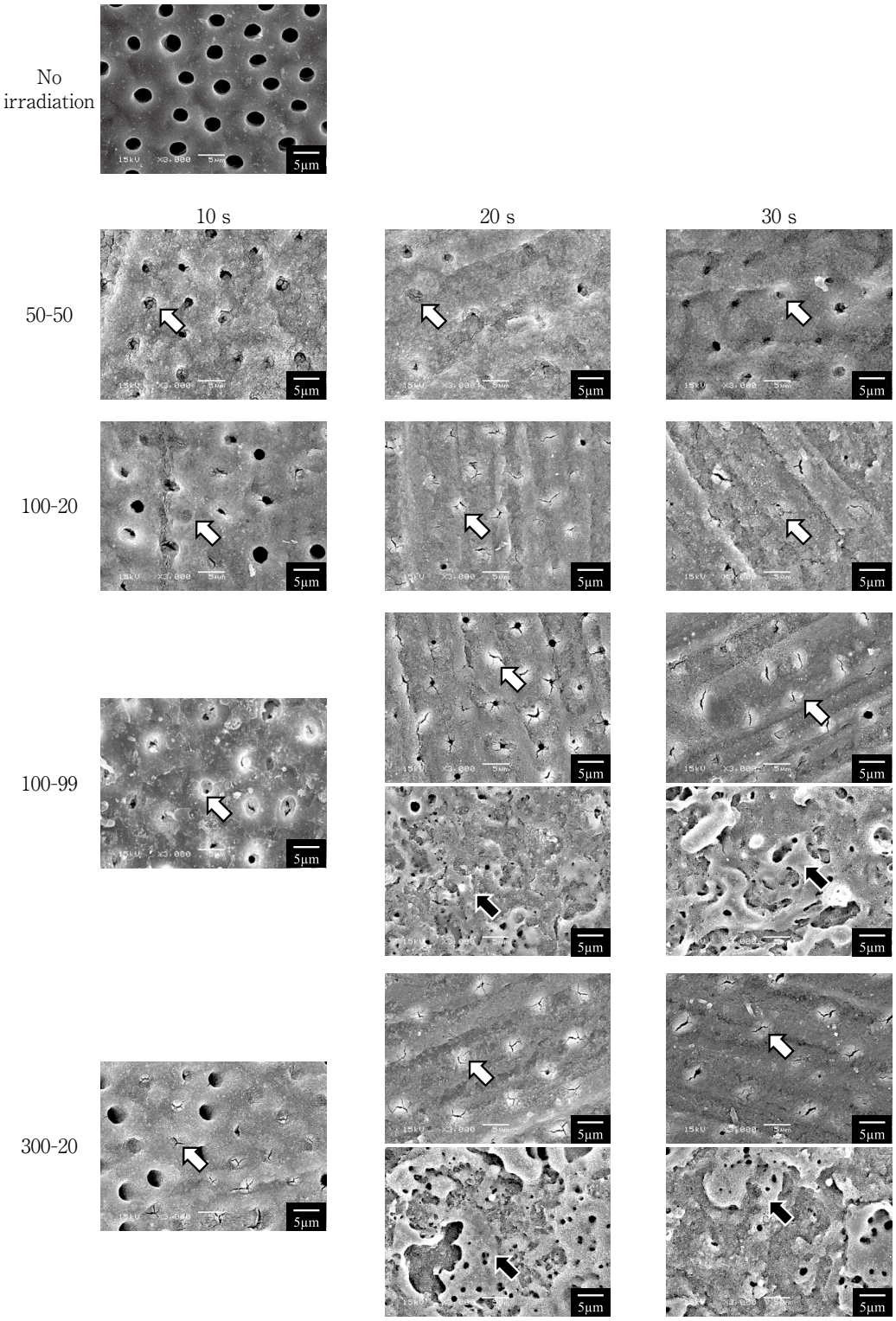

Fig. 5 SEM observations of the dentin specimen surfaces under each irradiation condition

The dentinal tubules were sealed by the cohesive structures and the layers formed by the reactants of TiO_2 solution and Nd: YAG laser (white arrow). In the 100-99 group (20 s and 30 s) and the 300-20 group (20 s and 30 s), the dentin surface was found to be melted in some areas (black arrow).

irritation to the fluid in the dentinal tubules[27,39]. In addition, the LLLT method is reported to reduce or alleviate pain by inhibiting the activity of Aδ or C fibers of peripheral nerves in the pulp using a GaAlAs semiconductor laser[23] or Nd: YAG laser[19] which are tissue penetrating lasers[40-43].

Dentin is a semi-permeable material with respect to the Nd: YAG laser light used in this experiment[44]. However, excessive laser irradiation can cause the laser light to reach the inside of the dentin, heating and possibly damaging the dental pulp[45]. In this experiment, the transmittance of Nd: YAG laser was attenuated to about 25% at a dentin disc thickness of 1.0 mm, regardless of the irradiation conditions. The transmittance of Nd: YAG laser decreased more with increasing thickness of the dentin disc. There are also reports indicating that the transmittance of the laser at a dentin thickness of 1.0 mm is about 25 to 45%[46,47]. Dentin is highly permeable to Nd: YAG laser due to the low absorption rate of hydroxyapatite, which is a component of dentin. Therefore, the irradiation energy can be attenuated by more than 80% if there is 3 mm or more of healthy dentin between the pulp and the laser. This means that even if there is 5 mm of healthy dentin, the energy cannot be completely absorbed[48].

When TiO_2 was used as a photoreactive agent during Nd: YAG laser irradiation, the transmittance of the laser was greatly attenuated to less than 5% regardless of the laser irradiation conditions. The photoreactive agent TiO_2 has anatase and rutile crystal structures. TiO_2 has two applications: it is used as a white pigment with suppressed photocatalysis, and it is also used for photocatalysis, each with different properties[49,50]. When photocatalytic activity is not required, the rutile type with low activity is used, whereas the anatase type with high activity is used when photocatalytic activity is required. TiO_2 with the rutile type crystalline structure was used in this experiment, as it is almost completely free of photocatalysis. In addition, the TiO_2 powder surface was coated with inert alumina (Al_2O_3) to suppress chemical reactivity, including photocatalysis. TiO_2 produces reactive oxygen species through photocatalysis, but the rutile type requires energy from short-wavelength light below 413 nm[50]. Thus, it is unlikely that the 1,064 nm Nd: YAG laser produces reactive oxygen species, and the possibility of photocatalytic reaction is extremely low. However, since TiO_2 absorbs laser light efficiently, the transmittance of the laser may have been attenuated due to the use of TiO_2 solution.

In this study, dentin disc specimens were prepared by forming from the occlusal surface based on the method of Zennyu etc.[27] to produce the specimen size and the open dentinal tubules. The results of the permeability inhibition ratio after laser irradiation combined with TiO_2 solution confirmed permeability inhibition under all conditions. SEM images showed that the open dentinal tubules on the dentin surface were sealed by the cohesive structures under all conditions. It has also been reported that when TiO_2 solution was irradiated with Nd: YAG laser, the temperature increased due to the heat generated with the absorption of laser light by TiO_2, rather than due to the effect of photocatalysis caused by the irradiation[25,51]. This suggests that the combination of Nd: YAG laser and TiO_2 solution caused thermal denaturation of proteins and other substances in the dentinal tubular fluid, thereby sealing the dentinal tubules. The significantly higher permeability inhibition ratio in the 100-99 group under the 10 s irradiation condition may be due to the effect of the higher energy density than in the other groups. It is assumed that the laser irradiated at a high energy density reacted efficiently with the TiO_2 solution to generate high thermal energy. Therefore, temperatures above 60°C[52] at which the proteins begin to denature and coagulate occurred, resulting in good dentinal tubule sealing abilities after a short irradiation time. However, the permeability inhibition ratio increased with increase of the irradiation time for the irradiation conditions of 50-50 and 100-20, and there was no significant difference in the permeability inhibition ratio of each group for the irradiation conditions of 20 s and 30 s. These results suggest that the reaction with the TiO_2 solution was accelerated and sufficient thermal energy was generated by prolonged irradiation time, even at low energy densities. Therefore, the same level of dentinal tubule sealing ability as that obtained by laser irradiation at high energy densities may have been obtained. However, the permeability inhibition ratio did not increase with increasing irradiation time under the irradiation conditions of 100-99 and 300-20. SEM images of the dentin surface showed images of dentin melting. There are reports that dentin denaturation occurs due to the generation and accumulation of higher thermal

energy at higher energy densities[9,10]. The thermal energy that occurred by high energy densities under the irradiation conditions of 100-99 and 300-20 was seemingly too high to seal the dentinal tubules by denaturation and coagulation of the proteins. Dentin degeneration is considered to influence the sealing ability of dentinal tubules. Hence, we concluded that the permeability inhibition ratio may have not increased in proportion to the increase of the energy density. Therefore, Nd: YAG laser irradiation combined with TiO_2 solution is effective for sealing dentinal tubules. However, in order to obtain good dentinal tubule sealing without dentin degeneration, care must be taken with the high energy density of the laser.

Furthermore, by investigating the flow rate of the TiO_2 solution, there is a possibility that Nd: YAG laser could provide a less invasive and more effective treatment method for dentin hypersensitivity by demonstrating the effect of HLLT in addition to the original LLLT effect of Nd: YAG laser.

Conclusions

The effects of dentin thickness or TiO_2, a photoreactive agent, on Nd: YAG laser penetration into dentin, and the effect of Nd: YAG laser irradiation with TiO_2 on the sealing of dentinal tubules were examined, and the following results were obtained.

1. The transmittance of Nd: YAG laser through dentin decreased with increasing dentin thickness.
2. The transmittance of Nd: YAG laser through dentin decreased significantly when TiO_2 was used in combination.
3. When Nd: YAG laser was irradiated with TiO_2, the sealing abilities of dentinal tubules improved. At 100 μs-100 mJ-99 pps, a high dentin permeability inhibition effect was observed after a short irradiation time. At 50 μs-50 mJ-50 pps and 100 μs-100 mJ-20 pps, the dentin permeability inhibition ratio increased with increasing irradiation time.

Conflict of Interest

The authors declare no conflict of interest in this study or report.

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Original Article

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Application of Intraoral Scanners in Dental Health Guidance

—Quantification and Visualization of Marginal Gingival Changes—

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Abstract

Introduction: We have been studying the effectiveness of the intraoral scanner (IOS) as a tool for dental health guidance. We have confirmed the possibility of scanning the gingiva with high precision and of capturing gingival changes quantitatively and visually by aligning the scan data. In the present study, we investigated whether the shape changes of marginal gingiva generated experimentally by inserting a gingival retraction cord into the gingival sulcus could be quantified and visualized by using IOS recording. In addition, we also studied the improvement of accuracy by alignment method. In other words, the objective of this study was to quantify and visualize the clinical shape changes of the gingiva with high precision by IOS, and to connect them to clinical applications.

Subjects and methods: The subjects were three faculty members of the Faculty of Health Sciences, Osaka Dental University. First, the oral cavity was scanned three times repeatedly. Then, gingival retraction cords were inserted into the gingival sulcus of four teeth, two in the maxilla and two in the mandible, for each study subject. The data was saved for IOS and as exported data in Standard Triangulated Language (STL) format. The STL data before and after gingival retraction were compared using three-dimensional inspection software.

Results: In the prealignment, which is the aligning of the scanned whole before and after gingival retraction, the deviation on the tooth surface was within ±0.5 mm in the interquartile range (IQR) for most of the measurement points. The deviation on the gingiva was slightly more variable than that on the tooth surface. For the local best-fit alignment on all tooth surfaces, the deviation on the tooth surface was smaller and less varied than that for the prealignment. On the other hand, the deviation at the gingiva in the local best-fit alignment on all tooth surfaces was larger and more varied than that in the prealignment. As for marginal gingival changes, 97.7% had bulging marginal gingiva and 89.4% had depressed gingival sulcus in the prealignment. In the local best-fit alignment on all tooth surfaces, 98.1% had distended marginal gingiva and 89.4% had depressed gingival sulcus. In the local best-fit alignment on the gingival retracted tooth surface, 97.7% had bulging marginal gingiva and 88.4% had depressed gingival sulcus.

Conclusions: The following conclusions were drawn from these results: 1. There was little difference in quantification or visualization between prealignment, which optimally aligned both tooth and gingival surfaces, and alignment, which optimally aligned all tooth surfaces in the dentition. 2. Although there is an error in the scanning of the oral cavity by IOS, it is precise enough to be used for dental health guidance. 3. Intraoral scans by IOS allow quantification and visualization of gingival changes.

Key words: intraoral scanner, dental health guidance, gingival change

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Introduction

Currently, intraoral scanners (IOS) are mainly used in the fabrication of prosthetic and orthodontic devices. We have been investigating the possibility of using this technology not only for the fabrication of prosthetic devices, but also for a wide range of dental treatments. In particular, we expect that intraoral scanned records captured by IOS will replace study casts and intraoral photographs in dental health guidance.

The color tone cannot be confirmed on study casts. In addition, intraoral photographs are not three-dimensional. Scanning and recording by IOS overcomes both of these issues and is considered to be of high value. Furthermore, the operation time is short[1], and less invasive for the patient. There is also no problem of space for storing the casts.

Therefore, we have been studying the usefulness of IOS for dental health guidance[2,3]. First, the accuracy of recording a complete denture was confirmed assuming an oral cavity, and the possibility of quantification and visualization of shape change was examined[2]. In addition, we applied film-type stomatitis patches to the gingival region in the actual oral cavity to determine the accuracy of the recording against changes in periodontal tissue caused by IOS[3].

In the previous study, the shape change was identified due to changes caused by the application of the film agent, and the gingiva was unchanged. Therefore, in this study, the shape of the marginal gingiva was experimentally changed by inserting a gingival retraction cord into the gingival sulcus. We then confirmed the quantification and visualization of slight changes in the gingiva and examined the extent to which the accuracy of quantification and visualization could be improved by changing the alignment method. The purpose of this study was to quantify and visualize the clinical shape changes in the gingiva with high accuracy by IOS, and to connect them to clinical applications.

Subjects and Methods

1. Subjects

The subjects were three faculty members of the Faculty of Health Sciences, Osaka Dental University. In a previous study[2], the maximum standard deviation of the distance between measurement points was 0.30 mm and its standard error was 0.12 mm. In another study[3], it was possible to quantify and visualize a film with a thickness of 0.11 mm applied to the gingiva. Based on the above, we considered that the objectives of the study could be achieved with three subjects based on the thickness of the retraction cords. Subjects excluded were those with three or more consecutive missing teeth, those who had extreme difficulty inserting gingival retraction cords into the gingival sulcus due to marginal gingival tension or severe pain during gingival retraction, and those who did not give their consent. The selection criteria were those who understood the study, agreed to participate in the study, and had no evidence of periodontal disease from an examination by a dental hygienist (not a JSP Dental Hygienist) with at least 15 years of practical experience. The subjects were one male and two females with a mean age of 48.0 years.

2. Scanning methods

TRIOS 3 (3shape, Copenhagen, Denmark) was used as the IOS. First, the oral cavity was scanned in triplicate. Following the procedures of the supplied measurement software, the intraoral cavity was scanned first in the mandible, then in the maxilla, and finally in the occlusion. Then, a total of four teeth were selected, two each in the maxilla and mandible, in which it was easy to insert the retraction cords in order to avoid subject fatigue and allow a longer time interval before and after retraction. Table 1 shows gingival retracted teeth and their probing depths in each subject. Bleeding at the time of insertion of the gingival retraction cord may affect IOS recording. Therefore, teeth that did not bleed during probing were selected as test teeth. Gingival retraction cords (Ultrapak cord, Ultradent Products, South Jordan, UT, USA) were inserted into the gingival sulcus of these teeth. The cords used were #000 (0.89 mm diameter) or #00 (1.04 mm diameter). The oral cavity was then scanned three times in the same manner as before gingival retraction.

3. Evaluation methods

The scanning data were stored as TRIOS 3-specific data and exported to STL format for storage. The STL data before and after gingival retraction were then compared using three-dimensional inspection software (GOM Inspect 2016, GOM, Braunschweig, Germany) in

Table 1 Gingival retracted teeth and their probing depths in each subject

Subject	Tooth		Probing depth (mm)				Tooth	Probing depth (mm)			
				Distal		Mesial			Mesial		Distal
Subject A	Maxilla	Right central incisor	Labial	2	2	2	Left central incisor	Labial	2	1	2
			Palatal	2	2	2		Palatal	2	2	2
				Distal		Mesial			Mesial		Distal
	Mandible	Right first molar	Lingual	3	2	3	Left second molar	Lingual	3	3	3
			Buccal	3	2	3		Buccal	3	2	3
				Mesial		Distal			Mesial		Distal
Subject B	Maxilla	Left central incisor	Labial	2	1	2	Left lateral incisor	Labial	1	1	2
			Palatal	2	1	1		Palatal	1	1	1
				Distal		Mesial			Mesial		Distal
	Mandible	Right second premolar	Lingual	1	1	1	Left second premolar	Lingual	2	1	2
			Buccal	1	1	3		Buccal	3	1	2
				Distal		Mesial			Mesial		Distal
Subject C	Maxilla	Right central incisor	Labial	2	2	2	Left first molar	Buccal	3	2	3
			Palatal	2	2	2		Palatal	2	2	2
				Distal		Mesial			Mesial		Distal
	Mandible	Right canine	Lingual	2	2	2	Left first premolar	Lingual	2	2	2
			Labial	2	2	2		Buccal	2	2	2

No bleeding on probing was observed on any of the teeth.

the following way.

The data before retraction and after retraction were read as CAD data (nominal elements) and mesh data (actual elements), respectively. The measurement points were then obtained on the surface of the mesh data, i. e., the data after gingival retraction. An example of the measurement points on the labial and buccal side of the mandible is shown in Figure 1. The measurement points were set with reference to the position of periodontal pocket measurement for future application to oral health guidance. A total of six measurement points were set on the labial, buccal, and lingual surfaces of each tooth: mesial, median, and distal, and six points on the keratinized gingival area corresponding to the measurement points on the tooth surface. The CAD and mesh data were then prealigned. Prealignment is the initial alignment, in which both the tooth surface and gingival surface are aligned for best fit. Deviations

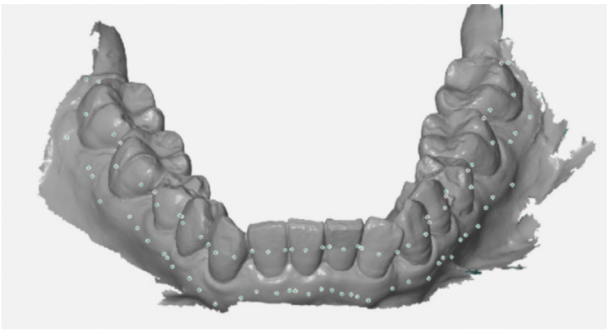

Fig. 1 Example of the measurement points on the labial and buccal side of the mandible

at the measurement points were calculated for the aligned images. Then, alignment was also performed on all tooth surfaces of the dentition, and the deviation at each measurement point was obtained. In addition, alignment was also performed on the tooth surfaces of gingival retracted teeth. One scanning data before gin-

Table 2 Rating criteria of gingival changes around gingivally retracted teeth by viewing surface comparison images before and after gingival retraction in surface comparison images

Degree of marginal gingival bulge		Degree of gingival sulcus depression	
Color indicating the deviation	Determination	Color indicating the deviation	Determination
Only green, light blue or blue	0	Only green, yellow or red	0
Yellow unclearly viewed	1	Light blue unclearly viewed	1
Yellow clearly viewed	2	Light blue clearly viewed	2
Red unclearly viewed	3	Blue unclearly viewed	3
Red clearly viewed	4	Blue clearly viewed	4

Blue is approximately −0.16 mm or less, light blue −0.15 mm to −0.06 mm, green −0.05 mm to +0.05 mm, yellow +0.06 mm to +0.15 mm, and red +0.16 mm or more.

gival retraction was aligned with three scanning data after retraction, respectively. Since three scans were also made before gingival retraction, the number of aligned combinations was nine multiplied by three times after retraction by three times before retraction in one subject.

Regarding color, the criteria for evaluating the visible changes in color on comparative images of the marginal gingiva of the gingival retracted teeth are shown in Table 2. The marginal gingiva was evaluated on a 5-point scale, with "4" for a bulge of +0.16 mm or more indicated in red and "0" for almost no bulge from −0.05 mm to +0.05 mm indicated in green. The gingival sulcus was evaluated on a 5-point scale, with "4" for a depression of −0.16 mm or less indicated in blue and "0" for almost no depression from −0.05 mm to +0.05 mm indicated in green.

4. Statistical analysis

Statistical analysis was performed as follows. The absolute value of the deviation was used as the error, and the influence of the site and alignment method on the error was analyzed by two-way ANOVA with region (tooth surface and gingiva) and alignment method (prealignment and local best-fit alignment on all tooth surfaces) as factors. The effect size was also calculated. In addition, the differences in respective errors between the tooth and gingival surfaces due to the different alignment methods were analyzed with a paired means *t*-test.

For the evaluation of marginal gingival changes, Friedman's test was performed using three different alignment methods as factors: prealignment, local best-fit alignment on all tooth surfaces, and alignment on the gingival retracted tooth surface. In addition, Wilcoxon's signed-rank test was performed for each combination, and the results were subjected to Bonferroni correction.

5. Ethical approval

This study was approved by the Osaka Dental University Medical Ethics Committee (Approval No. 111173-0). The materials and equipment used have been approved by the Ministry of Health, Labour and Welfare.

Results

1. Surface comparison in areas with no change

Examples of comparative images of intraoral records before and after gingival retraction scanned using IOS by prealignment, local best-fit alignment on all tooth surfaces, and local best-fit alignment on the gingival retracted tooth surface are shown in Figure 2. Three scans were taken before and after each gingival retraction, and the time interval recorded before and after gingival retraction was about 2 hours at most. Therefore, there was no shape change except for the marginal gingiva of the gingival retracted tooth and the movable portion of the gingiva. However, there was no record of a perfect match before and after gingival indentation. Visual comparison of the prealigned and aligned images on all tooth surfaces showed little difference in the comparative images before and after gingival retraction in any of the subjects.

On the other hand, morphological changes were observed in the marginal gingiva of the gingival retracted teeth in most of the images. In the mandible of the subject in Figure 2, gingival retraction was performed on the right canine and left first premolar. In Figure 2, changes in the marginal gingiva of the gingival retracted teeth were observed in both the prealign-

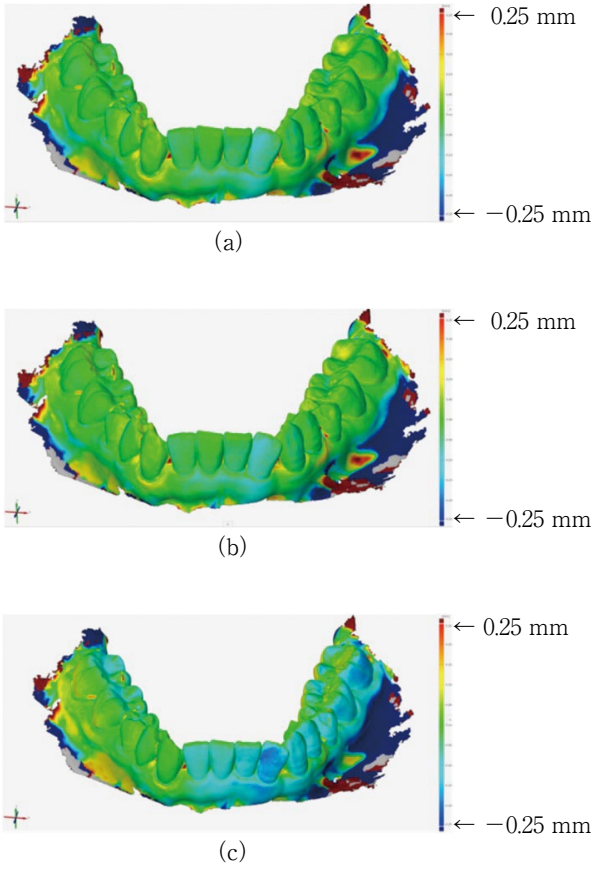

Fig. 2 Comparative images of records before and after gingival retraction scanned using IOS by alignment of the three methods

(a) Prealignment, (b) Local best-fit alignment on all tooth surfaces, (c) Local best-fit alignment on the gingival retracted tooth (in this figure, the mandibular right canine) surface.

The redder the deviation, the more positive the deviation, and the bluer the deviation, the more negative the deviation. A positive deviation indicates that the surface after gingival retraction is outside of the surface before retraction. A negative deviation indicates that the surface after gingival retraction is inside of the surface before retraction.

ment and the local best-fit alignment on comparison images of all tooth surfaces. Furthermore, when alignment was performed on the surface of the gingival retracted tooth—the right canine tooth in Figure 2 (c)—the deviation of the canine tooth surface was reduced and the marginal gingival changes appeared slightly different from the prealignment and local best-fit alignment on all tooth surfaces. On the other hand, deviations were larger in areas other than the canine teeth, especially on the contralateral side.

Figure 3 shows examples of comparative images around the gingival retracted maxillary central incisors by alignment of the three methods. The area with a large deviation from the brown band visible in the midline is the gingival retraction cords. The marginal gingiva of the gingival retracted tooth is indicated in red, indicating that the gingiva was augmented by approximately 0.2 mm. There was less gingival augmentation on the lingual side than on the labial side. In addition, the gingival sulcus indicated in blue shows that it was depressed after gingival retraction. The deviation values varied slightly depending on the alignment method.

Figure 4 shows box-and-whisker plots of the distribution of surface deviation compared by the prealignment of the IOS records before and after gingival retraction. The measurement points of the gingiva were set at the attached gingiva region. The number of data at each measurement point varied slightly, from 105 to 162, because some records had congenitally missing teeth or measurement points were not in the scanned range. Deviations of most measurement sites on the tooth surface were within ±0.5 mm in the interquartile range (IQR). On the other hand, there were a few outliers with deviations exceeding 0.20 mm. The deviation on the gingiva was slightly more variable than that on the tooth surface.

Figure 5 is a graph similar to Figure 4 with local best-fit alignment on all tooth surfaces. The deviation on the tooth surface was smaller than the prealignment and the variation was also smaller. On the other hand, the deviation in the gingival region was larger than the prealignment and the variability was also larger.

ANOVA results showed that both the factors of site (tooth surface and gingiva) and alignment method (prealignment and local best-fit alignment on all tooth surfaces) and the interaction of these two factors had significant effects ($p<0.01$). However, the effect sizes of the alignment methods were small: 0.080 for the tooth surface and −0.176 for the gingiva.

Figure 6 compares the absolute values of deviations by alignment method. When the prealignments were replaced by local best-fit alignment on all tooth surfaces, the error decreased from 0.036 mm to 0.034 mm on the tooth surface and increased from 0.044 mm to 0.052 mm on the gingiva. The results of the paired means *t*-test showed that the differences in errors were

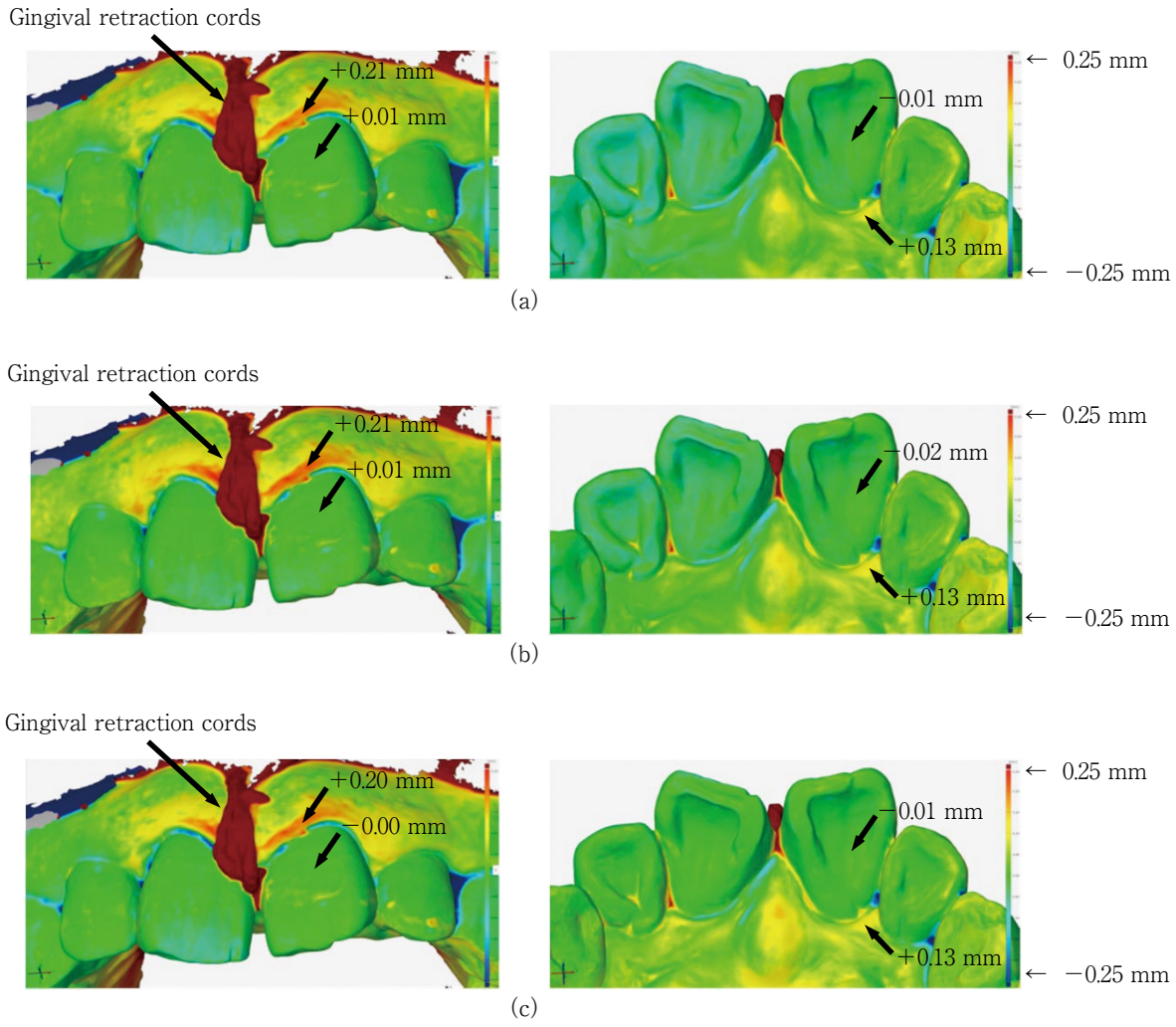

Fig. 3 Comparative images around the gingival retracted maxillary central incisor by alignment of the three methods

(a) Prealignment, (b) Local best-fit alignment on all tooth surfaces, (c) Alignment on the surface of the left central incisor.

The color display is the same as in Figure 2.

all significant at the 1% level of significance. On the other hand, the mean difference was 0.002 mm for the tooth surface and −0.008 mm for the gingiva, which were very small. The standard deviations were 0.030 mm for both prealignment and local best-fit alignment on all tooth surfaces on the tooth surface, and 0.044 mm for prealignment and 0.052 mm for local best-fit alignment on all tooth surfaces on the gingiva.

2. Marginal gingival changes in gingival retracted teeth

Table 3 shows the results of evaluating the changes in the marginal gingiva of the gingival retracted teeth by looking at the comparative images before and after gingival retraction. Of the 216 sites for which the data in Table 2 were tabulated by tooth alignment method, 211 (97.7%) had bulging marginal gingiva and 193 (89.4%) had depressed gingival sulcus in the prealignment. In the local best-fit alignment on all tooth surfaces, 212 (98.1%) had distended marginal gingiva and 193 (89.4%) had depressed gingival sulcus. In the local best-fit alignment on the gingival retracted tooth surface, 211 (97.7%) had bulging marginal gingiva and 191 (88.4%) had depressed gingival sulcus.

Most of the teeth with gingival retraction were anterior teeth in the maxilla and molars in the mandible.

Figure 7 compares the percentage of the evaluation results of the changes in the marginal gingiva before and after gingival retraction with the different align-

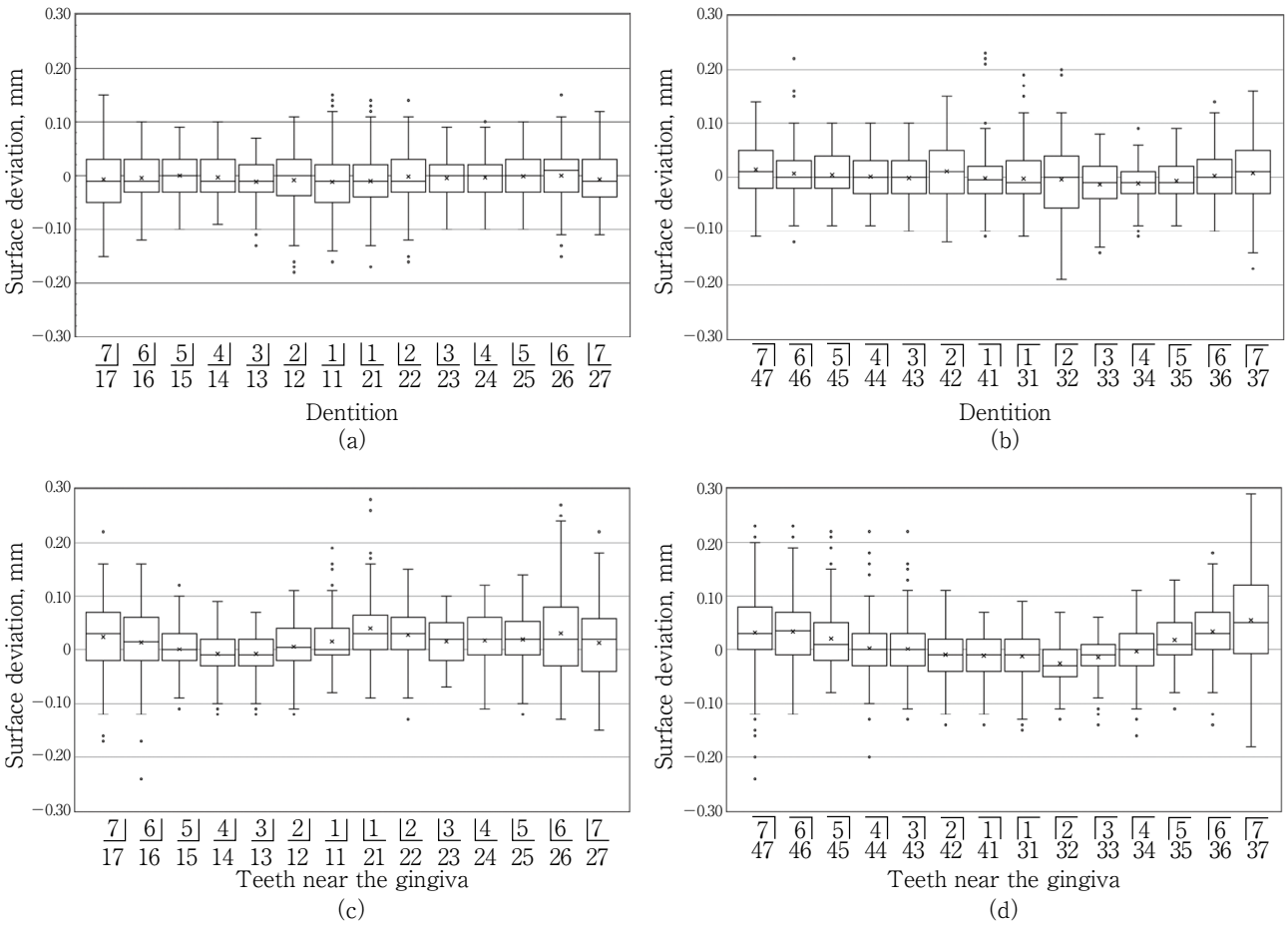

Fig. 4 Box-and-whisker plots showing surface in prealignment
(a) Maxillary tooth surface, (b) Mandibular tooth surface, (c) Maxillary gingival surface, (d) Mandibular gingival surface.

ment methods. About half of the patients had the highest rating of 4 for marginal gingival bulge. The ratings of 4 increased in the order of prealignment, local best-fit alignment on all tooth surfaces, and local best-fit alignment on the gingival retracted tooth surface. In contrast, in the gingival sulcus, the highest rating of 4 accounted for approximately 30%. In addition, the ratings of 4 decreased slightly in the order of prealignment, local best-fit alignment on all tooth surfaces, and local best-fit alignment on the gingival retracted tooth surface.

Bonferroni correction of the Wilcoxon signed-rank test results for the alignment method combinations showed that for marginal gingival bulge, local best-fit alignment on all tooth surfaces and local best-fit alignment on the gingival retracted tooth surface were rated higher than prealignment ($p<0.05$). No combination was significant at the 5% level of significance for the depression of the gingival sulcus. When the significance level was set at 10%, local best-fit alignment on the gingival retracted tooth surface was rated lower than prealignment.

Discussion

1. Scanning precision

There have been many studies on the trueness and precision of teeth and implants scanned by IOS[4–6], which have shown that scanning with IOS is clinically acceptable with excellent trueness and precision. In addition, there have recently been several studies on the use of IOS for gingival examination[7–9]. However, there have been few studies on the accuracy of scanning of gingiva[10,11]. Therefore, we measured the error of scanning in the gingival region of the same denture six times using a complete denture[2] and found that the recording precision was high even in the gingival region.

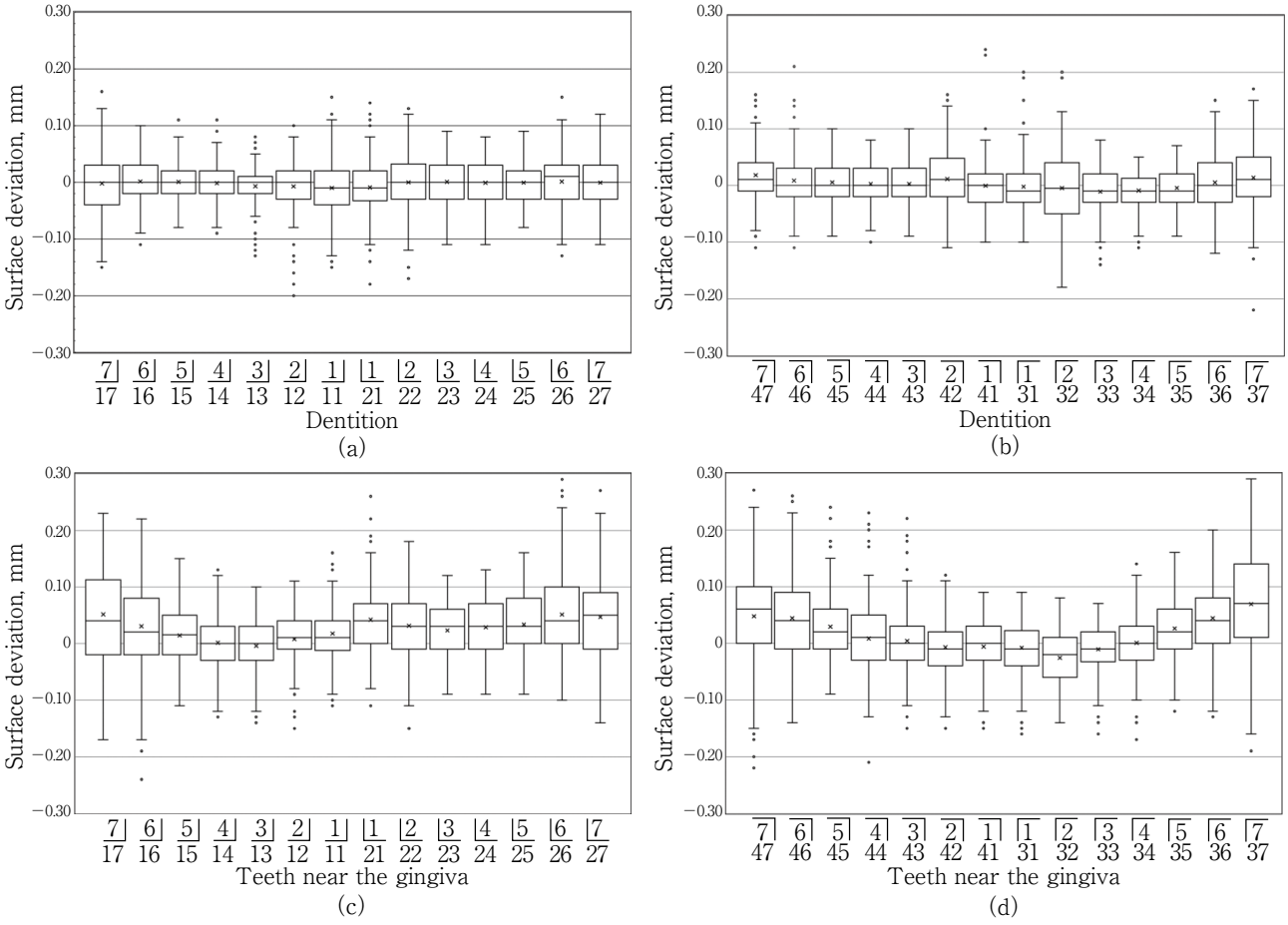

Fig. 5 Box-and-whisker plots showing surface deviations in alignment on the surface of the dentition
(a) Maxillary tooth surface, (b) Mandibular tooth surface, (c) Maxillary gingival surface, (d) Mandibular gingival surface.

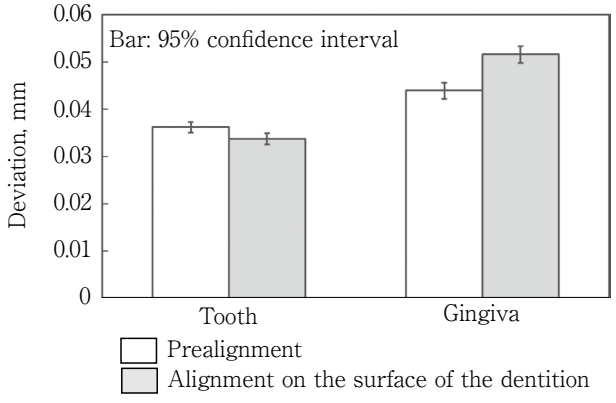

Fig. 6 Comparison of absolute values of deviation between prealignment and local best-fit alignment on all tooth surfaces

However, in intraoral scanning, the scanned object cannot be taken out of the mouth like a complete denture, so it is impossible to scan while holding the object in the hand and moving it. In addition, the recording precision may be reduced due to the presence of saliva and other intervening substances in the oral cavity. Therefore, in this study, the results of multiple intraoral scans were aligned and deviations were displayed to confirm the precision.

As shown in the example in Figure 2, even in the same oral cavity, areas other than the marginal gingiva of the right canine and left first premolar with gingival retraction were also found to be other than green, indicating less deviation. In other words, no records with perfectly matched scan data were obtained. Figure 4 shows that the deviations of each region were in the IQR, except for the gingival region of the mandibular left second molar, when prealigning was performed, i. e., the two entire 3D data sets were analyzed and aligned at the best-fitting position. That is, 50% of the measurements were within ±0.10 mm. When not including outliers, which are values more than 1.5 quadrants apart, all values were within ±0.20 mm, except for the maxil-

Table 3 Results of rating of gingival changes around gingivally retracted teeth by viewing surface comparison images before and after gingival retraction

Position		Alignment method	Side	Marginal gingival region						Gingival sulcus region					
				4	3	2	1	0	Total	4	3	2	1	0	Total
Maxilla	Anterior tooth	Prealignment	Labial	26	10	8	1	0	45	31	3	5	5	1	45
			Palatal	15	6	10	14	0	45	14	9	12	9	1	45
		On the surface of the dentition	Labial	23	11	10	1	0	45	30	5	5	4	1	45
			Palatal	18	3	13	11	0	45	12	6	14	12	1	45
		On the surface of the gingivally retracted tooth	Labial	30	5	8	0	2	45	32	2	6	4	1	45
			Palatal	19	3	10	13	0	45	15	3	10	16	1	45
	Posterior tooth	Prealignment	Buccal	0	5	3	1	0	9	0	7	0	2	0	9
			Palatal	3	1	5	0	0	9	1	2	3	3	0	9
		On the surface of the dentition	Buccal	3	3	3	0	0	9	0	7	0	2	0	9
			Palatal	6	2	1	0	0	9	0	3	0	6	0	9
		On the surface of the gingivally retracted tooth	Buccal	2	5	2	0	0	9	0	6	0	2	1	9
			Palatal	6	2	1	0	0	9	0	1	1	7	0	9
Mandible	Anterior tooth	Prealignment	Labial	2	1	5	0	1	9	0	0	2	5	2	9
			Lingual	0	2	5	1	1	9	0	0	1	6	2	9
		On the surface of the dentition	Labial	2	2	4	0	1	9	0	1	1	5	2	9
			Lingual	0	4	4	1	0	9	0	0	1	5	3	9
		On the surface of the gingivally retracted tooth	Labial	0	0	7	2	0	9	0	0	0	7	2	9
			Lingual	3	2	4	0	0	9	0	0	0	5	4	9
	Posterior tooth	Prealignment	Buccal	21	8	13	3	0	45	15	3	2	18	7	45
			Lingual	29	3	6	4	3	45	8	6	5	16	10	45
		On the surface of the dentition	Buccal	22	6	11	6	0	45	12	5	2	19	7	45
			Lingual	30	2	6	4	3	45	9	5	7	15	9	45
		On the surface of the gingivally retracted tooth	Buccal	25	7	12	1	0	45	10	7	1	20	7	45
			Lingual	27	5	9	1	3	45	8	7	7	14	9	45

lary right second molar and mandibular left second molar gingival areas. Since deformation occurs during alginate impression and plaster hardening even in the study cast, IOS scanning is considered to be accurate enough for dental health guidance.

On the other hand, outliers were also found. On the tooth surfaces, more outliers were observed on the anterior teeth than on the molars. This may be due to the fact that the incisal area of the anterior teeth is narrower than the occlusal surface of the molars, making it difficult to superimpose the images during scanning, especially on the labial and lingual sides. In the gingival region, outliers were also common in the molar region, especially in the mandible. This may be because the distance from the gingival margin of molars to the mucobuccal fold and the distance to the floor of the oral cavity were short, and the measurement point was included in the movable range of the gingiva.

Since it is the gingiva that is changed by dental health guidance, it seems reasonable to align the scanned image on all tooth surfaces, i. e., on all remaining tooth surfaces, rather than on the entire image. Comparing (a) and (b) in Figure 2, visually little difference was discernible between the two comparative images. The same was true for the other images examined in this study. Comparing the results of local best-fit alignment on all tooth surfaces (Figure 5) with the results of prealignment (Figure 4), the IQR was slightly less on the tooth surfaces. In contrast, in the gingival region, on the contrary, the IQR was slightly widened. The absolute value of the deviation shown in Figure 6, i. e., the error, was also smaller on the tooth surface and

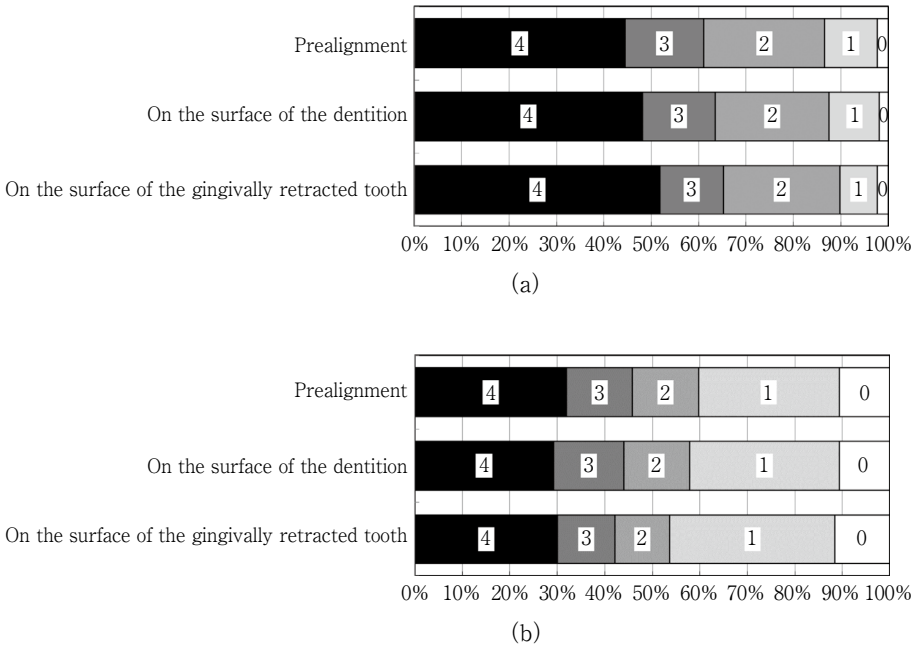

Fig. 7 Comparison of the percentage of the evaluation results of the changes in the marginal gingiva before and after gingival retraction with the different alignment methods
(a) Marginal gingival region, (b) Gingival sulcus region.

larger in the gingival region. These results are considered to be reasonable because of the alignment in the dentition tooth plane. On the other hand, although there was a significant difference in the mean values, the effect of converting the prealigning to the local best-fit alignment on all tooth surfaces is considered to be small because the difference was small: the smallest unit of deviation was 1/100 mm, and the standard deviation of each mean value was 0.030 to 0.052 mm, which is larger than the difference in the mean values.

In Figure 4 (d) and Figure 5 (d), the deviation was larger for the posterior teeth in the mandible. In other words, the gingiva was slightly swollen after the gingival retraction. The cause of this phenomenon will be examined in the future.

On the other hand, it is known that when a complete arch is scanned by IOS, the trueness and precision are worse than when a partial arch is scanned[12]. This is because IOS creates an overall image by gradually overlapping the scanned images and enlarging them into an overall image[13]. Therefore, in order to examine the changes in the gingival area more accurately, it is considered necessary to align the data by block or by each tooth surface. We would like to study this issue in the future.

2. Quantification and visualization of gingival changes

In a previous study[3], we investigated whether changes caused by the film could be quantified and visualized by aligning scan data before and after a 0.11-mm-thick film was applied to the gingiva. As a result, the thickness of the applied film could be accurately quantified and visualized. On the other hand, although the films were very thin, the boundaries were clear, so it was necessary to evaluate the actual gingival changes in the actual oral cavity to see if they could be clarified. Therefore, in this study, the gingival retraction cord was inserted into the gingival sulcus to alter the gingiva.

As shown in Figures 2 and 3, the marginal gingiva of gingival retracted teeth often had a bulge of approximately 0.2 mm or less. The diameter of the gingival retraction cord used was 0.89 mm for #000 and 1.04 mm for #00, according to the attachment. Therefore, the bulge of the marginal gingiva was considerably smaller than the diameter of the gingival retraction cord. This may be due to the fact that the cords become thinner due to compression of the receding gingiva and that the

gingiva is also deformed by elasticity.

The deviation of the gingival bulge often slightly varied depending on the method of aligning. In the example shown in Figure 3, the prealigning showed a labial gingival bulge of 0.21 mm, whereas the local best-fit alignment on the gingival retracted tooth surface showed a ridge of 0.20 mm. If aligning is performed only on the tooth surface of a tooth that has been retracted, the deviation will be large in the area away from the aligned tooth surface as shown in Figure 2(c), so it should only be used to determine the gingiva of the tooth in question or its vicinity. Many of the gingival retracted teeth also showed depressed changes in the gingival sulcus, indicated by the blue color. This may be due to the expansion of the gingival sulcus by gingival retraction.

The visualization of gingival changes could be confirmed in approximately 98% of the cases for marginal gingival bulge and in approximately 89% of the cases for gingival sulcus depressions, as shown in Table 3, therefore it is considered to be sufficient for clinical use. In Figure 7, the percentage of 4, which is the easiest for examining the marginal gingival bulge, increased in the order of prealignment, alignment on the dentition, and alignment on the gingival surface of the compressed tooth, indicating that alignment on the tooth surface makes the change easier to examine. However, the gingival sulcus depression was rated less frequently than the marginal gingival bulge, i. e., it was difficult to examine. With respect to the depression of the gingival sulcus, the alignment method had little effect on visualization. The cause may be that the gingival retraction cord is near the gingival margin or does not form a depression if it protrudes from the gingival sulcus, or conversely, if the gingival retraction cord is inserted too deeply into the gingival sulcus, the gingival sulcus closes. Another possible reason is that the light of IOS does not reach the gingival sulcus sufficiently.

3. Clinical significance and future studies

Scanning with IOS provides a three-dimensional view of the shape of the oral cavity. Furthermore, the results of our previous studies and this study indicate that changes in oral shape can be quantitatively captured on the order of 1/100th of a millimeter. In addition, shape changes could be visualized on a color display.

These indicate that gingival changes that could only be expressed as “better” or “worse” in dental health guidance can be expressed numerically. For example, the degree of gingival swelling can be expressed as a numerical value based on breadth and height. Furthermore, changes over time can also be expressed numerically. Visualization facilitates understanding for patients as well as dentists and dental hygienists.

The ability to display changes in the oral cavity on a scale of 1/100 th of a millimeter may also allow clinicians and researchers to find changes that were previously unknown.

Shape changes are determined by aligning the intraoral scanning record. Aligning methods are important to obtain changes accurately because of errors in recording. We think that further study of alignment methods in clinical applications is needed.

Probing depth was the only method that could quantitatively represent changes in gingival shape in health guidance. With the use of IOS, detailed changes over time can be represented quantitatively and visually in an easy-to-understand manner. Therefore, in the future, gingival changes in health guidance will be mainly recorded by IOS. On the other hand, probing will continue to be an important examination because probing depth cannot be recorded.

In addition, areas that are difficult to observe on intraoral photographs, such as the lingual and palatal surfaces, can be easily observed. It is predicted that the clarity of images recorded by IOS will improve in the future. Therefore, IOS records are likely to be used as an alternative to intraoral photographs.

In the future, we plan to study how gingival changes can be examined by using IOS in clinical dental health guidance, and to verify whether dental health guidance using IOS will be easier for patients to understand than conventional guidance methods, and whether it will be useful for dentists and dental hygienists who provide such guidance.

Conclusions

The possibility of quantifying and visualizing gingival changes for the purpose of using IOS for oral health guidance was investigated.

By repeated scanning of the oral cavity before and after gingival retraction with IOS and evaluating the results of aligning the obtained records, the following conclusions were obtained:

1. There was little difference in quantification or visualization between prealignment, which optimally aligned both tooth and gingival surfaces, and alignment, which optimally aligned all tooth surfaces in the dentition.

2. Although there is an error in the scanning of the oral cavity by IOS, it is precise enough to be used for dental health guidance.

3. Intraoral scans by IOS allow quantification and visualization of gingival changes.

Part of this study was presented at the 65th Spring Meeting of the Japanese Society of Periodontology (June 4, 2022, Tokyo).

Conflict of Interest

The authors declare no conflicts of interest associated with this manuscript.

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1) Clark AB, Erickson D, Hamilton FG. Tensile bond strength and modulus of elasticity of several composite resins. J Dent Res 1992; 37: 618-621.

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2) Phillips RW. Skinner's science of dental materials. 9th ed. WB Saunders: Philadelphia; 1991. 219-221.

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3) Torneck CD. Dentin-pulp complex. Ten Cate AR. Oral histology. 5th ed. Mosby: St. Louis; 1998. 150-196.

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4) Marais JT. Cleaning efficacy of a new root canal irrigation material. J Dent Res 1998; 77: 669, Abst. No. 300.

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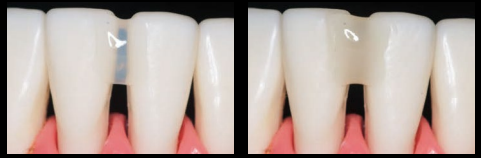
患者さんの症例に合わせて
クリア、TC（トゥースカラー）を選択


クリア　TC（トゥースカラー）

患者さんにやさしい

嫌な**ニオイがなく**
ナノフィラーによる
滑沢表面性状で
プラークがつきにくい

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ブレない品質、五つ星

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機器の分類：管理医療機器（クラスⅡ）
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Concept

SMOOTH 急な引き込まれを大幅軽減

FLEXIBLE 本来の根管から逸脱しづらい
刃部構造と柔軟性

SIMPLE Ⅰ・Ⅱ・Ⅲの３本で終了
簡単な手順、使用方法を採用

ホームページより
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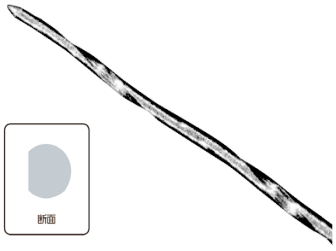
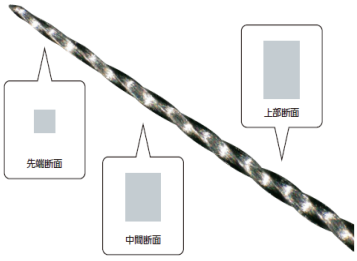
D ファインダー



- 過剰な切削・食い込みを軽減できる。
- 刃部強度を高め、穿通力を UP。
- 石灰化、狭窄した根管にも折れにくく有効。

グライドファインダー



穿通性 + 切削性

- 先端部テーパーを強化し力が伝わりやすい。
- 断面形状の変化による優れた切削性と柔軟性。
- 穿通のみならずグライドパス形成も可能。

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