IN VITRO VALIDATION OF A LASER FLUORESCENCE-BASED SUBGINGIVAL CALCULUS DETECTION INSTRUMENT.

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MASTER OF SCIENCE

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ABSTRACT

Objectives: Because subgingival dental calculus in periodontal pockets is associated in the etiopathogenesis of progressive human periodontitis, and is difficult to accurately detect with conventional manual explorers and probing instruments, there is an urgent clinical need for more reliable diagnostic methods for the detection and localization of subgingival dental calculus. A low-power (< 1 milliwatt) diode laser emitting visible red laser fluorescence at a 655 nm wavelength in the near-infrared electromagnetic spectrum (Diagnodent Pen, Kavo Dental Corp., Charlotte, NC USA), and fitted with a periodontal probe-like rigid cylindrical sapphire tip, is approved for clinical patient care by the United States Food and Drug Administration and commercially marketed for subgingival dental calculus detection, but has received relatively little research attention. This study assessed the in vitro reproducibility and accuracy of this visible red laser fluorescence-emitting instrument for dental calculus detection on root surfaces of extracted human teeth.

Methods: A total of 50 extracted single and multi-rooted human teeth (11 incisors, 4 canines, 7 premolars, and 28 molars) with a range of visually-evident dental calculus deposits were initially evaluated with a SZX10 research stereomicroscope (Olympus America, Inc., Center Valley, PA USA) at 10x magnification for the presence of dental calculus on tooth root surfaces, which was recognized by its dark color and raised surface morphology. One dental calculus-positive and one dental calculus-negative root surface was selected per tooth as test surfaces for further evaluation. The presence and nature of dental calculus deposits on each test root surface was scored on a 0-2 scale with a modified Subgingival Calculus Index (SCI) by an experienced, board-certified

periodontist using an 11/12 Old Dominion University dental explorer. Two independent dentist examiners with varied educational and clinical experience backgrounds (one a board-certified specialist in periodontics with 35 years of clinical dental care experience, and the other a general dentist in an advanced general dentistry residency program with 6 years of clinical dental care experience), each assessed the test root surfaces with the visible red laser fluorescence-emitting instrument using two different evaluation protocols. In the first evaluation protocol, each examiner perpendicularly directed the visible red laser fluorescence-emitting instrument tip twice along test root surfaces, and recorded the maximum laser fluorescence intensity values obtained from each pass, which potentially ranged from 0-99. In the second evaluation protocol, each examiner assessed test root surfaces twice for maximum laser fluorescence intensity values with the laser instrument tip directed parallel to the tooth root surface and advanced in an apical direction from the tooth cementoenamel junction, similar to how a periodontal probe is introduced in vivo into periodontal pockets. Correlation coefficient analysis evaluated intra- and inter-examiner reproducibility of visible red laser fluorescence intensity values obtained with both evaluation protocols. A two-tailed, independent samples, Student's t-test evaluated mean visible red laser fluorescence intensity values measured between dental calculus-positive and dental calculus-negative root surfaces, and also statistically compared mean visible red laser fluorescence intensity scores recorded on dental calculus-positive tooth root surfaces exhibiting a modified SCI score = 2, as compared to a modified SCI score = 1. Sensitivity, specificity, positive predictive value, negative predictive value, and odds ratio analysis assessed the occurrence of dental calculus-positive and -negative root surfaces associated with two visible red laser fluorescence

intensity threshold levels recommended for clinical diagnostic purposes by the manufacturer (≥ 5 and > 40).

Results: A total of 50 root surfaces exhibited a modified SCI score = 0 (no root surface dental calculus detected), whereas 19 root surfaces revealed modified SCI scores = 1 (root surface dental calculus detected in thin deposits, but not in a markedly-raised ledge), and 31 root surfaces had modified SCI scores = 2 (root surface dental calculus detected in a markedly-raised ledge). A high level of both intra- and inter-examiner reproducibility of visible red laser fluorescence intensity readings was found with both tooth root evaluation protocols, despite the marked differences between the two dentist examiners in their educational backgrounds and length of clinical dental care experience, with correlation coefficient values ranging from r = 0.948 to r = 0.999 for duplicate assessments made by the two independent examiners themselves and between them. Mean visible red laser fluorescence intensity values recorded by the two independent examiners with the instrument perpendicularly directed along tooth root surfaces (first evaluation protocol) were 98.9 (standard deviation ± 0.4) and 99.0 (standard deviation ± 0.0), respectively, on dental calculus-positive root surfaces, which were significantly greater than mean values of 10.9 (standard deviation ± 6.0) and 12.3 (standard deviation ± 8.1), respectively, recorded on dental calculus-negative root surfaces ($P < 0.0001$ for each examiner; two-tailed, independent samples, Student's t-test). Similarly, mean visible red laser fluorescence intensity values recorded by the two independent examiners with the instrument directed apical and parallel to the tooth root surface (second evaluation protocol) were 76.9 (standard deviation ± 26.4) and 79.7 (standard deviation ± 23.8), respectively, on dental calculus-positive root surfaces, which were significantly

greater than mean values of 4.2 (standard deviation ± 2.7) and 4.9 (standard deviation ± 4.1), respectively, on dental calculus-negative root surfaces ($P < 0.0001$ for each examiner; two-tailed, independent samples, Student's t-test). Significantly greater visible red laser fluorescence intensity scores were found on dental calculus-positive root surfaces with modified SCI scores = 2, as compared to modified SCI scores = 1, but only when the visible red laser fluorescence-emitting instrument tip was directed parallel to the tooth root surface and advanced apically like a periodontal probe. A threshold level of ≥ 5 for visible red laser fluorescence intensity readings provided 100% sensitivity, 68% specificity, a 75.8% positive predictive value, a 100% negative predictive value, and an odds ratio relationship of 20.1 [95% confidence interval = 8.8, 45.8] for the presence of dental calculus on tooth root surfaces. In comparison, a threshold level of > 40 for visible red laser fluorescence intensity values offered 90% sensitivity, 100% specificity, a 100% positive predictive value, a 90.9% negative predictive value, and an odds ratio relationship of 36.6 [95% confidence interval = 16.7, 80.2] for the presence of dental calculus on tooth root surfaces.

Conclusions: These in vitro findings document, for the first time, a high level of intra- and inter-examiner reproducibility of visible red laser fluorescence intensity measurements on human tooth root surfaces, regardless of the whether the instrument is directed either perpendicular or parallel to extracted tooth root surfaces. Dental calculus-positive root surfaces on extracted teeth exhibited significantly higher visible red laser fluorescence intensity scores than dental calculus-negative root surfaces, particularly when dental calculus deposits were present in markedly-raised ledges. In addition, a threshold level of > 40 for visible red laser fluorescence intensity readings offered greater

diagnostic accuracy than a threshold level of ≥ 5 for identification of dental calculus on root surfaces of extracted teeth. These findings provide further in vitro validation for use of the visible red laser fluorescence-emitting instrument for detection of dental calculus on root surfaces of human teeth. Additional validation studies, conducted clinically in vivo, on the visible red laser fluorescence-emitting instrument are warranted.

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CHAPTER 1

INTRODUCTION

Dental calculus formation on human tooth root surfaces is a complex process that occurs with precipitation of calcium phosphate salts, originating mainly from gingival crevicular fluid flow into gingival sulci and periodontal pockets, which mineralize pre-existing subgingival bacterial biofilms adherent and growing on teeth (Roberts-Harry & Clerehugh 2000). Subgingival dental calculus usually has a dark brown-black color, in contrast to the grey-white color of supragingival dental calculus (Jepsen et al. 2011). Subgingival dental calculus tenaciously adheres to tooth root surfaces by calcifying organic pellicle glycoproteins on teeth, and mechanically locking calcified crystals into resorption lacunae and other irregularities on subgingival tooth surfaces (Roberts-Harry & Clerehugh 2000).

Subgingival dental calculus has been shown to harbor a number of periodontopathic bacterial species within its surface-associated structural lacunae and unmineralized channels (Calabrese et al. 2007). The major periodontal bacterial pathogen *Treponema denticola*, in particular, was found on and within nearly all subgingival dental calculus samples examined in a transmission electron microscopic study using immunogold staining with *T. denticola* species-specific polyclonal antibodies (Calabrese et al. 2007). In addition, the non-shedding outer surfaces of subgingival dental calculus facilitate dental plaque growth, and are invariably coated with bacterial biofilms in the absence of antimicrobial chemotherapy (Jepsen et al. 2011). In moderate-to-severe chronic periodontitis patients with overtly-detectable subgingival dental

calculus deposits, greater subgingival levels of motile rods and cultivable black-pigmented anaerobic rods were recovered than were found in similar periodontitis patients with little or no detectable subgingival dental calculus (Brown et al. 1991).

Subgingival dental calculus may also harbor bacterial toxins within surface porosities, such as phosphorylated dihydroceramide lipids from the major periodontal bacterial pathogen *Porphyromonas gingivalis*, which can alter gingival fibroblast morphology and promote their secretion of potentially gingival tissue-damaging pro-inflammatory cytokines (Nichols & Rojanasomsith 2006). In this regard, subgingival calculus with its overlying bacterial populations has been described as analogous to a "toxic waste dump site" and "a slow release device delivering pathogenic products" (Mandel & Gaffar 1986).

Interestingly, dental calculus by itself without viable dental plaque biofilm coatings exhibits a relatively low pathogenic potential in animal model testing when sterilized free of living microorganisms (Allen & Kerr 1965), and can even provide an adherent surface for junctional epithelium in rhesus monkeys when its outer surface is disinfected with 2% chlorhexidine gluconate (Listgarten & Ellegaard 1973).

Nevertheless, the presence of subgingival dental calculus has been repeatedly associated with progressive periodontitis disease activity in humans. In a longitudinal study of tea laborers in Sri Lanka who were without any professional or home dental care over a period of 15 years, it was found that teeth with subgingival dental calculus exhibited a significantly greater rate of clinical periodontal attachment loss as compared to teeth that were free of dental calculus (Anerud et al. 1991). Similarly, subgingival

dental calculus was significantly associated with progressive periodontal attachment loss over a 6-year time period in patients with early-onset periodontitis (Albandar et al. 1998).

Conventional detection of subgingival dental calculus in humans with dental explorers and probes is considered problematic. Explorers and probes are reliant upon the non-visual, tactile discrimination of the clinician to feel for root surface irregularities indicative of the presence of dental calculus, as compared to a clinically smooth feel characteristic of dental calculus-negative root surfaces. However, clinical studies have shown a high false negative diagnostic outcome with conventional manual explorer detection of subgingival dental calculus, with 77.4% of root surfaces of teeth still positive for subgingival dental calculus after periodontal root instrumentation being erroneously scored with explorers as calculus-free (Sherman et al. 1990). Similarly, the level of agreement between two clinical examiners in subgingival dental calculus detection after periodontal root instrumentation was found to relatively poor, with the examiners less than 50% of the time in agreement on the presence of subgingival dental calculus when it was actually present (Pippin & Feil 1992).

Because subgingival dental calculus in periodontal pockets is associated with the etiopathogenesis of progressive human periodontitis (Mandel & Gaffar 1986), and since it is difficult to accurately detect with conventional manual explorers and probing instruments (Sherman et al. 1990, Pippin & Feil 1992), there is an urgent clinical need for more reliable diagnostic methods for the detection and localization of subgingival dental calculus.

In this regard, a low-power (< 1 milliwatt) diode laser emitting visible red laser fluorescence at a 655 nm wavelength in the near-infrared electromagnetic spectrum (Diagnodent Pen, Kavo Dental Corp., Charlotte, NC USA), and fitted with a periodontal probe-like rigid cylindrical sapphire tip, is approved for clinical patient care by the United States Food and Drug Administration, and is commercially marketed for subgingival dental calculus detection (Figure 1).

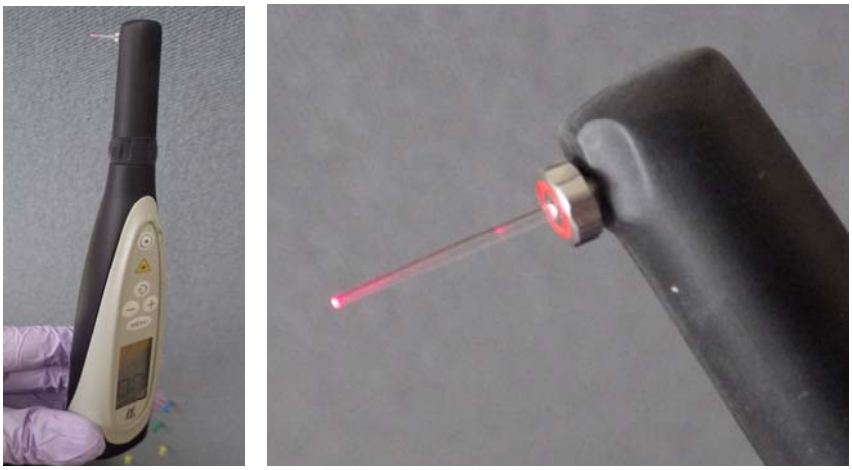

Figure 1. Visible red laser fluorescence-emitting instrument (left) with a periodontal probe-like rigid cylindrical sapphire tip (right).

However, the instrument to date has received relatively little research attention. Folwaczny et al. (2002) examined 30 extracted human teeth with dental calculus with the visible red laser fluorescence-emitting instrument, and found greater fluorescence intensity scores on dental calculus vs. tooth cementum devoid of dental calculus. In addition, lower intensity scores were measured by the visible red laser fluorescence-emitting instrument on tooth root surfaces in the presence of blood, as compared to in air or in an electrolyte solution (Folwaczny et al. 2002).

Krause et al. (2003) examined 20 extracted human teeth with dental calculus with the visible red laser fluorescence-emitting instrument, and also found greater fluorescence intensity scores on dental calculus as compared to tooth cementum. However, in contrast to the findings of Folwaczny et al. (2002), no influence of blood or physiological saline was detected on fluorescence intensity scores.

In a follow-up study, Folwaczny et al. (2004) mounted 40 extracted human teeth with subgingival dental calculus into a manikin head. Subgingival dental calculus was removed on some teeth with a clinical endpoint determined with a dental explorer, whereas on other teeth subgingival dental calculus removal was continued until a fluorescence intensity score of < 5 was measured with the visible red laser fluorescence-emitting instrument. Between these two subgingival dental calculus removal protocols, it was found that less residual subgingival calculus was present post-treatment on molar teeth when the subgingival root instrumentation was guided to an endpoint by a fluorescence intensity score of < 5 (Folwaczny et al. 2004).

Additionally, Shakibaie & Walsh (2014) mounted 30 extracted human teeth with subgingival dental calculus into manikin heads with silicone gingiva, and found fluorescence intensity scores with the visible red laser fluorescence-emitting instrument to have a better correlation to subgingival dental calculus volume than to subgingival dental calculus surface area.

To date, the in vitro reproducibility of the visible red laser fluorescence-emitting instrument has yet to be determined, and the accuracy of the instrument in subgingival

dental calculus detection when directed perpendicular vs. parallel to tooth root surfaces remains unknown.

As a result, the purpose of this study was to to assess the in vitro reproducibility and accuracy of the visible red laser fluorescence-emitting instrument for dental calculus detection when directed perpendicular, as compared to parallel, to root surfaces of extracted human teeth.

CHAPTER 2

MATERIALS AND METHODS

Laboratory Facilities

All study procedures were performed in the Oral Microbiology Testing Service (OMTS) Laboratory, located in Room 365-A of Building 600, which is part of the Temple University Maurice H. Kornberg School of Dentistry on the Temple University Health Sciences Center campus in Philadelphia, Pennsylvania. Since the data for the present non-clinical, laboratory-based, study was obtained without any intervention or interaction with living individuals, and did not involve any identifiable private information, the research activity did not involve human subjects, as defined by United States Department of Health and Human Services regulations at 45 CFR part 46.116(f), and did not require a human subjects institutional review board approval, per a written determination issued by the Temple University Human Subjects Protections Institutional Review Board.

Test Tooth Root Surfaces

A total of 50 extracted single and multi-rooted human teeth (11 incisors, 4 canines, 7 premolars, and 28 molars) with a range of visually-evident dental calculus deposits were obtained from a collection of extracted teeth maintained by a Health Ministry dental clinic in Kuwait. No data was available as to the identification or demographics of the patients from whom the teeth were removed, the reasons for the tooth removal, or clinical dental status of the patient, including periodontal probing depths and the relationship of the free gingival margin to the cemenoenamel junction of

the teeth. The teeth were washed free of blood and loose debris with tap water and a toothbrush, and subsequently stored dry in a plastic bag.

The teeth were initially evaluated at 10-fold magnification with an Olympus SZX10 dissecting research stereomicroscope (Olympus America, Inc., Center Valley, PA USA), equipped with a Fostec Ace I fiberoptic light source, for the presence of dental calculus on tooth root surfaces, which was recognized by its dark color and raised surface morphology (Figure 2).

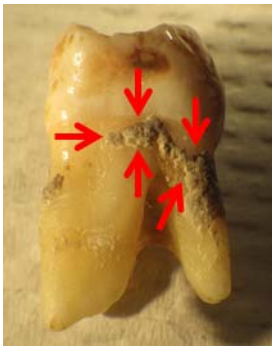

Figure 2. Human molar tooth with root surface dental calculus (red arrows).

One dental calculus-positive, and one dental calculus-negative, root surface from non-furcation smooth tooth surfaces was selected per tooth as test surfaces for further evaluation. On some teeth where no areas could be identified as being devoid of root surface calculus, a periodontal curette was used to thoroughly remove a portion of the presenting root surface dental calculus to create a limited root surface area that could be designated as dental calculus-negative. The selected dental calculus-positive and dental calculus-negative test root surfaces were each marked with a black felt-tipped pen to aid study examiners with identification and localization of areas to perform assessments, and each was photographed for research documentation.

The presence and nature of dental calculus deposits on each of the selected test root surfaces was scored in vitro on a 0-2 scale with a modified Subgingival Calculus Index (SCI) (Watanabe et al. 1982) by an experienced, board-certified periodontist (Thomas E. Rams, DDS, MHS, PhD) using an 11/12 Old Dominion University dental explorer with a #6 satin steel handle (Hu-Friedy Manufacturing Co., Chicago, IL USA). The criteria for the modified SCI were as follows:

0 = no root surface calculus detected

1 = root surface dental calculus detected in thin deposits, but not in a markedly-raised ledge

2 = root surface dental calculus detected in a markedly-raised ledge

Visible Red Laser Fluorescence Testing

Two independent dentist examiners with varied educational and clinical experience backgrounds, one a board-certified specialist in periodontics with 35 years of clinical dental care experience (Thomas E. Rams, DDS, MHS, PhD), and the other a general dentist in an advanced general dentistry residency program with 6 years of clinical dental care experience (Abdulaziz Alwaqyan, BDS), evaluated the selected test tooth root surfaces with a hand-held visible red laser fluorescence-emitting instrument fitted with a periodontal probe-like rigid cylindrical sapphire tip (Diagnodent Pen, Kavo Dental Corp., Charlotte, NC USA), after the instrument was calibrated as specified by manufacturer guidelines.

Each study examiner assessed the test tooth root surfaces using two different evaluation protocols. In the first evaluation protocol, each examiner perpendicularly

directed the visible red laser fluorescence-emitting instrument tip twice along test root surfaces, and recorded the maximum laser fluorescence intensity values obtained from each pass, which potentially ranged from 0-99 (Figures 3 and 4).

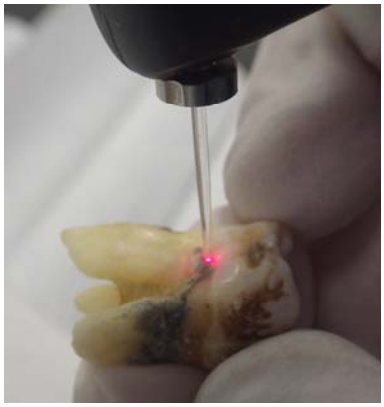

Figure 3. Visible red laser fluorescence-emitting instrument tip directed perpendicular to a dental calculus-positive tooth root surface.

Figure 4. Visible red laser fluorescence-emitting instrument tip directed perpendicular to a dental calculus-negative tooth root surface.

In the second evaluation protocol, each examiner assessed test root surfaces twice for maximum laser fluorescence intensity values, which potentially ranged from 0-99, with the laser instrument tip directed parallel to the tooth root surface and advanced in an

apical direction from the tooth cementoenamel junction, similar to how a periodontal probe is introduced in vivo into periodontal pockets (Figures 5 and 6).

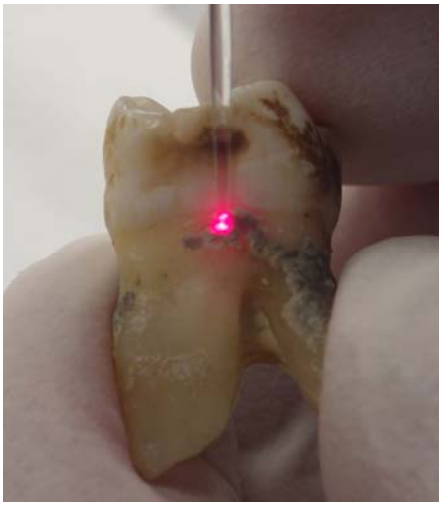

Figure 5. Visible red laser fluorescence-emitting instrument tip directed parallel to a dental calculus-positive tooth root surface.

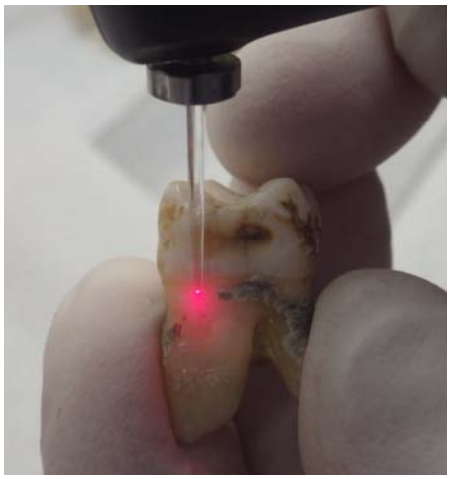

Figure 6. Visible red laser fluorescence-emitting instrument tip directed parallel to a dental calculus-negative tooth root surface.

The tooth root surface evaluations were performed in air on a laboratory benchtop, without the presence of any oral fluids, such as saliva, gingival crevicular fluid,

or blood, coating the tooth root surfaces, dental calculus deposits, or the tip of the visible red laser fluorescence-emitting instrument, during fluorescence testing.

Data Analysis

Calculation of the mean plus standard error for duplicate visible red laser fluorescence intensity readings, and Pearson correlation coefficients, evaluated intra- and inter-examiner reproducibility of visible red laser fluorescence intensity values obtained with both evaluation protocols. An independent samples Student's t-test evaluated mean plus standard deviation tabulations of visible red laser fluorescence intensity values measured between dental calculus-positive and dental calculus-negative root surfaces. An independent samples Student's t-test was also used to statistically compare mean visible red laser fluorescence intensity scores recorded on dental calculus-positive tooth root surfaces exhibiting a modified SCI score = 2, as compared to a modified SCI score = 1. A two-tailed *P*-value of ≤ 0.05 used as a critical threshold for determining the statistical significance of Student's t-test outcomes.

Using 2x2 contingency table analysis (McNeil et al. 1975), sensitivity, specificity, positive predictive value, negative predictive value, and odds ratio analysis assessed the occurrence of dental calculus-positive and -negative root surfaces associated with two visible red laser fluorescence intensity threshold levels recommended for clinical diagnostic purposes by the manufacturer (≥ 5 and > 40). Sensitivity was defined as the probability that visible red laser fluorescence intensity threshold levels or higher will be measured when root surface dental calculus is detected (true positive rate). Specificity was defined as the probability that visible red laser fluorescence intensity threshold levels

or higher will not be measured when root surface dental calculus is not detected (true negative rate). Positive predictive value was defined as the probability that root surface dental calculus is detected when visible red laser fluorescence intensity threshold levels or higher are detected. Negative predictive value was defined as the probability that root surface dental calculus is not detected when visible red laser fluorescence intensity threshold levels or higher are not detected.

Due to the occurrence of zero event cells in 2x2 contingency table analysis, Peto odds ratios and their 95% confidence intervals (CI) (Brockhaus et al. 2014), as determined using an on-line calculator (http://www.hutchon.net/peto%20vers%202.htm), were used to estimate true odds ratios in assessing the relationship between the two visible red laser fluorescence intensity threshold levels and detection of root surface dental calculus.

A PC-based, 64-bit, statistical software package (JMP® Pro 10.0.2, SAS Institute, Inc., Cary, NC USA) was used in the data analysis.

CHAPTER 3

RESULTS

Test Tooth Root Surfaces

A total of 50 root surfaces exhibited a modified SCI score = 0 (no root surface dental calculus detected), whereas 19 root surfaces revealed modified SCI scores = 1 (root surface dental calculus detected in thin deposits, but not in a markedly-raised ledge), and 31 root surfaces had modified SCI scores = 2 (root surface dental calculus detected in a markedly-raised ledge). Figures 7 through 12 provide photographic documentation of a representative subset of test tooth root surfaces evaluated in this study.

Figure 7. Premolar tooth with a dental calculus-negative root surface (above left black line) (modified SCI score = 0), and a dental calculus-positive root surface (above right black line) scored with a modified SCI score = 1.

Figure 8. Molar tooth with a dental calculus-negative root surface (above left black line) (modified SCI score = 0), and a dental calculus-positive root surface (above right black line) scored with a modified SCI score = 2.

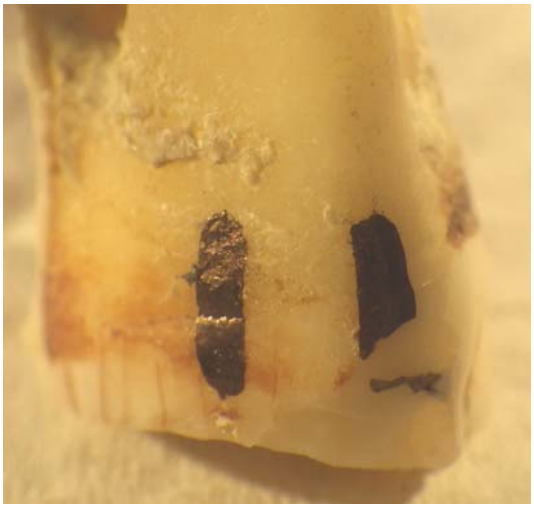

Figure 9. Molar tooth with a dental calculus-negative root surface (above right black line) (modified SCI score = 0), and a dental calculus-positive root surface (above left black line) scored with a modified SCI score = 1.

Figure 10. Molar tooth with a dental calculus-negative root surface (above right black line) (modified SCI score = 0), and a dental calculus-positive root surface (above left black line) scored with a modified SCI score = 1.

Figure 11. Molar tooth with a dental calculus-positive root surface (above black line) scored with a modified SCI score = 2.

Figure 12. Molar tooth with a dental calculus-negative root surface (above right black line) (modified SCI score = 0), and a dental calculus-positive root surface (above left black line) scored with a modified SCI score = 2.

Visible Red Laser Fluorescence Testing

A high level of both intra- and inter-examiner reproducibility of visible red laser fluorescence intensity readings was found with both tooth root evaluation protocols, despite the marked differences between the two dentist examiners in their educational backgrounds and length of clinical dental care experience, with Pearson correlation coefficient values ranging from r = 0.948 to r = 0.999 for duplicate assessments made by the two independent examiners themselves and between them (Tables 1 and 2).

Table 1. Intra-examiner reproducibility of duplicate laser fluorescence intensity scores

Perpendicular root surface evaluation protocol	Mean visible red laser fluorescence intensity readings ± standard error: First reading	Second reading	Pearson correlation coefficient (r)
Examiner #1	55.7 ± 4.4	55.6 ± 4.4	+ 0.999
Examiner #2	55.0 ± 4.4	54.8 ± 4.4	+ 0.999
Parallel root surface evaluation protocol	**First reading**	**Second reading**	**Pearson correlation coefficient (r)**
Examiner #1	40.5 ± 4.1	40.7 ± 4.1	+ 0.987
Examiner #2	41.9 ± 4.2	42.6 ± 4.2	+ 0.960

Table 2. Inter-examiner reproducibility of duplicate laser fluorescence intensity scores

Root surface evaluation protocol	Mean visible red laser fluorescence intensity readings ± standard error: Examiner #1	Examiner #2	Pearson correlation coefficient (r)
#1 - perpendicular	55.7 ± 4.4	54.9 ± 4.4	+ 0.995
#2 - parallel	40.5 ± 4.1	42.2 ± 4.1	+ 0.948

Mean visible red laser fluorescence intensity values recorded by the two independent examiners with the instrument perpendicularly directed along tooth root surfaces (first evaluation protocol) were 98.9 (standard deviation ± 0.4) and 99.0 (standard deviation ± 0.0), respectively, on dental calculus-positive root surfaces, which were significantly greater than mean values of 10.9 (standard deviation ± 6.0) and 12.3 (standard deviation ± 8.1), respectively, recorded on dental calculus-negative root surfaces ($P < 0.0001$ for each examiner; two-tailed, independent samples, Student's t-test). Similarly, mean visible red laser fluorescence intensity values recorded by the two independent examiners with the instrument directed apical and parallel to the tooth root surface (second evaluation protocol) were 76.9 (standard deviation ± 26.4) and 79.7 (standard deviation ± 23.8), respectively, on dental calculus-positive root surfaces, which

were significantly greater than mean values of 4.2 (standard deviation ± 2.7) and 4.9 (standard deviation ± 4.1), respectively, on dental calculus-negative root surfaces ($P < 0.0001$ for each examiner; two-tailed, independent samples, Student's t-test) (Table 3).

Table 3. Visible red laser fluorescence intensity values on dental calculus-positive and -negative tooth root surfaces

	Mean visible red laser fluorescence intensity readings ± standard deviation (range)		
Perpendicular root surface evaluation protocol	Root surface dental calculus present	Root surface dental calculus absent	*P*-value
Examiner #1	99.0 ± 0.0 (99)	12.3 ± 8.1(2-34.5)	< 0.0001
Examiner #2	99.0 ± 0.4 (96.5-99)	10.9 ± 6.0 (4.5-27.5)	< 0.0001
Parallel root surface evaluation protocol	Root surface dental calculus present	Root surface dental calculus absent	*P*-value
Examiner #1	76.9 ± 26.4 (18.5-99)	4.2 ± 2.7 (1-14.8)	< 0.0001
Examiner #2	79.7 ± 23.8 (13-99)	4.9 ± 4.1 (1-18.5)	< 0.0001

Table 4 presents mean visible red laser fluorescence intensity readings on dental calculus-positive tooth root surfaces stratified by modified SCI scores.

Table 4. Visible red laser fluorescence intensity values on dental calculus-positive tooth root surfaces with varying modified SCI scores

	Mean visible red laser fluorescence intensity readings ± standard deviation (range)		
Perpendicular root surface evaluation protocol	Root surfaces with modified SCI score = 2	Root surfaces with modified SCI score = 1	*P*-value
Examiner #1	99.0 ± 0.0 (99)	99.0 ± 0.0 (99)	> 0.05
Examiner #2	98.9 ± 0.1 (96.5-99)	99.0 ± 0.0 (99)	> 0.05
Parallel root surface evaluation protocol	Root surfaces with modified SCI score = 2	Root surfaces with modified SCI score = 1	*P*-value
Examiner #1	90.0 ± 3.1 (31.5-99)	55.6 ± 5.7 (18.5-99)	< 0.0001
Examiner #2	89.0 ± 2.6 (44.5-99)	64.4 ± 6.5 (13-99)	0.0018

Significantly higher mean visible red laser fluorescence intensity scores by both study examiners were recorded on dental calculus-positive tooth root surfaces exhibiting a modified SCI score = 2, as compared to a modified SCI score = 1, when the visible red laser fluorescence-emitting instrument tip was directed parallel to the tooth root surface and advanced apically like a periodontal probe ($P < 0.0001$ and $P = 0.0018$ for the two study examiners, respectively; two-tailed, independent samples, Student's t-test) (Table 4). No similar statistically significant differences in laser fluorescence intensity values between dental calculus-positive tooth root surfaces exhibiting a modified SCI score = 2, as compared to a modified SCI score = 1, were found when the visible red laser fluorescence-emitting instrument tip was directed perpendicular to the tooth root surface (Table 4).

Table 5 presents a 2x2 contingency table distribution of tooth root surfaces by a laser fluorescence threshold level of ≥ 5 and presence or absence of dental calculus.

Table 5. Distribution of tooth root surfaces by a laser fluorescence threshold level of ≥ 5 and presence or absence of dental calculus

		Root surface dental calculus present	Root surface dental calculus absent
Laser fluorescence threshold	≥ 5	50	16
	< 5	0	34

Based on this distribution, use of a diagnostic threshold level of ≥ 5 for visible red laser fluorescence intensity readings provided 100% sensitivity, 68% specificity, a 75.8% positive predictive value, a 100% negative predictive value, and an odds ratio relationship of 20.1 [95% confidence interval = 8.8, 45.8] for the presence of dental calculus on tooth root surfaces.

In comparison, Table 6 presents a 2x2 contingency table distribution of tooth root surfaces by a laser fluorescence threshold level of > 40 and presence or absence of dental calculus.

Table 6. Distribution of tooth root surfaces by a laser fluorescence threshold level of > 40 and presence or absence of dental calculus

		Root surface dental calculus present	Root surface dental calculus absent
Laser fluorescence threshold	> 40	45	0
	≤ 40	5	50

Based on this distribution, use of a higher diagnostic threshold level of > 40 for visible red laser fluorescence intensity values offered 90% sensitivity, 100% specificity, a 100% positive predictive value, a 90.9% negative predictive value, and an odds ratio relationship of 36.6 [95% confidence interval = 16.7, 80.2] for the presence of dental calculus on tooth root surfaces.

CHAPTER 4

DISCUSSION

The present study found, for the first time, a high level of intra- and inter-examiner reproducibility associated with visible red laser fluorescence intensity measurements on human root surfaces, regardless of the whether the visible red laser fluorescence-emitting instrument tip was directed either perpendicular or parallel to the extracted tooth root surfaces. Moreover, the reproducibility of the visible red laser fluorescence intensity measurements was not significantly influenced by the differing educational background or length of clinical dental experience of the two examiners. Both independent examiners, who had markedly varied dental backgrounds and experience, nevertheless produced very high Pearson correlation coefficients between their own duplicate visible red laser fluorescence intensity measurements, and between comparison measurements made by each other. Such reproducibility assessments have not been previously reported for the visible red laser fluorescence-emitting instrument, and provide new insight and positive support into the possible clinical value of the device. However, it is important to stress that the present study was conducted in vitro under ideal examination conditions without the presence of complicating factors associated with human intraoral use of a dental instrument. Thus, it will be critical to further assess the reproducibility of the visible red laser fluorescence-emitting instrument clinically in vivo under a variety of conditions commonly encountered in clinical dental practices. While it is not known how in vivo reproducibility evaluations will turn out when they are conducted, the present in vitro study findings at least demonstrate a

favorable potential for the reliability of the visible red laser fluorescence-emitting instrument, since if it were to have been unreliable in reproducibility evaluations under ideal test conditions in vitro, it would be unlikely to demonstrate better reproducibility outcomes when subjected to more challenging in vivo clinical conditions.

Another major finding from the present study is that dental calculus-positive root surfaces on extracted human teeth exhibited significantly higher fluorescence intensity scores than dental calculus-negative root surfaces, particularly when dental calculus deposits were present in markedly-raised ledges. It is particularly important to note that significantly higher fluorescence intensity scores on dental calculus-positive root surfaces were recorded with both evaluation protocols, including where the visible red laser fluorescence-emitting instrument tip was directed parallel to test root surfaces, similar to the clinical situation in vivo where a periodontal probe is apically advanced into periodontal pockets. Because the visible red light from the diode laser instrument is emitted straight out of the periodontal probe-like rigid cylindrical sapphire tip with an unknown amount of lateral dispersion, it is not surprising that higher fluorescence intensity values were measured when the instrument tip was directed perpendicular onto subgingival dental calculus deposits as compared to a parallel direction. In a perpendicular orientation the full extent of the visible red laser fluorescence light energy would potentially be absorbed by subgingival dental calculus located within the spot size of the laser beam. In comparison, subgingival dental calculus detection with the laser instrument tip advanced parallel to the subgingival tooth root surface was reliant upon calculus absorption of the laterally dispersed visible red laser fluorescence light energy

coming from the instrument tip, particularly when calculus deposits were thin in dimension on root surfaces. Evidence for this is found in the present study results demonstrating that significantly higher fluorescence intensity scores were found on calculus-positive tooth root surfaces exhibiting a modified SCI score = 2, as compared to a modified SCI score = 1, when the diode laser was directed parallel to the root surface. Since subgingival dental calculus deposits scored with a modified SCI value = 2 exhibit a markedly-raised ledge from tooth root surfaces, which would then be more within the central portion of the diode laser beam spot side, it is not surprising that relatively high mean fluorescence intensity scores of 89-90, similar to those found with the instrument tip directed perpendicular to root surfaces, were found with these types of root surface dental calculus deposits.

More important from a potential clinical application aspect are the mean fluorescence intensity scores of 55.6 to 64.5 obtained using the instrument tip parallel to root surfaces on subgingival dental calculus deposits scored with a modified SCI value = 1, which were thin dental calculus deposits not presenting as a markedly-raised ledge. These scores are fortunately much higher than mean fluorescence intensity measurements of 4.2-4.9 that were recorded from subgingival dental calculus-free root surfaces with the diode laser tip directed parallel to tooth root surfaces. This suggests that the visible red laser fluorescence-emitting instrument may have clinical utility in successfully distinguishing between root surfaces with thin layers of subgingival dental calculus, and those without subgingival dental calculus. However, as can be seen in Figures 7, 9, and 10 of the present study results section, a range of subgingival dental calculus deposits

were scored with a modified SCI value = 1. Some of the subgingival dental calculus deposits appeared to have a more raised and prominent surface morphology, and covered a greater root surface area, than other subgingival dental calculus deposits. As a result, it is not known how well the visible red laser fluorescence-emitting instrument will perform on root surfaces where subgingival dental calculus is present only as thin, non-confluent and dispersed small islands. Additional studies are indicated to specifically test the performance outcomes of the diode laser on such subgingival dental calculus-affected root surfaces in relation to those that are dental calculus-free.

Another important finding from the present study was the observation that a threshold level of > 40 for fluorescence intensity readings offered greater diagnostic accuracy than a threshold level of ≥ 5 for identification of dental calculus on root surfaces of extracted teeth. These two threshold values were studied because of their designation by the instrument manufacturer as important in distinguishing between dental calculus-positive vs. dental calculus-negative tooth root surfaces. For a diagnostic threshold level of ≥ 5 for fluorescence intensity readings, it was found to provide 100% sensitivity, 68% specificity, a 75.8% positive predictive value, a 100% negative predictive value, and an odds ratio relationship of 20.1 [95% confidence interval = 8.8, 45.8] for the presence of dental calculus on root surfaces. These values indicate that in circumstances where fluorescence intensity values reach or exceed a level of 5, then there is a strong likelihood of dental calculus being present.

However, an even stronger relationship between the presence or absence of subgingival dental calculus was found for a diagnostic threshold level of > 40 for

fluorescence intensity measurements. This threshold value provided 90% sensitivity, 100% specificity, a 100% positive predictive value, a 90.9% negative predictive value, and an odds ratio relationship of 36.6 [95% confidence interval = 16.7, 80.2] for the presence of dental calculus on root surfaces. With the markedly higher odds ratio values for the > 40 threshold level of 36.6, as compared to that of the ≥ 5 threshold designation of 20.1, a greater degree of clinical confidence is likely to be attained that subgingival dental calculus is in fact present on a tooth root surface if a fluorescence intensity score of > 40 is obtained at a particular tooth root surface site.

Finally, it is important to note that the visible red laser fluorescence-emitting instrument displayed some undesired performance characteristics that, while not formally evaluated in the present study design, may detract from its overall clinical utility and value. First, the instrument needed to be carefully placed along tooth root surfaces without touching the tip to non-test root surfaces, such as the surface of non-vinyl dental examination gloves or carious lesions, which may have the capability of absorbing the visible red laser fluorescence light energy and producing a false-positive instrument reading. Second, after scoring each tooth root surface, the visible red laser fluorescence-emitting instrument needed to be re-set back to a zero level prior to further scoring of additional root surfaces, which proved to be time-consuming and somewhat labor-intensive as compared to the more rapid conventional detection of subgingival dental calculus with dental explorers along multiple tooth root surfaces. Future studies in vivo are needed to determine whether the information obtained by the visible red laser fluorescence-emitting instrument, in comparison to that obtained by conventional dental

explorers in the hands of experienced clinicians, outweighs the disadvantages of the instrument in terms of its initial purchase cost, periodic replacement costs for the cylindrical sapphire instrument tip, which is subject to breakage, and likely increased chair time for performing subgingival dental calculus detection with the instrument as compared to a conventional dental explorer.

CHAPTER 5

CONCLUSIONS

These in vitro findings document, for the first time, a high level of intra- and inter-examiner reproducibility of visible red laser fluorescence intensity measurements on human tooth root surfaces, regardless of the whether the instrument is directed either perpendicular or parallel to extracted tooth root surfaces. Dental calculus-positive root surfaces on extracted teeth exhibited significantly higher visible red laser fluorescence intensity scores than dental calculus-negative root surfaces, particularly when dental calculus deposits were present in markedly-raised ledges. In addition, a threshold level of > 40 for visible red laser fluorescence intensity readings offered greater diagnostic accuracy than a threshold level of ≥ 5 for identification of dental calculus on root surfaces of extracted teeth. These findings provide further in vitro validation for use of the visible red laser fluorescence-emitting instrument for detection of dental calculus on root surfaces of human teeth. Additional validation studies, conducted clinically in vivo, on the visible red laser fluorescence-emitting instrument are warranted.

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