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DETERMINANTS OF THE CANARY SYSTEM™ EFFICACY IN EARLY CARIES DETECTION: AN IN VITRO STUDY

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ABSTRACT

Aim: This study aimed to investigate how specific technical and oral factors influence the efficacy of the Canary System in detecting and quantifying early caries lesions

Methodology: Forty extracted human teeth with early caries lesions were examined. Twenty teeth had lesions on the occlusal surfaces, while the remaining twenty had lesions on smooth surfaces. The lesions were assessed using the Canary System under various conditions to evaluate its accuracy. The following conditions were tested for the accuracy of the machine readings. (1) Presence of moisture, (2) the influence of the distance of the optical tip of the hand-piece to the lesion surface, (3) bacterial biofilm on the lesion, and (4) staining of the lesion, were investigated.

Results: Tukey's Multiple Comparison test revealed that moisture and staining significantly affected the Canary Numbers. Moisture presence resulted in significantly lower readings (28.63 ± 16.0, $p < 0.01$), while staining caused significantly higher readings (71.93 ± 21.9, $p < 0.01$) compared to dry-state examinations. In contrast, bacterial biofilm and the distance of the optical tip had no significant impact on the Canary Numbers. A similar trend was observed for lesions on occlusal surfaces. For lesions on smooth surfaces, only staining caused significantly higher readings (65.70 ± 16.89, $p < 0.01$) compared to the dry state

Conclusions: The findings suggest that the efficacy of the Canary System in caries detection may be influenced by lesion staining on all surfaces and by the presence of moisture on occlusal surfaces.

KEYWORDS: dental caries, smooth surface caries, occlusal caries, early carious lesion, initial caries, demineralization, biofilm, Canary system, laser fluorescence, efficacy, accuracy, caries detection.

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INTRODUCTION

Dental caries is recognized as the most common disease affecting the oral cavity, posing a significant health challenge for individuals of all ages. This condition, which is influenced by various factors, involves the demineralization and deterioration of tooth structure due to the presence of fermentable dietary sugars and acid-producing bacteria[1]. However, a primary challenge faced by dentists is the early detection of dental caries. Identifying caries at its initial stages allows for the implementation of preventive and remineralizing interventions to potentially reverse, and, halt the progression of the disease [2]. Consequently, accurate and early detection of initial caries lesions enables healthcare providers to assess the extent of the damage and deliver appropriate treatments to arrest or even reverse decay, ultimately preserving the integrity of the affected tooth structure [3].

Methods for detecting caries lesions have evolved over the years, progressing from visual, tactile and radiographic examinations, to x-ray free diagnostics, and the use of laser fluorescence devices such as the Canary System (CS). The CS employs photothermal radiometry-luminescence technology to directly evaluate the tooth crystal structure by converting laser light into thermal infrared and optical emissions, which are then captured and analyzed to determine the extent of demineralization and calculate the Canary number. As the remineralization advances and enamel prisms begin to reconstruct their proper structure, the thermal and luminescence properties gradually revert to those typical of a healthy tooth structure. Throughout the therapeutic process, a decrease in the Canary number is anticipated if the lesion is undergoing remineralization (improving), and an increase if the lesion is experiencing further demineralization (deteriorating) [4].

Previous research had examined the efficiency of emerging technologies in identifying and measuring carious lesions [5–8]. However, the outcomes of both in vivo and in vitro studies have shown inconsistency, leading to uncertainty regarding their overall diagnostic utility in clinical settings. Therefore, the current study aimed to explore how specific technical and oral factors may impact the efficacy of CS to detect and quantify early caries lesions. The study assessed the precision of the CS readings under different circumstances, the presence of moisture, the varying distances between the lesion's surface and the optical tip of the device, the extent of bacterial biofilm covering the lesion, and the existence of stains on the lesions.

MATERIALS AND METHODS

Ethical Considerations

This study was carried out at the department of Comprehensive Dentistry of the School of Dentistry of the University of Texas Health San Antonio (UTHSA) after the approval of the proposed protocol by the UTHSA Institutional Review Board and the Research and Ethical committees at MIU and was given an IRB code HSC20080233N and an IRB# MIU-IRB-1920-016.

Grouping:

Forty freshly extracted human molar teeth (N=40) appropriately disposed from various dental clinics in UTHSA School of Dentistry were collected. Teeth with initial caries lesions were selected and stored in 0.1% thymol solution until used. Teeth were randomly assigned to two groups according to the site of the detected caries lesions: smooth surface (n=20) or occlusal pits/fissures (n=20).

Teeth Preparation

Upon specimens' preparation, any remaining soft tissues were removed. Then the teeth were cleansed by toothbrushing with wet pumice to remove remaining dental plaque from the enamel surface. The teeth were then washed thoroughly

and dried using an air-water syringe to visualize the white caries lesion. The early caries lesions were identified and marked with a circle on each tooth for recognition. The specimens were prepared by using sticky wax to mount each tooth on a Lego block in an orientation that exposed the detected caries lesion to face the optical tip of the CS directed perpendicularly to the lesion. Teeth with smooth surface caries lesions were mounted on their side on red and black Lego blocks, while teeth with occlusal surface caries lesions were mounted vertically in blue and white Lego blocks. Each Lego block was carved with a given specimen number.

Experimental Set-Up

The experimental setup included a mounting jack and the CS is shown in figure 1. A multipurpose clamp was used to firmly hold the optical tip of the CS. The bottom floor of the jack contained double-sided sticky tape to hold the Lego block firmly, with the caries lesion under examination facing upwards towards and at a right angle to the optical tip of the CS. The distance between the optical tip and the surface of the lesion was measured with a meter stick in millimeters and can be varied by moving the jack upwards and downward.

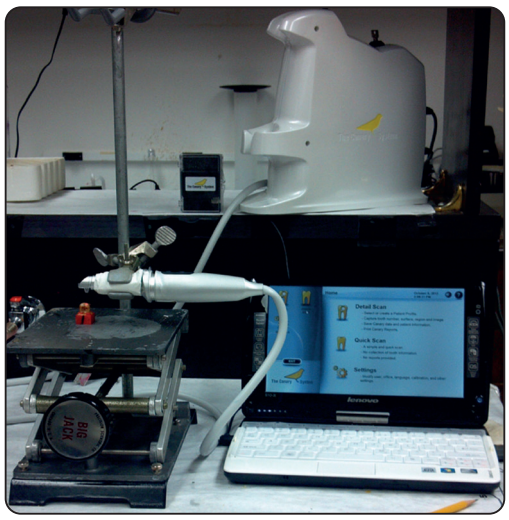

Fig. (1) The Experiment Setup

Exposure to Potential Influencing Factors

Hydration state of the lesion (Wet and Dry state):

Prior to examination, all teeth were immersed in water at room temperature for two hours to enable water to infiltrate the pores of the caries lesion. Upon examination, each tooth was taken out of the water, shaken by hand to remove excess water, then mounted to the floor of the mounting jack and caries lesion was scanned with the CS under wet conditions. The tooth was then dried for 10 seconds using an air-water syringe, and the caries lesion was re-scanned under dry conditions. In both conditions, the mounting Jack was lowered until the optical tip of the CS came into contact with the surface of the caries lesion to be scanned in accordance with the manufacturer's instruction. The CS generated a Canary number that corresponds to the severity of the caries lesion. Readings were recorded under both wet and dry conditions.

Influence of the lesion distance from the optical tip

The lesion scanning under dry condition was repeated with the optical tip of the CS being at 1, 2, 3, 4 and 5 mm away from the caries lesion. The generated Canary readings at each scanning position were recorded.

Influence of bacterial biofilm:

Subsequent to the aforementioned examinations, all teeth were submerged in 70% ethanol for a duration of 1.5 hours for the purpose of cold sterilization and were subsequently positioned under the air vent for an equivalent duration to eliminate the ethanol residue. The strain Streptococcus mutans UA159 was acquired from the Institute of Stomatology at Wenzhou Medical University. For the cultivation of planktonic S. mutans, Brain-heart infusion (BHI, OXOID, Basingstoke, UK) was employed, whereas biofilm formation was facilitated using BHIS (BHI augmented with 1% sucrose, m/v). The culture conditions were maintained at a temperature of 37°C

in an atmosphere containing 5% CO_2.[9] All teeth were then placed in a Brain Heart Infusion (BHI) broth microbiological culture medium inoculated with S.mutans (at a concentration of approximately 10^7 CFU/ml in 10 ml). Biofilm was grown for 48 hours with change of BHI broth every 12 hours. After biofilm formation, the teeth were dried using an air syringe, and subsequently examined using the CS, with the resultant Canary number being documented.

Influence of lesion staining

The bacterial biofilm was removed using an electric toothbrush (Braun Oral-B Plaque Remover, Proctor & Gamble, Cincinnati, OH, USA), then the teeth were re-disinfected by immersion in 70% ethanol for 1.5 hours. Teeth were then positioned under the air vent for an equivalent duration to eliminate the ethanol residue. The lesions were stained by immersing all teeth in coffee solution for 72 hours at room temperature. The coffee was prepared by brewing 5 ounces of Colombian coffee in 2 liters of water and allowing the coffee to condense and cool for 3 hours in an open container. This process gave 1.5 liters of condensed rich coffee. For examination, each caries lesion was dried with the air-syringe and scanned using the CS, and the Canary number was recorded.

Statistical analysis

Using SPSS version 19.1 (SPSS Inc. IBM Company, United States), the data collected from above examinations were analyzed statistically by One-Way repeated ANOVA test followed by Tukey's test. Analysis was first performed on the entire data irrespective of the lesion location, then on the smooth surface (sample 1 – 20) and then the occlusal surfaces (samples 21-40). Furthermore, Tukey's Multiple Comparison test using a critical value of 10.03 with 152 degrees of freedom was performed on the smooth surfaces then the occlusal surfaces. For the statistical analysis, the data from the dry state examination was used as the baseline data and was compared with the data from the other examination conditions. This is because examination of the lesions following 10 seconds drying is the recommended condition of examination when using the Canary System under clinical condition. P values <0.05 were considered statistically significant.

RESULTS

The results of the statistical analysis of the data generated from all examinations were tabulated in Table 1.

Effect of Moisture on CS Readings

Regarding the effect of moisture on the CS readings, with all data together irrespective of the tooth surface (smooth & occlusal), there was a statistically significant difference ($p \leq 0.05$) between the mean values of the Canary numbers for the dry (41.25±17.2) and the wet (28.63 ±16.0) lesions. Tukey's Multiple Comparison test showed that there was a statistically significant difference ($p \leq 0.05$) between dry (39.30±20.576) and wet (17.35±9.98) CS readings for occlusal caries only, but not for smooth surfaces.

Effect of Distance on CS Readings

The results also showed that there was no statistically significant difference between the CS readings at different distances (1 - 5 mm) between the tip of the devise and the surface of the lesion, irrespective of the type of surface (smooth & occlusal). Similar trend was observed for smooth surface ($p=0.8128$) and occlusal surface lesions ($p=0.1016$).

Effect of Biofilm on CS Readings

There was no statistically significant difference ($p=0.1192$) between dried clean samples and samples covered with biofilm for either smooth or occlusal surface lesions.

TABLE (1) Mean Values ±Standard Deviation for each condition.

Condition of Examination	Overall (all surfaces)	Comparison with Dry recommend-ed condition	Occlusal surfaces	Comparison with Dry recommend-ed condition	Smooth surfaces	Comparison with Dry recommend-ed condition
Dry tooth surface (recommend-ed usage condition)	41.25 ±17.2	-	39.300±20.576	-	43.200 ±13.348	-
Wet	28.63 ±16.0	Significant	17.35±9.98	Significant	39.90±12.7	Not significant
Biofilm	47.15 ±16.3	Not significant	47.65±19.37	Not significant	46.65±12.91	Not significant
Stain	71.93±21.9	Significant	78.15±24.84	Significant	65.70±16.89	Significant
1 mm	45.20±21.8	Not significant	46.65±28.94	Not significant	43.75±11.74	Not significant
2 mm	48.08±21.5	Not significant	52.40±25.68	Not significant	43.75±15.74	Not significant
3 mm	48.15±18.5	Not significant	47.55±21.88	Not significant	48.75±14.81	Not significant
4 mm	46.72±18.6	Not significant	47.80±23.49	Not significant	45.65±12.50	Not significant
5 mm	39.15±17.7	Not significant	32.70±16.06	Not significant	45.60 ±17.34	Not significant

Effect of Stains on CS Readings

Irrespective of the type of surface, there was statistically significant difference ($p \leq 0.01$) in the CS readings between the stained (71.93±21.9) and the unstained (41.25 ±17.2) lesions. Similar trend was observed for both smooth (stained 65.70±16.89 vs unstained 43.20 ±13.35) and occlusal (78.15±24.84 vs 39.30±20.58) surface lesions at ($p \leq 0.01$).

DISCUSSION

The emphasis on caries management has increasingly leaned towards a preventive and minimally invasive strategy, highlighting the importance of defining efficacious, precise and reliable diagnostic instruments for early detection of caries. In a similar vein, the value of these tools in tracking the efficacy of preventive interventions over time has grown to be just as essential as their accuracy [10].

The CS has been utilized for the quantitative evaluation of the demineralization as well as remineralization of initial caries lesions on enamel following the application of a non-invasive therapeutic intervention [11]. It serves as a crucial diagnostic tool that relies on a unique combination of frequency-domain laser-induced infrared photothermal radiometry and modulated luminescence. By employing photothermal radiometry-luminescence, an innovative energy conversion technology, the CS can directly evaluate the condition of the tooth crystal structure. This state-of-the-art device operates by directing intensity-modulated laser light at a constant frequency onto the tooth, inducing luminescence (LUM) and subsequently converting the light into both heat and light, photo-thermal radiation (PTR) of longer wavelength, thereby producing thermal infrared and optical emissions at the identical frequency. These emitted signals are captured by specialized detectors, and then demodulated by two lock-in amplifiers. This results in the extraction of two amplitudes and two phases.

The distinctive characteristics of the tooth tissue, as revealed by these amplitudes and phases, undergo changes in response to demineralization of the tooth, with the extent of alteration being contingent upon the level of demineralization. The initial mineral loss in the tooth, known as incipient caries, triggers subtle modifications in the ultrastructure, leading to the creation of a more porous and less dense environment. Consequently, this process amplifies the generated PTR signals while diminishing the LUM signals emanating from the tooth, culminating in a proportional escalation in the Canary number. This intricate process elucidates the progressive nature of caries detection, and underscores the significance of advanced technological applications in enhancing diagnostic capabilities [12].

Despite the positive outcomes observed, the application of the CS in clinical settings remains delicate due to the impact of various factors on its evaluation of caries lesions [13]. It has been recommended that establishing a reliable standardization is crucial in order to prevent inaccurate positive diagnoses [14]. Therefore, the primary objective of the present study was to investigate the influence of the moisture level (dry or wet) of caries lesions, the distance between the probe tip of the device and the surfaces of lesions, the presence of bacterial biofilm on lesion surfaces, and the staining of lesions, on the assessment of caries lesions by the CS.

The study results revealed that moisture on the surface of lesions located in occlusal pits and fissures significantly reduced the Canary number, indicating less carious affection. This has important implications in clinical practice, as it may result in early-stage caries lesions not being detected by the CS, and lesions at advanced stages being mistakenly perceived as amenable to remineralization rather than requiring restorative treatment. This adverse effect of moisture could be attributed to the pooling of water (puddle) in deep occlusal pits and fissures. Nevertheless, there was no impact of moisture on smooth surfaces, as there was no statistically significant distinction between the dry and wet conditions, possibly due to absence of pits and fissures.

The staining investigation outcomes indicated that regardless of the location of the caries lesion on the tooth surface (smooth or occlusal), staining of the lesion led to a significant increase in the Canary number, falsely indicating a more severe condition. This could have significant implications in the clinical use of the CS for monitoring caries lesions during treatment. The Canary number is expected to decrease during treatment if the lesion is remineralizing and increase if the lesion is further demineralizing. A caries lesion that is assessed and treated during the initial clinical visit may wrongly appear to progress in subsequent visits if stained by chromogens from food substances like coffee or tea. Therefore, the CS may not be suitable for patients with caries lesions prone to staining, such as tobacco users or heavy coffee and tea consumers.

Interestingly, the results of the study showed that the readings of the CS were not affected by factors such as the presence of biofilm, and the measuring distance between the probe tip of the devise and the surface of the lesion up to 5 mm investigated in the present study. The outcomes of the present study can be explained by the capability of the CS to collect data from a hemispherical region measuring 1.5 mm in diameter, located up to 5 mm beneath the surface of the tooth and not just confined to the surface of the tooth. These findings corroborate the notion that an area extending at least 5 mm below the enamel surface can be assessed and examined when the wavelength and modulation frequency of the PTR signal are optimized[15]. Moreover, the results of the study came in agreement with a previous study which reported that the CS has the ability to accurately detect caries even beneath the pits and fissure sealants with high specificity of 94% when compared to DIAGNOdent [16].

In summary, this research validated the efficacy of the Canary System, utilizing Frequency Domain Photothermal Radiometry and Modulated Luminescence (FD-PTR and LUM), as a reliable tool for detecting and monitoring caries lesions. Its efficacy is unaffected by the distance of the optical tip (1mm to 5mm), biofilm, and moisture (smooth surfaces/moisture affecting occlusal surfaces) but is notably influenced by stains. Hence, establishing the Canary System as a proficient method for identifying and tracking tooth decay without the requirement for dental x-rays or invasive procedures, provided the lesions are free from stains and dry to ensure accurate quantification of dental caries lesions.

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