UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

ANÁLISE OBJETIVA DE LESÕES CERVICAIS NÃO CARIOSAS ATRAVÉS DE ESCANEAMENTO DIGITAL TRIDIMENSIONAL

Caroline de Farias Charamba

2021

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia, da Universidade Federal da Paraíba, como parte dos requisitos para obtenção do título de Mestre em Odontologia

Orientador: Prof. Dr. Anderson Takeo Hara
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João Pessoa - PB
2021

Catalogação de Publicação na Fonte. UFPB - Biblioteca Central

C469a Charamba, Caroline de Farias.
Análise objetiva de lesões cervicais não cariosas através de escaneamento digital tridimensional / Caroline de Farias Charamba. - João Pessoa, 2021.
49 f. : il.

Orientação: Anderson Takeo Hara.
Coorientação: Frederico Barbosa de Sousa.
Dissertação (Mestrado) - UFPB/CCS.

1. Abrasão dentária. 2. Dentifrício. 3. Tecnologia odontológica. I. Hara, Anderson Takeo. II. Sousa, Frederico Barbosa de. III. Título.

UFPB/CCS CDU 616.314-001.4(043)

Elaborado por ANNA REGINA DA SILVA RIBEIRO - CRB-15/024

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DEDICATÓRIA

Dedico este trabalho aos meus pais, por sempre me ensinarem que a educação é o melhor caminho e por fazerem de tudo para que eu sempre alçasse voos mais altos. Às minhas irmãs, que sempre me incentivaram. Ao meu namorado por acreditar no meu melhor. Aos meus sobrinhos que trouxeram mais alegria para minha vida.

AGRADECIMENTOS

Aos meus pais (André e Sara) que não medem esforços para que eu realize meus sonhos. Obrigada por toda dedicação ao longo de minha vida, por cada conselho, por cada renúncia, sem vocês não seria nem metade do que sou. Às minhas irmãs (Andrea, Ana Luiza e Amanda) que são minhas companheiras de vida, que vibram comigo cada conquista como se fosse delas. Obrigada pelo amor ofertado. Aos meus sobrinhos (Malu, Alice e João) que me mostraram uma nova face do amor.

Aos meus avós (José e Benedita) que foram primordiais em minha educação, mesmo ausentes sei que se alegrariam de ver aonde cheguei. À minha madrinha (Antonete), que sempre se fez presente desde minha infância e que contribuiu para quem eu sou hoje. Aos meus cunhados (Danilo, Rafael e Abelardo) que sempre estão dispostos a me ajudar.

Ao meu namorado, Neto, o qual presenciou desde minha graduação meu desejo de ingressar no mestrado. Obrigada por me incentivar a crescer, a buscar o melhor na vida pessoal e profissional, obrigada por me mostrar que sou capaz quando muitas vezes nem eu acredito. À família Leal, que torce por cada conquista minha e me acolheu muito bem em suas vidas.

Ao meu orientador, Anderson. Considero-me sortuda por ter tido um profissional tão capacitado para me orientar ao longo desses dois anos. Mais do que o arcabouço intelectual ofertado, o senhor sempre foi uma pessoa incrível, ensinando, sendo paciente, compreensivo e responsável. Levarei sua dedicação e responsabilidade como exemplo.

Ao meu coorientador, Frederico. Sem dúvidas o senhor demonstra um amor muito grande pela docência e pesquisa, sua responsabilidade e dedicação são exemplares. Obrigada por me receber no LABIAL, tomarei o senhor como exemplo de profissionalismo.

À todos os professores do PPGO, pelos ensinamentos ofertados e pelo exemplo de dedicação a vida acadêmica.

À minha eterna orientadora, Ana Karina, que na graduação despertou meu interesse para a pesquisa e docência. Obrigada por cada conselho, ajuda e por estar sempre presente.

Ao professor André que me acompanha desde a graduação. Obrigada por ceder o LABIO e por aceitar participar da avaliação do meu trabalho. O senhor faz parte de tudo isso. À professora Raquel que gentilmente aceitou participar deste momento.

Às minhas amigas queridas, Louandrys e Fernanda, que escutam cada sonho meu e me incentivam a ir atrás de consegui-los. Que possamos sempre fazer isso umas pelas outras.

Agradeço pelo suporte financeiro concedido pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

“Ninguém vence sozinho, nem no campo, nem na vida”.

Papa Francisco

RESUMO

O objetivo deste estudo foi explorar o uso do escaner intraoral 3D para a detecção e monitorização de lesões cervicais não cariosas (LCNCs) simuladas *in vitro*. Foram analisadas 288 LCNCs de diferentes severidades, simuladas em um modelo laboratorial onde variou-se a rigidez da escova dental (macia, média e dura) e a abrasividade do slurry abrasivo (baixa, média, alta e controle negativo). As impressões dentárias foram feitas ao início e depois de 35.000 e 65.000 ciclos de escovação, e depois digitalizadas com um escaner CEREC Omnicam, para análise da perda volumétrica nas LCNCs. Esses resultados foram comparados com os da perfilometria óptica 3D, considerada como padrão de ouro. Os dados foram analisados usando os testes ANOVA e PLSD de Fisher (alfa=0,05), e a concordância entre métodos usando o coeficiente de correlação intraclasse e gráfico de Bland-Altman. Escovas dentais dura e média causaram maior perda dental do que as macias, quando associadas ao slurry mais abrasivo, após 35000 e 65000 ciclos ($p<0,001$). A variação da abrasividade do slurry causou diferenças na perda dental (controle<baixa<média<alta, $p<0,0001$) após 35000 e 65000 ciclos, independentemente do tipo de escova dental, exceto após 35000 ciclos, quando controle=baixo ($p=0,55$). Menor perda dental foi observada após 35000 em relação a 65000 ciclos para todos slurries ($p<0,0001$), exceto para o controle. O coeficiente de correlação intraclasse para a concordância entre os métodos de teste e o padrão ouro foi de 0,85. A análise de imagens 3D do escaner intraoral pode detectar e monitorar a progressão das NCCLs, embora esta capacidade seja limitada em lesões incipientes. Foi encontrada uma boa concordância geral entre o método de teste e a perfilometria óptica. O método sugerido apresentou potencial para detectar e monitorizar LCNCs clinicamente.

Palavras-chave: Abrasão dentária, dentifrícios, tecnologia odontológica.

ABSTRACT

To explore the use of 3D intraoral scanner/image analysis for the detection and monitoring of simulated non-carious cervical lesions (NCCLs) *in vitro*. A total of 288 NCCLs of different severities and simulated using a laboratorial model associating toothbrush stiffness (soft, medium and hard) and toothpaste abrasivity (low, medium, high and negative control) were analyzed. Dental impressions were taken from specimens before and after 35K and 65K brushing strokes, and then scanned with a CEREC Omnicam scanner. 3D models were analyzed for volumetric tooth loss. 3D optical profilometry was considered as gold-standard. Data were analyzed using ANOVA and Fisher's PLSD tests (alpha=0.05), and agreement between methods by using intraclass correlation coefficient. Toothbrushes of hard and mid stiffness caused higher tooth loss than soft when associated with the highest abrasive, at 35K and 65K strokes ($p<0.001$). Variation in slurry abrasivity led to differences in tooth loss (with control<low<medium<high, $p<0.0001$) after both 35K and 65K strokes, regardless of the type of toothbrush used, except at 35K, wherein control=low ($p=0.55$). 35K strokes caused less tooth loss than 65K for all abrasive slurries ($p<0.0001$) except controls. The intra-class correlation coefficient for agreement between the test and gold-standard methods was 0.85. Analysis of 3D images from intraoral scanner could detect and monitor NCCLs progression, although this ability was limited on incipient lesions. Overall good agreement was found between the test method and optical profilometry. The suggested method may be applicable to detect and monitor NCCLs clinically.

Keywords: non-carious cervical lesions, toothbrush, toothpaste, dentifrice, abrasivity, dental technology.

LISTA DE ABREVIATURAS E SIGLAS

UFPB	Universidade Federal da Paraíba
ANOVA	Análise de Variância
LCNCs	Lesões Cervicais Não Cariosas
3D	Tridimensional
PLSD	Diferenças menos significativas protegidas
mm	Milímetros
mm^3	Milímetros cúbicos
g	Gramas
Min	Minutos
ISO	Organização Internacional de Normatização

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1. INTRODUÇÃO

Lesões cervicais não cariosas (LCNCs) caracterizam-se pela presença de defeitos na região do colo dental resultantes da perda de tecido mineral, cuja origem não está relacionada com a cárie[1,2]. LCNCs apresentam prevalência média de 60%[3,4], comprometem a estrutura dental e a estética, estão associadas à hipersensibilidade dentinária e podem progredir expondo a polpa. Desta forma, estas lesões são bastante relevantes do ponto de vista da saúde bucal, principalmente a longo prazo[1]. A importância desse tema se faz mais específica para a América do Sul, que dentre os continentes é o que apresenta prevalência relativamente mais alta. Estudos prévios indicam que o Brasil se sobressai com uma prevalência média observada de LCNCs em torno de 69%[5].

A prevalência de LCNCs aumenta com a idade, atinge mais homens, acomete mais a face vestibular do dente, sendo os mais susceptíveis a esse tipo de lesão o primeiro pré-molar superior, segundo pré-molar superior, caninos, seguido pelos molares[3,4,6-9]. As LCNCs estão relacionadas com recessão gengival e com o nível ósseo alveolar, que em situações de doença periodontal está diminuído contribuindo para a exposição radicular, que ficará mais susceptível aos eventos causadores das LCNCs. Portanto, manter a saúde periodontal é um fator que contribui para prevenção desse tipo de lesão[10]

A etiologia das LCNCs ainda permanece controversa[1], mas a literatura mostra associação com escovação horizontal, força aplicada na escovação, dureza das cerdas da escova de dente, frequência de escovação, abrasividade do dentifrício, consumo de comidas e bebidas ácidas, e condições sistêmicas que levam à presença de suco gástrico na cavidade bucal [3,4,7,9,11,16]. Frente a isso, as lesões podem ser originadas por processos: 1) corrosivos (usualmente conhecidos por erosão dental), quando a perda de estrutura dental é devida à exposição a ácidos de origem não bacteriana e agentes quelantes[12]; 2) de fadiga (geralmente denominada abfração), quando a perda de tecido se relaciona a cargas oclusais excessivas[13]; e 3) abrasivos, causada por insultos mecânicos como a escovação[14].

No tocante ao desgaste dental, é imperativo que se realize um diagnóstico precoce e correto. Assim, é primordial a existência de métodos que qualifiquem e quantifiquem as lesões de modo que seja possível avaliar sua severidade e monitorar sua progressão. Em estudos laboratoriais isso é possível através de

métodos como perfilometria, radiomicrografia, microdureza[15]. A perfilometria é considerada o padrão-ouro nos estudos *in vitro* e é utilizada em diversos experimentos[16-18]. Porém, esses métodos são restritos aos laboratórios de pesquisa, fazendo com que sua aplicação clínica não seja possível[19,20].

A detecção de LCNCs clinicamente pode ser feita através de diversos índices, incluindo o *Tooth Wear Evaluation System* (TWES). Esse método consiste em primariamente avaliar sinais clínicos para classificar a lesão em erosão, abrasão ou atrição. O segundo passo, seria o que os autores chamam de quantificar, ação que consiste em avaliar as faces dentais e aplicar escores que designam a presença ou não de desgaste dental e quais tecidos são envolvidos. Em lesões atingindo dentina propõe-se medir a coroa dental com auxílio de uma sonda periodontal, mas existe um viés já que alguns desgastes não levam a diminuição cervico-incisal do elemento dental. O último passo desse método seria o registro da lesão, seja pela confecção de modelos ou fotografias[15]. Outro índice comumente utilizado é o *Basic Erosive Wear Examination* (BEWE), que classifica o grau de severidade em cada superfície dental em uma escala ordinal que varia de 0 (hígido) à 3 (perda substancial de estrutura dental em mais de 50% da face dental). É possível também pontuar os sextantes com o escore mais severo e em seguida somar os valores obtidos, para se chegar a uma classificação que definirá a abordagem terapêutica a ser seguida[21].

Porém, o que se observa é que os índices clínicos são subjetivos, necessitam de um profissional experiente para resultados confiáveis e possuem o viés de interpretação do examinador[22].Nesse cenário, vem sido sugerido na literatura o uso do escaner intraoral como uma ferramenta objetiva que permite um diagnóstico e acompanhamento das LCNCs. Os escaners apresentam a vantagem de serem utilizados na cadeira odontológica e por permitirem uma detecção precoce da lesão[19,23,24].

Um recente estudo utilizou o escaner intraoral para obter imagens de elementos dentais e restaurações feitas de zircônia. Não foi realizada uma investigação no tocante ao volume de LCNCs, mas as imagens obtidas foram utilizadas para calcular o desgaste dental/da zircônia através da superimposição de imagens. Esse método mostrou um bom coeficiente de correlação com o

microtomógrafo, revelando que o escaner intraoral é uma ferramenta em potencial para mensurar o desgaste dental[25].

Com o intuito de explorar essa nova tecnologia, o presente estudo objetivou utilizar o escaner intraoral 3D e análise de imagens para detecção e monitorização da progressão de LCNCs simuladas *in vitro*. Com isso, esse estudo pode contribuir para o diagnóstico precoce desse tipo de lesão, monitoramento de sua progressão e na orientação para que a conduta profissional seja a mais adequada de acordo com a severidade, uma vez que fornece uma metodologia mais objetiva de avaliação da LCNC.

2. CAPÍTULO 1

O manuscrito a seguir foi publicado no periódico "Clinical Oral Investigations" (Fator de Impacto: 2.812 / Clin Oral Investig. 2021 Jan 19. doi: 10.1007/s00784-020-03737-z. Online ahead of print).

Objective Assessment of Simulated Non-Carious Cervical Lesion by Tridimensional Digital Scanning

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Keywords: Non-carious cervical lesions, toothbrush, toothpaste, dentifrice, abrasivity, dental technology.

ABSTRACT

OBJECTIVE: To explore the use of 3D intraoral scanner/image analysis for the detection and monitoring of simulated non-carious cervical lesions (NCCLs) *in vitro*.
MATERIALS AND METHODS: A total of 288 NCCLs of different severities and simulated using a laboratorial model associating toothbrush stiffness (soft, medium and hard) and toothpaste abrasivity (low, medium, high and negative control) were analyzed. Dental impressions were taken from specimens before and after 35K and 65K brushing strokes, and then scanned with a CEREC Omnicam scanner. 3D models were analyzed for volumetric tooth loss. 3D optical profilometry was considered as gold-standard. Data were analyzed using ANOVA and Fisher's PLSD tests (alpha=0.05), and agreement between methods by using intraclass correlation coefficient.
RESULTS:Toothbrushes of hard and mid stiffness caused higher tooth loss than soft when associated with the highest abrasive, at 35K and 65K strokes ($p<0.001$). Variation in slurry abrasivity led to differences in tooth loss (with control<low<medium<high, $p<0.0001$) after both 35K and 65K strokes, regardless of the type of toothbrush used, except at 35K, wherein control=low ($p=0.55$). 35K strokes caused less tooth loss than 65K for all abrasive slurries ($p<0.0001$) except controls. The intra-class correlation coefficient for agreement between the test and gold-standard methods was 0.85.
CONCLUSIONS: Analysis of 3D images from intraoral scanner could detect and monitor NCCLs progression, although this ability was limited on incipient lesions. Overall good agreement was found between the test method and optical profilometry.
CLINICAL RELEVANCE: The suggested method may be applicable to detect and monitor NCCLs clinically.

INTRODUCTION

Non-carious cervical lesions (NCCLs) can be defined as loss of tooth structure at the cemento-enamel junction area not related to dental caries [1,2]. They can directly impact tooth form, function and esthetics, and be associated with pain, rendering NCCLs very relevant when considering long-term oral health [1]. The prevalence of NCCLs varies, but typically occur in approximately 60% of dental patients [3,4]. Prevalence increases with age, is higher in males and on buccal dental surfaces, and affects mostly upper premolars and canines, followed by molars [3-8].

The etiology of NCCLs remains a topic of debate and ongoing research [1], with reports suggesting associations to horizontal toothbrushing, toothbrushing force, toothbrushing frequency, toothbrush bristle hardness, consumption of acidic foods and drinks and/or esophageal reflux [3,4,6,8,9]. The proposed processes responsible for NCCLs can be parsed into three principal types: 1) corrosion (usually known as dental erosion), when the loss of dental structure is due to exposure to acids of non-bacterial origin and chelating agents [10]; 2) fatigue (usually referred as dental abfraction), when tissue loss is related to excessive occlusal forces [11], although this has been a cause of controversy [1]; and 3) dental abrasion, which is caused by mechanical insults such as toothbrushing [12].

Dental practitioners agree on the paramount importance of early clinical detection and longitudinal assessment and monitoring of tooth wear levels [13]. The distinction between physiological and pathological rates of tooth wear impacts the implementation of preventive and therapeutic measures [14]. NCCLs are conventionally monitored clinically by subjective means, including analysis of photographs and study cast models, as well as by the use of tooth wear indices, like the Basic Erosive Wear Examination (BEWE) [15]. Although these methods are useful, they each have shortcomings. Clinical photographs are limited by difficulty in standardizing settings (positioning, lighting, etc) for longitudinal comparisons and study casts require substantial space for physical storage [14]. Further, the BEWE index [16] is subjective and results are dependent on examiner experience [17]. Therefore, the development of an objective evaluation tool performed in real-time at chair-side would be extremely beneficial [18].

Recent technological advances have positively impacted digital dentistry. For instance, intraoral scanners have allowed the rapid acquisition of 3D models of teeth with several applications in diverse areas such as prosthodontics, orthodontics, dental surgery, aesthetic dentistry and dental implantology [19]. Recent studies have also suggested that intraoral scanners can be promising tools for the early diagnostic and assessment of tooth wear given their ease of use and growing ubiquity in dental practices today; however testing and further development is needed [14,18,20*]*. In order to further explore and advance this field, our aim in this study was to test the use of 3D intraoral scanner and image analysis for the detection and monitoring of the progression of in vitro simulated NCCLs. The null hypothesis was that there is no difference in the values of the dental volumes obtained when using the intraoral 3D scanner when compared to those obtained by optical profilometry.

MATERIALS AND METHODS

Experimental Design

This study employed dental impressions previously created and analyzed by optical profilometry [21]. In the current study, they were scanned with the 3D intraoral scanner test system and image analysis was subsequently performed to determine volumetric tooth loss. All the procedures were performed in random and blind conditions. In the previous original study, NCCL severity levels were simulated by the association of three experimental factors: 1) toothbrush stiffness (soft, medium and hard), 2) toothpaste abrasivity (low, medium, high and negative control), 3) number of simulated brushing strokes (35K and 65K). A total of 24 groups (n = 24 samples), with different levels of volumetric tooth loss, stemmed from the associations of the study factors. The newly generated data were analyzed considering the experimental factors tested, and also correlated with the data original data from the previous study.

Specimen Preparation, Treatment and Impression

This study used the specimens' impressions created in the original study, where details of their preparation and treatment are fully described [21]. In brief, 288 upper first premolars were randomized into 12 stiffness×abrasivity groups (n=24).

They were brushed in an automated V-8 toothbrushing machine for 35K and 65K double strokes, using toothbrushes of different stiffness (soft, medium, hard, Lactona Corp., Bergen op Zoom, The Netherlands) associated to slurries containing hydrated silica of different abrasive levels (low/Zeodent 113, medium/Zeodent 124, high/Zeodent 103, Huber Engineered Materials, Havre de Grace, MD, USA), prepared according to the ISO11609 guidelines for dentifrice abrasivity testing. A total of three impressions were made for each specimen (at baseline and after 35K and 65K brushing strokes), with an elastomeric impression material (hydrophilic vinyl polysiloxane, Examix NDS Monophase, GC America, Alsip, IL, USA). The impressions were labeled and kept stored in dry conditions at room temperature.

Specimen's Impression Evaluation

In the original study, each specimen's impression was scanned with an optical profilometer using a type S65/10 sensor (resolution of 0.30 µm, Proscan 2000, Scantron, Taunton, UK) and analyzed with the Proform Software (Scantron) using the superimposition and subtraction functions to obtain the tooth volume loss data after each brushing period [21].

In the present study, the dental impressions were scanned with a CEREC Omnicam 4.6.1 (Dentsply-Sirona, Bensheim, Germany) intraoral scanner with white light IOS and triangulation technology to produce digital impressions. Based on root-mean-square evaluation criterion, the scanner's reported accuracy parameters for single tooth scan are: trueness of 13.8 µm (± 1.4 µm) and precision of 12.5 µm (± 3.7 µm) [22]. A previously trained examiner performed all the scans to ensure consistency. Scanner calibration and use followed the manufacturer's instructions. The scanner was allowed to warmup for 20 minutes prior to use. The impressions were scanned paying attention to the sound emitted by the equipment, which helps in carrying out the correct procedure and maintaining ideal measuring distance (0-15 mm) between the camera and tooth surface. Visual inspection was performed to verify that all surfaces of each specimen were properly captured (Fig. 1), and scans were saved in stereolithography (.STL) format for subsequent analysis.

Tooth Volume Loss Analysis

The .STL output files were opened in Geomagic Wrap 2017 (3D Systems, Rock Hill, SC, USA) 3D modelling software. The volume of each model was

recorded using the software. Wrap's lasso tool was then applied to select surfaces affected by the simulated lesions, and those surfaces were deleted from the model. The resulting holes were then filled using the curvature fill function, which interpolates the surface using a nearest neighbor polygon algorithm as a guide. Resulting surfaces were compared with original baseline surfaces to confirm the accuracy of the fill algorithm using models of specimens scanned during the original study. This task was first performed independently for each tooth and resultant tooth loss volume was computed. The difference in volume between the original model and that filled was used to calculate volume loss associated with each NCCL simulation.

Statistical Analysis

A mixed-model ANOVA was used to analyze volumetric loss, with fixed effects for toothpaste abrasivity, toothbrush stiffness, brushing strokes, and their interactions, a random effect for the right-left pairing within specimens, and a repeated effect for brushing strokes within the specimens. Pairwise comparisons utilize Fisher's Protected Least Significant Differences method. Agreement between the tooth structure loss measurements from the digital intraoral scanner and the lab optical profilometer was assessed using a Bland-Altman plot and an intra-class correlation coefficient. A 5% significance level was used for all tests. Analyses were performed using SAS version 9.4.

RESULTS

Due to non-normality, a log (base 10) transformation was performed on the volumetric loss data prior to the ANOVA. Overall, the effects of toothpaste abrasivity, toothbrush stiffness, and brushing strokes were significant, as were the slurry-toothbrush and slurry-strokes interactions; whereas the toothbrush-strokes and slurry-toothbrush-strokes were not significant.

After 35K (Table 1), brushed samples evinced significantly less tooth structure loss than after 65K (Table 2) for high, medium and low abrasives slurries ($p<0.0001$), but not for control ($p=0.16$). Toothbrush stiffness hard and mid had significantly higher tooth structure loss than soft for the high abrasive slurry ($p<0.001$). No other significant differences were found between toothbrushes

(p>0.05). Deionized water (DIW, negative control) had significantly less tooth structure loss than high and medium abrasives slurries. DIW had also significantly less tooth structure loss than low abrasive for 65K strokes (p<0.0001) but not for 35K strokes (p=0.55). Low abrasive had significantly less tooth surface loss than high and medium abrasives (p<0.001). Medium abrasive had significantly less tooth surface loss than high abrasive (p<0.001).

The intraclass correlation coefficient for agreement between the digital intraoral scanner and the optical profilometer was 0.85 (Fig. 2). Disagreements ranged from 8.0 mm^3 lower to 13.9 mm^3 higher for the digital intraoral scanner than the optical profilometer, with an overall average difference of 0.2 mm^3 higher for the digital intraoral scanner. The limits of agreement on the Bland-Altman plot, mean difference +/- 2 standard deviations, were at +4.0 mm^3 and -4.3 mm^3. The plot suggests that the digital intraoral scanner underestimated tooth structure loss for measurements less than ~2.5 mm^3 and overestimated larger tooth structure loss compared to the optical profilometer. Additionally, the plot shows that the differences in the measured volume loss between the two methods increases in magnitude with the amount of structure loss.

DISCUSSION

The fill-algorithm analysis of 3D models obtained using the intraoral scanner detected significant effects for toothpaste abrasivity, toothbrush stiffness and brushing strokes, as well as for the slurry-toothbrush and slurry-stroke interactions. Overall, the increase of toothbrushing strokes led to an increase of dentin volume loss, or progression of the lesion. There were significant differences in volume loss for all groups brushed with abrasive slurries between 35K and 65K strokes. This result was expected considering the development pattern of NCCLs and reinforce the importance of toothbrushing strokes when designing a NCCL experimental model, as better differentiation of abrasive effects was observed in advanced lesions at 65K strokes, compared to the early lesions at 35K strokes.

Toothbrushes of hard and mid stiffness showed significantly higher tooth structure loss than those of soft stiffness, but only when associated with the highest abrasive (Z103), after both 35K and 65K strokes. The toothbrush filament stiffness seems to impact how the abrasives particles from the toothpaste interact with the

tooth surface [23]. Previous investigations have observed similar results where higher abrasive slurries and hard toothbrush stiffness cause higher tooth wear [21, 23]. However, other studies have shown opposite results, which are explained by the fact that soft toothbrushes are more capable of carrying the abrasive particles [24]. The difference in these results may be related to differences between the experimental methods. For instance, the current study used a toothbrushing load of 200 g and frequency of 260 strokes/min, whereas Alshehab et al. (2018) [24] used a load of 250g with frequency of 150 strokes/min, which might have emphasized more the interactions among toothbrush, abrasives and tooth. In addition, different toothbrushing simulation times were used, with final brushing times of 65K strokes in the present study compared to 4.2K strokes in the previous one. Therefore, more severe lesions were simulated in the present work.

The 3D analysis performed differentiated surface loss resulting from different abrasive slurries used after both 35K and 65K strokes regardless of the type of toothbrush used. Our results are in accordance with previous studies able to directly correlate tooth wear with slurry abrasivity [25,26]. At 35K, no differences were found between the low abrasive slurry and the negative control group. This can be explained by the fact that the low abrasive slurry may not generate detectable lesions at 35K, but at 65K these lesions would be sufficiently pronounced to be detected using this method. This finding may also be related to a limitation of our analytical protocol involving virtual fill of the lesion. In fact, incipient lesions may be of insufficient depth and volume loss to be measured by filling.

The main goal of this study was to compare the results obtained in a previous study [21] with a method based on the use of 3D intraoral scanners and image analysis. In the current study, there was no significant difference between the brushing strokes (35K vs 65K) when brushing with water (negative control); whereas in the previous study, higher volume loss was observed at 65K. A second difference was that the present data shows tooth volume loss when brushing with the medium abrasive (Z124) not affected by the stiffness of the toothbrush, whereas in the previous study hard and mid stiffness were significantly higher than soft. A third difference was that hard toothbrush caused significantly higher dental structure loss than the mid toothbrushes when brushed with the more abrasive slurry (Z103), when using optical profilometry. A final difference was that in the previous study, there

was significant difference in the tooth volume loss between groups brushed with water (control) and groups brushed with low abrasive slurry (Z113), for both 35K and 65K strokes. This contrast in results seem to be directly related to limitations in sensitivity of the current method for detecting and measuring incipient lesions, as pointed out above.

When testing the correlation between the current and previous (original) data at both 35K and 65K, we observed that the overall values for the more initial lesions (groups brushed with DIW) were numerically lower; whereas the values for the more advanced lesions (groups brushed with high abrasive slurry) were numerically higher. This can be visualized through the Bland-Altman plot that shows the intraoral scanner to underestimate the tooth structure loss less than ~2.5mm³ and to overestimate the tooth structure loss for larger structure losses. This underscores limitations of measuring the most incipient lesions using the intraoral scanning protocol. For the advanced lesions, differences may relate to limitations in the digital filling algorithm, and lack of the original dental surface to be used as a reference. Differences between the current and original methods are also evident from variation in volume differences between 35K and 65K, as we observed an average increase rate of 11% and 48% for the groups brushed with DIW in the profilometry and intraoral scanner studies, respectively. Despite these specific differences, though, the comparison between the two sets of data showed a high correlation coefficient agreement of 0.85 [27]. Our results suggest that relatively smaller simulated lesions were not properly detected/measured by the intraoral scanner, indicating a limitation of this method. A previous in vitro investigation similarly concluded that smaller volume changes (approximately 1.5 mm^3) measured by intraoral scanner were inconsistent at this level [20].

While the optical profilometry method [21] is more sensitive for documenting incipient NCCLs, the approach is unfeasible for direct clinical assessment [20,27]. In contrast, the intraoral scanning protocol can be applied routinely by practitioners at chairside, it is non-invasive, and the required instrumentation is becoming increasingly common in dental practices. Moreover, unlike the optical profilometry protocol, the fill algorithm used with the intraoral approach does not require a baseline measurement for subtraction of volume. This therefore can be used to quantify and monitor NCCLs once they are deep enough to be visualized by the

dentist. And the lack of sensitivity of the intraoral scanner to measure incipient NCCLs is less of an issue at that point, as only the smallest lesions cannot be detected using the currently presented approach. This fits nicely with the suggestion of O'Hara and Millar (2020) [14] for the use of 3D images to monitor the development and progression of tooth wear, as a potential way to overcome the barriers of the current clinical subjective methods. Additional advantages of the intraoral scanners are their reasonable cost and the reduced time to scan the dental surfaces and lesions (seconds per tooth).

Despite the above mentioned potential value of this method for the monitoring of NNCLs, we acknowledge that there is a need for further investigations to determine cut-off values or thresholds for the detection and progression of these lesions clinically. In the current study only two time points were considered for the analysis of the NCCLs, which limited this understanding. Therefore, more detailed laboratorial and clinical longitudinal studies using more evaluation time points will be valuable for assessing the ultimate potential of this new approach to monitoring and documenting NCCL progression.

ACKNOWLEDGMENT

This research was part of CFC's dissertation to be submitted in partial fulfillment of the MSD degree in Dentistry, from the Graduate Program in Dentistry at the Federal University of Paraiba, Brazil and part of JN's thesis submitted in partial fulfillment of honors requirements in Biomedical Engineering at the University of Arkansas.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: Caroline F. Charamba declares that she has no conflict of interest. James Needy declares that he has no conflict of interest. Peter S. Ungar declares that he has no conflict of interest. Frederico B. de Souza declares that he has no conflict of interest. George J. Eckert declares that he has no conflict of interest. Anderson T. Hara declares that he has no conflict of interest.

Funding: This study was partially supported by Nacional Council for Scientific and Technological Development (CNPq), Brazil; and by the Erosive Tooth Wear Research Program of the Indiana University School of Dentistry.

Ethical approval: All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: For this type of study, formal consent is not required.

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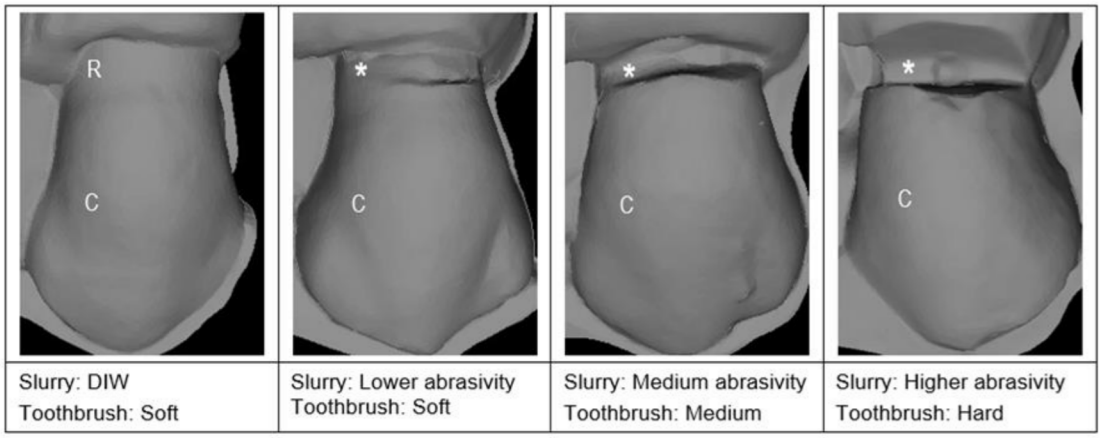
FIGURES & TABLE

Fig. 1. Representative images captured by the intra-oral scanner illustrating the range of simulated NCCLs, according to the slurry abrasivity and toothbrush stiffness levels (C: crown; R: root, *NCCL).



Slurry: DIW Toothbrush: Soft	Slurry: Lower abrasivity Toothbrush: Soft	Slurry: Medium abrasivity Toothbrush: Medium	Slurry: Higher abrasivity Toothbrush: Hard

Fig. 2. Agreement between Digital Intra-oral Scanner and Lab Optical Profilometer for measuring tooth structure loss.

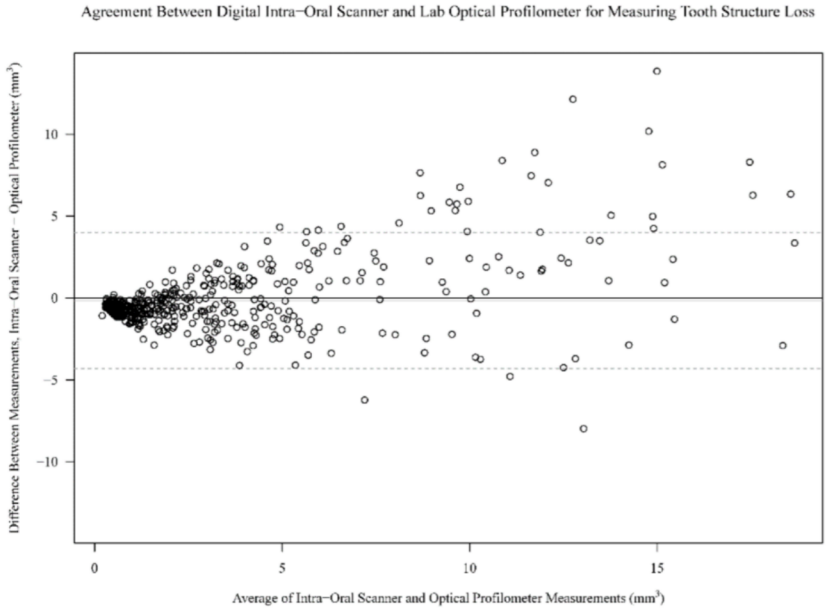


Table 1. Interaction between different toothbrushes (soft, medium, hard) and abrasives levels (control, low, medium, high) at 35K and 65K strokes. Volume loss (mm^3) means (standard-deviations) are presented.

Strokes	Slurry abrasivity	Soft	Medium	Hard
35,000	Lower	0.27 (0.25) Aa	0.37 (0.59) Aa	0.27 (0.38) Aa
	Medium	1.44 (0.63) Ba	1.96 (1.14) Ba	2.20 (0.91) Ba
	Higher	2.03 (1.39) Ca	4.64 (1.92) Cb	7.00 (3.87) Cb
	DI water (control)	0.21 (0.14) Aa	0.21 (0.14) Aa	0.26 (0.17) Aa
65,000	Lower	0.86 (0.36) Ba	1.00 (0.73) Ba	0.84 (0.59) Ba
	Medium	3.22 (1.37) Ca	4.42 (1.77) Ca	4.47 (1.36) Ca
	Higher	7.59 (3.20) Da	12.00 (4.75) Db	14.59 (4.52) Db
	DI water (control)	0.21 (0.13) Aa	0.24 (0.16) Aa	0.31 (0.20) Aa

Different letters represent significant differences ($p<0.05$): upper-case within rows; lower-case within columns.

3. CONSIDERAÇÕES GERAIS

Nossos resultados mostraram que a análise das imagens obtidas através do escaner intraoral foi capaz de detectar o aumento do volume de perda dental em todos os grupos quando comparado os dois tempos de escovação de 35000 e 65000 ciclos. Esse achado é reforçado pela literatura, que mostra que a formação das LCNCs se caracteriza pela perda incremental da estrutura dentária na região cervical ao longo do tempo afetando, portanto, principalmente adultos e idosos[9]. Estudos anteriores de Lintonjua et al.[26] (2004) e Sabrah et al.[17] (2018) mostraram resultados semelhantes. O grupo controle que não utilizou abrasivo não mostrou nenhuma diferença no desgaste dental ao longo do tempo. Estudos prévios não encontraram a formação de LCNCs quando a escovação simulada foi realizada apenas com água, com investigação feita visualmente[18] ou por perfilometria[27]. Esse comportamento é justificado pela ausência de abrasivos durante a escovação.

Os dados encontrados também permitiram identificar o comportamento das LCNCs sob influência dos filamentos da escova de dente. Filamentos de consistência média e dura foram os que causaram maior perda de tecido dental. Estudar essa variável é importante uma vez que a consistência das cerdas da escova afeta o modo que o abrasivo é transportado e interage com a superfície dental[28]. Também foi possível observar o efeito da abrasividade do slurry com o desgaste dental, sendo uma relação diretamente proporcional. Apesar disso, em grupos que formaram uma lesão menor, o método objetivo proposto de análise não foi capaz de diferenciá-los.

Apesar dessa limitação encontrada em lesões incipientes, o uso do escaner intraoral apresentou um alto coeficiente de correlação com a perfilometria, possuindo como vantagens o fato de poder ser utilizado clinicamente, ser de fácil uso e não invasivo. Portanto, este estudo evidenciou o potencial para uso clínico do escaner intraoral e análise digital das imagens para a detecção e monitoramento das LCNCs. Mais estudos laboratoriais e clínicos são necessários, sobretudo que utilizem mais pontos na linha do tempo para analisar melhor a sensibilidade deste novo método de quantificação das LCNCs.

4. CONCLUSÃO

A análise de imagens 3D obtidas a partir de um escaner intraoral pode detectar e monitorar a progressão das LCNCs, embora essa capacidade tenha demonstrado ser limitada em lesões incipientes. Em geral, foi encontrado um bom coeficiente de concordância entre o método de teste e a perfilometria óptica.

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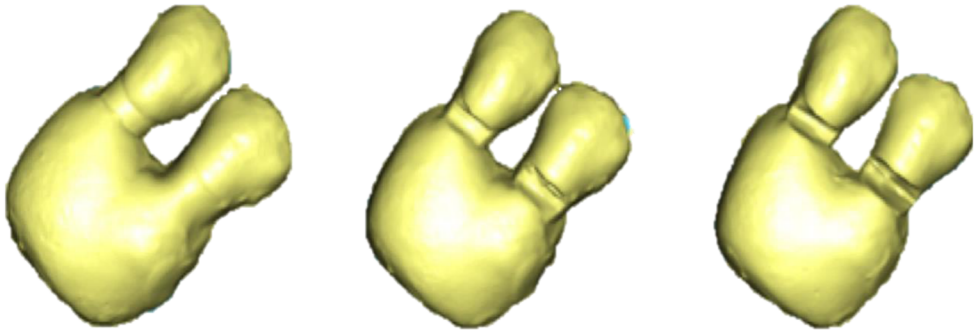
APÊNDICE A- Imagens obtidas através do Escaner intraoral

Figura 1- Imagem dos espécimes visualizados no programa *Geomatic*



Legenda: É possível ver o mesmo espécime em tempos de análise diferentes, sendo da esquerda para direita após 0 ciclos, 35k ciclos e 65k ciclos de escovação.

Figura 2- Restauração virtual dos espécimes

Legenda: Espécimes restaurados virtualmente. É possível observar primeiro a restauração do elemento dental à direita e depois do elemento dental à esquerda.

APÊNDICE B- Imagens obtidas através da perfilometria no estudo original.

Figura 1- Imagem tridimensional dos espécimes obtida após escaneamento com perfilometro ótico.

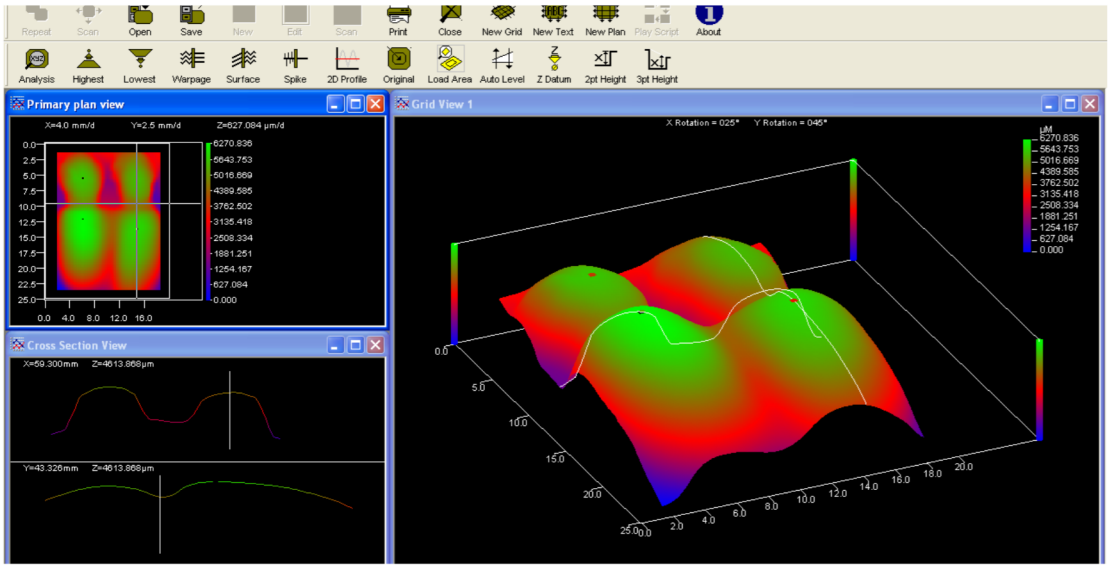
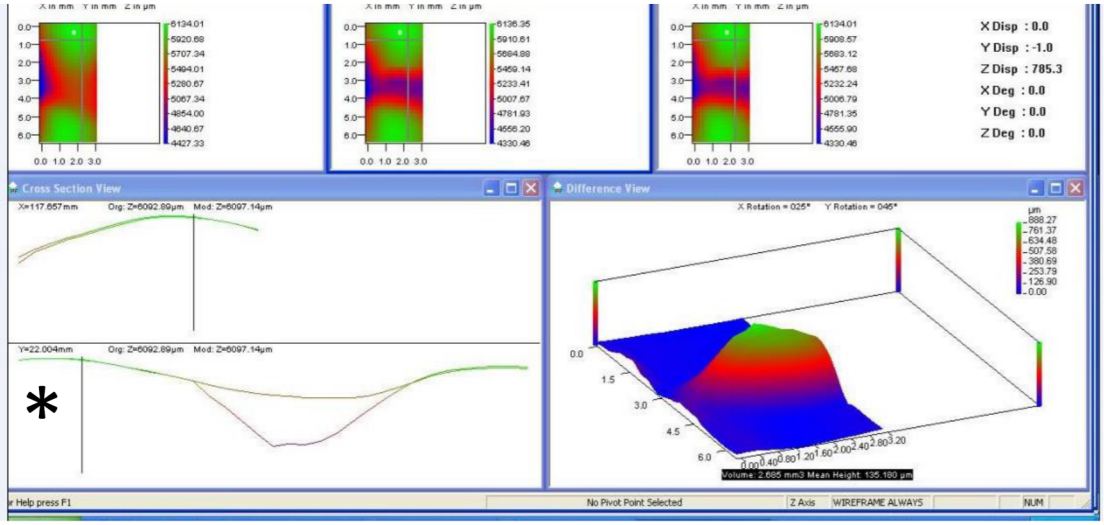


Figura 2- Análise de subtração de imagem da Lesão Cervical Não Cariosa



Legenda: *Cálculo da diferença do volume entre o escaneamento original e após a escovação.

ANEXO A- Certidão do Comitê de Ética do estudo original.

IUPUI/CLARIAN INSTITUTIONAL REVIEW BOARD (IRB) REVIEW

APPLICATION FOR RESEARCH NOT SUBJECT TO FDA OR COMMON RULE DEFINITIONS OF HUMAN SUBJECTS RESEARCH N50911-07

Please type only in the gray boxes. To mark a box as checked, double-click the box, select "checked", and click "OK".

SECTION I: PERSONNEL INFORMATION

Principal Investigator: Domenick Zero, DDS **Department:** Oral Health Research Institute

Building/Room No.: OH Phone: 317-274-8822 E-Mail: dzero@iupui.edu

Contact Information: Name: Sue Kelly, CCRC Address: OH 106

Phone: 274-3954 Fax: 274-5425 E-Mail: sakelly@iupui.edu

If this is a Student Protocol, List Name of the Student: Phone:

Study Title: **Collection of Human Teeth for In Vitro and In Situ Research Studies (03-D-158)**

Sponsor/Funding Agency: n/a Grant/Sponsor No.: /

Sponsor Protocol #/Grant #: Period: From: Nov 2009 to indefinite

Sponsor Type: ☐ Federal ☐ State ☐ Industry ☐ Not-for-Profit ☒ Unfunded; ☐ Internally Funded

Grant Title (if different from project title):

SECTION II: PROJECT TYPE

Refer to the **Checklist for Determining Whether an Activity Requires Review by the IUPUI/Clarian IRB** for additional information.

☐ **Research Involving Data on Decedent PHI.** Please indicate that the following criteria are satisfied:

- ☐ The use is solely for research on the identifiable health information of decedents.
- ☐ The PHI sought is necessary for the purposes of the research; and
- ☐ Upon request, the covered entity disclosing the data may require the investigator to provide documentation of the death of the individual(s) about whom information is being sought.

☐ **Limited Data Set.** The research uses or discloses PHI as a limited data set for research purposes.

This project type may only be selected if the following is true: Your data set excludes 16 specified identifiers that are listed in the regulations, including: name, street address, telephone and fax numbers, e-mail address, social security number, certificate/license number, vehicle identifiers and serial numbers, URLs and IP addresses, and full face photos and other comparable images. The limited data set could include the following identifiable information: admission, discharge, and service dates, date of death, age (including age 90 and older), and five digit zip code.

Indicate from where the data will be obtained:

- ☐ The data will be provided from a covered entity (e.g. division, department, or practice plan) separate from that of the investigator. **NOTE: A data use agreement must be established between the entity(ies) providing the data and the investigator.** See the **Confidentiality and Privacy SOP** for additional information.
- ☐ The data will be obtained from within the investigator's own covered entity (e.g. his/her own data or that of the department). **No data use agreement is required.**
- ☐ Other, please explain:

☐ **De-Identified Health Information.** The research involves the use or disclosure of de-identified health information.

This project type may only be selected if the following is true: The health information excludes all of the following: (1) Name; (2) All geographic subdivisions smaller than a state, including street address, city, county, precinct, zip codes if the geographic unit of combining all the same three initial digits contains more than 20,000 people; (3) All elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death; and all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated in a single

category of age 90 or older; (4) Telephone numbers; (5) Fax numbers; (6) Electronic mail addresses; (7) Social security numbers; (8) Medical record numbers; (9) Health plan beneficiary numbers; (10) Account numbers; (11) Certificate/license numbers; (12) Vehicle identifiers and serial numbers, including license plate numbers; (13) Device identifiers and serial numbers; (14) Web universal resource locators (URLs); (15) Internet protocol (IP) address numbers; (16) Biometric identifiers, including finger and voice prints; (17) Full face photographic images and any comparable images; and (18) Any other unique identifying number, character, or code.

☒ **Coded Private Information or Biological Specimens**. The research involves only coded private information or specimens.

To qualify for this type of review, the private information or specimens cannot be linked to specific individuals by the investigator(s) either directly or indirectly through coding systems. To qualify, both of the following conditions must be met:

☒ The private information or specimens were **not** collected specifically for this proposed research project through an interaction or intervention with living individuals. **NOTE: If this condition is not met, then your research involves human subjects and requires a human subjects research submission.**

AND

☒ The investigator(s) cannot readily ascertain the identity of the individuals to whom the private information or specimens pertain because: (mark which option(s) applies)

☐ the key to decipher the code will be destroyed before the research begins
☐ the investigator(s) and the holder of the key will enter into an agreement prohibiting the release of the key to the investigator(s) under any circumstances, until the individuals are deceased
☒ Other. Please explain: Human teeth are needed for in situ and in vitro studies. Dentists send teeth in large jars that are completely deidentified. The jars are emptied into vats that contain hundreds of teeth that cannot be identified.

For additional information on research with coded private information or biological specimens, please refer to the OHRP Guidance on Research Involving Coded Private Information or Biological Specimens (October 16, 2008) at: http://www.hhs.gov/ohrp/humansubjects/guidance/cdebiol.pdf.

SECTION III: PROJECT DESCRIPTION

1. Provide a brief description, in lay terms, of the purpose of the proposed project and the procedures to be used.

   The purpose of this project is to collect human teeth from oral surgeons around the country for development into enamel specimens for in situ and in vitro research studies. Specimens are received in bulk from dental offices and placed into large vats for sorting and development of specimens. No identifiers are provided from the oral surgeons collecting the teeth.

2. Provide a list of all data points that will be collected below or attach a data collection sheet.

   na

Statement of Principal Investigator. By submitting this form, the Principal Investigator acknowledges that he/she has personally reviewed this report and agrees with the above assessment.

For IRB Use Only:

☒ Accepted

☐ Denied
- ☐ Separate human subjects application must be submitted.
- ☐ Project doesn't meet ethical principles
- ☐ Other action required:

Authorized Signature: Regina Mininger Date: NOV 27 2009

ANEXO B- Artigo publicado na resista *Clinical Oral Investigations*

Clinical Oral Investigations
https://doi.org/10.1007/s00784-020-03737-z

ORIGINAL ARTICLE

Objective assessment of simulated non-carious cervical lesion by tridimensional digital scanning

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Received: 1 June 2020 / Accepted: 8 December 2020

Abstract

Objective To explore the use of 3D intraoral scanner/image analysis for the detection and monitoring of simulated non-carious cervical lesions (NCCLs) in vitro.

Materials and methods A total of 288 NCCLs of different severities and simulated using a laboratorial model associating toothbrush stiffness (soft, medium, and hard) and toothpaste abrasivity (low, medium, high, and negative control) were analyzed. Dental impressions were taken from specimens before and after 35K and 65K brushing strokes, and then scanned with a CEREC Omnicam scanner. 3D models were analyzed for volumetric tooth loss. 3D optical profilometry was considered as the gold standard. Data were analyzed using ANOVA and Fisher's PLSD tests (alpha = 0.05), and agreement between methods by using intraclass correlation coefficient.

Results Toothbrushes of hard and mid stiffness caused higher tooth loss than soft when associated with the highest abrasive, at 35K and 65K strokes ($p < 0.001$). Variation in slurry abrasivity led to differences in tooth loss (with control < low < medium < high, $p < 0.0001$) after both 35K and 65K strokes, regardless of the type of toothbrush used, except at 35K, wherein control = low ($p = 0.55$). 35K strokes caused less tooth loss than 65K for all abrasive slurries ($p < 0.0001$) except controls. The intraclass correlation coefficient for agreement between the test and gold standard methods was 0.85.

Conclusions Analysis of 3D images from intraoral scanner could detect and monitor NCCL progression, although this ability was limited on incipient lesions. Overall good agreement was found between the test method and optical profilometry.

Clinical relevance The suggested method may be applicable to detect and monitor NCCLs clinically.

Keywords Non-carious cervical lesions · Toothbrush · Toothpaste · Dentifrice · Abrasivity · Dental technology

Introduction

Non-carious cervical lesions (NCCLs) can be defined as loss of tooth structure at the cemento-enamel junction area not related to dental caries [1, 2]. They can directly impact tooth form, function, and esthetics, and be associated with pain, rendering NCCLs very relevant when considering long-term oral health [1]. The prevalence of NCCLs varies, but typically occurs in approximately 60% of dental patients [3, 4]. Prevalence increases with age, is higher in males and on buccal dental surfaces, and affects mostly upper premolars and canines, followed by molars [3–8].

The etiology of NCCLs remains a topic of debate and ongoing research [1], with reports suggesting associations with horizontal toothbrushing, toothbrushing force, toothbrushing frequency, toothbrush bristle hardness, consumption of acidic foods and drinks, and/or esophageal reflux [3, 4, 6, 8, 9]. The proposed processes responsible for NCCLs can be parsed into three principal types: (1) corrosion (usually known as dental erosion), when the loss of dental structure is due to exposure to acids of non-bacterial origin and chelating agents [10]; (2)

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Published online: 19 January 2021

fatigue (usually referred to as dental abfraction), when tissue loss is related to excessive occlusal forces [11], although this has been a cause of controversy [1]; and (3) dental abrasion, which is caused by mechanical insults such as toothbrushing [12].

Dental practitioners agree on the paramount importance of early clinical detection and longitudinal assessment and monitoring of tooth wear levels [13]. The distinction between physiological and pathological rates of tooth wear impacts the implementation of preventive and therapeutic measures [14]. NCCLs are conventionally monitored clinically by subjective means, including analysis of photographs and study cast models, as well as by the use of tooth wear indices, like the Basic Erosive Wear Examination (BEWE) [15]. Although these methods are useful, they each have shortcomings. Clinical photographs are limited by difficulty in standardizing settings (positioning, lighting, etc) for longitudinal comparisons and study casts require substantial space for physical storage [14]. Further, the BEWE index [16] is subjective and results are dependent on examiner experience [17]. Therefore, the development of an objective evaluation tool performed in real time at chairside would be extremely beneficial [18].

Recent technological advances have positively impacted digital dentistry. For instance, intraoral scanners have allowed the rapid acquisition of 3D models of teeth with several applications in diverse areas such as prosthodontics, orthodontics, dental surgery, esthetic dentistry, and dental implantology [19]. Recent studies have also suggested that intraoral scanners can be promising tools for the early diagnostic and assessment of tooth wear given their ease of use and growing ubiquity in dental practices today; however, testing and further development are needed [14, 18, 20]. In order to further explore and advance this field, our aim in this study was to test the use of 3D intraoral scanner and image analysis for the detection and monitoring of the progression of in vitro simulated NCCLs. The null hypothesis was that there is no difference in the values of the dental volumes obtained when using the intraoral 3D scanner when compared to those obtained by optical profilometry.

Materials and methods

Experimental design

This study employed dental impressions previously created and analyzed by optical profilometry [21]. In the current study, they were scanned with the 3D intraoral scanner test system and image analysis was subsequently performed to determine volumetric tooth loss. All the procedures were performed in random and blind conditions. In the previous original study, NCCL severity levels were simulated by the association of three experimental factors: (1) toothbrush stiffness (soft, medium, and hard), (2) toothpaste abrasivity (low, medium, high, and negative control), (3) number of simulated brushing strokes (35K and 65K). A total of 24 groups (n = 24 samples), with different levels of volumetric tooth loss, stemmed from the associations of the study factors. The newly generated data were analyzed considering the experimental factors tested, and also correlated with the data original data from the previous study.

Specimen preparation, treatment, and impression

This study used the specimens' impressions created in the original study, where details of their preparation and treatment are fully described [21]. In brief, 288 upper first premolars were randomized into 12 stiffness×abrasivity groups (n = 24). They were brushed in an automated V-8 toothbrushing machine for 35K and 65K double strokes, using toothbrushes of different stiffness (soft, medium, hard; Lactona Corp., Bergen op Zoom, The Netherlands) associated with slurries containing hydrated silica of different abrasive levels (low/Zeodent 113, medium/Zeodent 124, high/Zeodent 103, Huber Engineered Materials, Havre de Grace, MD, USA), prepared according to the ISO11609 guidelines for dentifrice abrasivity testing. A total of three impressions were made for each specimen (at baseline and after 35K and 65K brushing strokes), with an elastomeric impression material (hydrophilic vinyl polysiloxane, Examix NDS Monophase, GC America, Alsip, IL, USA). The impressions were labeled and kept stored in dry conditions at room temperature.

Specimen's impression evaluation

In the original study, each specimen's impression was scanned with an optical profilometer using a type S65/10 sensor (resolution of 0.30 μm, Proscan 2000, Scantron, Taunton, UK) and analyzed with the Proform Software (Scantron) using the superimposition and subtraction functions to obtain the tooth volume loss data after each brushing period [21].

In the present study, the dental impressions were scanned with a CEREC Omnicam 4.6.1 (Dentsply-Sirona, Bensheim, Germany) intraoral scanner with white light IOS and triangulation technology to produce digital impressions. Based on root-mean-square evaluation criterion, the scanner's reported accuracy parameters for single tooth scan are the following: trueness of 13.8 μm (± 1.4 μm) and precision of 12.5 μm (± 3.7 μm) [22]. A previously trained examiner performed all the scans to ensure consistency. Scanner calibration and use followed the manufacturer's instructions. The scanner was allowed to warm up for 20 min prior to use. The impressions were scanned paying attention to the sound emitted by the equipment, which helps in carrying out the correct procedure and maintaining ideal measuring distance (0–15 mm) between the camera and tooth surface. Visual inspection was

performed to verify that all surfaces of each specimen were properly captured (Fig. 1), and scans were saved in stereolithography (.STL) format for subsequent analysis.

Tooth volume loss analysis

The .STL output files were opened in Geomagic Wrap 2017 (3D Systems, Rock Hill, SC, USA) 3D modelling software. The volume of each model was recorded using the software. Wrap's lasso tool was then applied to select surfaces affected by the simulated lesions, and those surfaces were deleted from the model. The resulting holes were then filled using the curvature fill function, which interpolates the surface using a nearest neighbor polygon algorithm as a guide. Resulting surfaces were compared with original baseline surfaces to confirm the accuracy of the fill algorithm using models of specimens scanned during the original study. This task was first performed independently for each tooth and resultant tooth loss volume was computed. The difference in volume between the original model and that filled was used to calculate volume loss associated with each NCCL simulation.

Statistical analysis

A mixed-model ANOVA was used to analyze volumetric loss, with fixed effects for toothpaste abrasivity, toothbrush stiffness, brushing strokes, and their interactions, a random effect for the right-left pairing within specimens, and a repeated effect for brushing strokes within the specimens. Pairwise comparisons utilize Fisher's Protected Least Significant Differences method. Agreement between the tooth structure loss measurements from the digital intraoral scanner and the lab optical profilometer was assessed using a Bland-Altman plot and an intraclass correlation coefficient. A 5% significance level was used for all tests. Analyses were performed using SAS version 9.4.

Results

Due to non-normality, a log (base 10) transformation was performed on the volumetric loss data prior to the ANOVA. Overall, the effects of toothpaste abrasivity, toothbrush stiffness, and brushing strokes were significant, as were the slurry-toothbrush and slurry-strokes interactions, whereas the toothbrush-strokes and slurry-toothbrush-strokes were not significant.

After 35K (Table 1), brushed samples evinced significantly less tooth structure loss than after 65K (Table 1) for high, medium, and low abrasive slurries ($p < 0.0001$), but not for control ($p = 0.16$). Toothbrush stiffness hard and mid had significantly higher tooth structure loss than soft for the high abrasive slurry ($p < 0.001$). No other significant differences were found between toothbrushes ($p > 0.05$). Deionized water (DIW, negative control) had significantly less tooth structure loss than high and medium abrasive slurries. DIW had also significantly less tooth structure loss than low abrasive for 65K strokes ($p < 0.0001$) but not for 35K strokes ($p = 0.55$). Low abrasive had significantly less tooth surface loss than high and medium abrasives ($p < 0.001$). Medium abrasive had significantly less tooth surface loss than high abrasive ($p < 0.001$).

The intraclass correlation coefficient for agreement between the digital intraoral scanner and the optical profilometer was 0.85 (Fig. 2). Disagreements ranged from 8.0 mm^3 lower to 13.9 mm^3 higher for the digital intraoral scanner than the optical profilometer, with an overall average difference of 0.2 mm^3 higher for the digital intraoral scanner. The limits of agreement on the Bland-Altman plot, mean difference ± 2 standard deviations, were at + 4.0 mm^3 and − 4.3 mm^3. The plot suggests that the digital intraoral scanner underestimated tooth structure loss for measurements less than ~ 2.5 mm^3 and overestimated larger tooth structure loss compared to the optical profilometer. Additionally, the plot shows that the

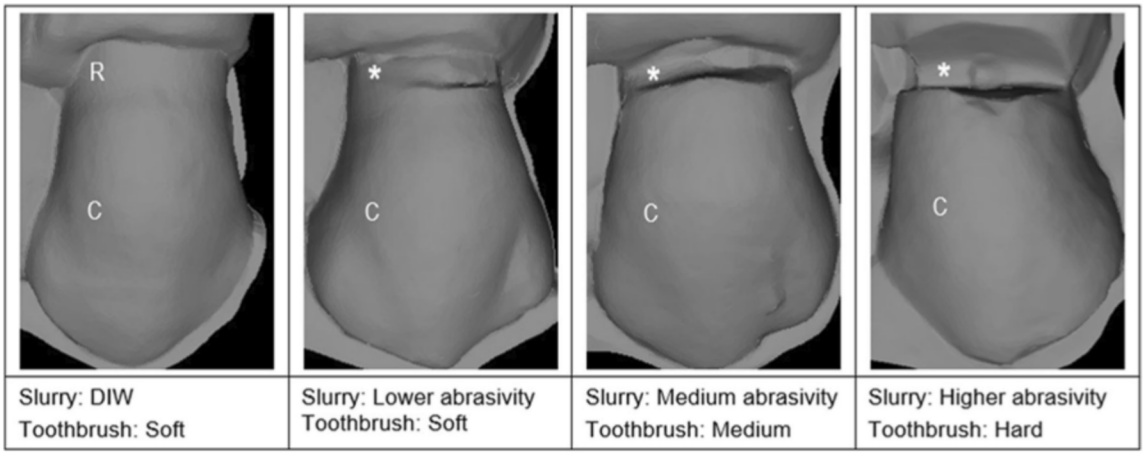

Fig. 1 Representative images captured by the intraoral scanner illustrating the range of simulated NCCLs, according to the slurry abrasivity and toothbrush stiffness levels (C, crown; R, root; *, NCCL)

Table 1 Interaction between different toothbrushes (soft, medium, hard) and abrasives levels (control, low, medium, high) at 35K and 65K strokes. Volume loss (mm^3) means (standard deviations) are presented

Strokes	Slurry abrasivity	Soft	Medium	Hard
35,000	Lower	0.27 (0.25)Aa	0.37 (0.59)Aa	0.27 (0.38)Aa
	Medium	1.44 (0.63)Ba	1.96 (1.14)Ba	2.20 (0.91)Ba
	Higher	2.03 (1.39)Ca	4.64 (1.92)Cb	7.00 (3.87)Cb
	DI water (control)	0.21 (0.14)Aa	0.21 (0.14)Aa	0.26 (0.17)Aa
65,000	Lower	0.86 (0.36)Ba	1.00 (0.73)Ba	0.84 (0.59)Ba
	Medium	3.22 (1.37)Ca	4.42 (1.77)Ca	4.47 (1.36)Ca
	Higher	7.59 (3.20)Da	12.00 (4.75)Db	14.59 (4.52)Db
	DI water (control)	0.21 (0.13)Aa	0.24 (0.16)Aa	0.31 (0.20)Aa

Different letters represent significant differences ($p < 0.05$): uppercase within columns (for 35,000 and 65,000 strokes independently); lowercase within rows

differences in the measured volume loss between the two methods increase in magnitude with the amount of structure loss.

Discussion

The fill algorithm analysis of 3D models obtained using the intraoral scanner detected significant effects for toothpaste abrasivity, toothbrush stiffness, and brushing strokes, as well as for the slurry-toothbrush and slurry-stroke interactions.

Overall, the increase of toothbrushing strokes led to an increase of dentin volume loss, or progression of the lesion. There were significant differences in volume loss for all groups brushed with abrasive slurries between 35K and 65K strokes. This result was expected considering the development pattern of NCCLs and reinforced the importance of toothbrushing strokes when designing a NCCL experimental model, as better differentiation of abrasive effects was observed in advanced lesions at 65K strokes, compared to the early lesions at 35K strokes.

Toothbrushes of hard and mid stiffness showed significantly higher tooth structure loss than those of soft stiffness, but only

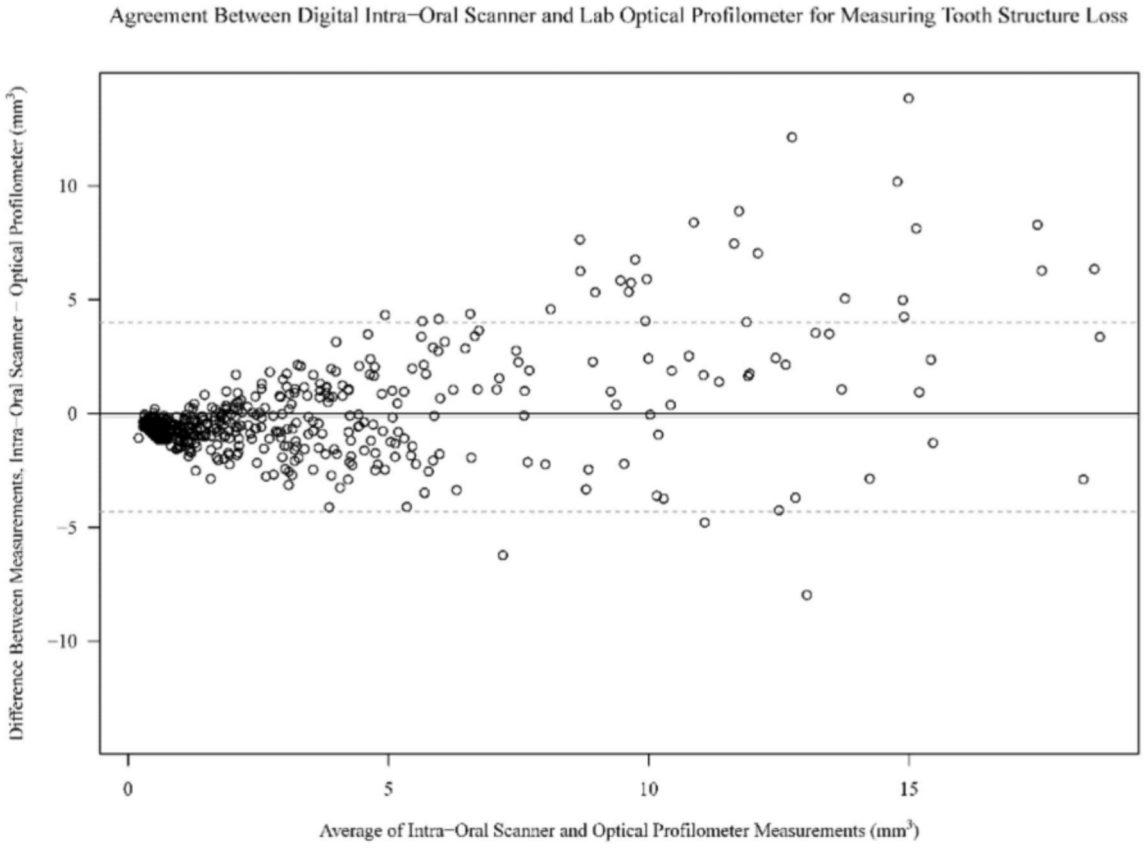

Fig. 2 Agreement between digital intraoral scanner and lab optical profilometer for measuring tooth structure loss

when associated with the highest abrasive (Z103), after both 35K and 65K strokes. The toothbrush filament stiffness seems to impact how the abrasive particles from the toothpaste interact with the tooth surface [23]. Previous investigations have observed similar results where higher abrasive slurries and hard toothbrush stiffness cause higher tooth wear [21, 23]. However, other studies have shown opposite results, which are explained by the fact that soft toothbrushes are more capable of carrying the abrasive particles [24]. The difference in these results may be related to differences between the experimental methods. For instance, the current study used a toothbrushing load of 200 g and frequency of 260 strokes/min, whereas Alshehab et al. [24] used a load of 250 g with frequency of 150 strokes/min, which might have emphasized more the interactions among toothbrush, abrasives, and tooth. In addition, different toothbrushing simulation times were used, with final brushing times of 65K strokes in the present study compared to 4.2K strokes in the previous one. Therefore, more severe lesions were simulated in the present work.

The 3D analysis performed differentiated surface loss resulting from different abrasive slurries used after both 35K and 65K strokes regardless of the type of toothbrush used. Our results are in accordance with previous studies able to directly correlate tooth wear with slurry abrasivity [25]. At 35K, no differences were found between the low abrasive slurry and the negative control group. This can be explained by the fact that the low abrasive slurry may not generate detectable lesions at 35K, but at 65K, these lesions would be sufficiently pronounced to be detected using this method. This finding may also be related to a limitation of our analytical protocol involving virtual fill of the lesion. In fact, incipient lesions may be of insufficient depth and volume loss to be measured by filling.

The main goal of this study was to compare the results obtained in a previous study [21] with a method based on the use of 3D intraoral scanners and image analysis. In the current study, there was no significant difference between the brushing strokes (35K vs 65K) when brushing with water (negative control), whereas in the previous study, higher volume loss was observed at 65K. A second difference was that the present data shows tooth volume loss when brushing with the medium abrasive (Z124) not affected by the stiffness of the toothbrush, whereas in the previous study, hard and mid stiffness were significantly higher than soft. A third difference was that hard toothbrush caused significantly higher dental structure loss than the mid toothbrushes when brushed with the more abrasive slurry (Z103), when using optical profilometry. A final difference was that in the previous study, there was significant difference in the tooth volume loss between groups brushed with water (control) and groups brushed with low abrasive slurry (Z113), for both 35K and 65K strokes. This contrast in results seem to be directly related to limitations in sensitivity of the current method for detecting and measuring incipient lesions, as pointed out above.

When testing the correlation between the current and previous (original) data at both 35K and 65K, we observed that the overall values for the more initial lesions (groups brushed with DIW) were numerically lower, whereas the values for the more advanced lesions (groups brushed with high abrasive slurry) were numerically higher. This can be visualized through the Bland-Altman plot that shows the intraoral scanner to underestimate the tooth structure loss less than ~ 2.5mm^3 and to overestimate the tooth structure loss for larger structure losses. This underscores limitations of measuring the most incipient lesions using the intraoral scanning protocol. For the advanced lesions, differences may relate to limitations in the digital filling algorithm, and lack of the original dental surface to be used as a reference. Differences between the current and original methods are also evident from variation in volume differences between 35K and 65K, as we observed an average increase rate of 11% and 48% for the groups brushed with DIW in the profilometry and intraoral scanner studies, respectively. Despite these specific differences, though, the comparison between the two sets of data showed a high correlation coefficient agreement of 0.85 [26]. Our results suggest that relatively smaller simulated lesions were not properly detected/measured by the intraoral scanner, indicating a limitation of this method. A previous in vitro investigation similarly concluded that smaller volume changes (approximately 1.5 mm^3) measured by intraoral scanner were inconsistent at this level [20].

While the optical profilometry method [21] is more sensitive for documenting incipient NCCLs, the approach is unfeasible for direct clinical assessment [20, 26]. In contrast, the intraoral scanning protocol can be applied routinely by practitioners at chairside, it is non-invasive, and the required instrumentation is becoming increasingly common in dental practices. Moreover, unlike the optical profilometry protocol, the fill algorithm used with the intraoral approach does not require a baseline measurement for subtraction of volume. This therefore can be used to quantify and monitor NCCLs once they are deep enough to be visualized by the dentist. And the lack of sensitivity of the intraoral scanner to measure incipient NCCLs is less of an issue at that point, as only the smallest lesions cannot be detected using the currently presented approach. This fits nicely with the suggestion of O'Hara and Millar [14] for the use of 3D images to monitor the development and progression of tooth wear, as a potential way to overcome the barriers of the current clinical subjective methods. Additional advantages of the intraoral scanners are their reasonable cost and the reduced time to scan the dental surfaces and lesions (seconds per tooth).

Despite the above-mentioned potential value of this method for the monitoring of NNCLs, we acknowledge that there is a need for further investigations to determine cutoff values or thresholds for the detection and progression of these lesions clinically. In the current study only two time points were considered for the analysis of the NCCLs, which limited this

understanding. Therefore, more detailed laboratorial and clinical longitudinal studies using more evaluation time points will be valuable for assessing the ultimate potential of this new approach to monitoring and documenting NCCL progression.

Acknowledgments This research was part of CFC's dissertation to be submitted in partial fulfillment of the MSD degree in Dentistry, from the Graduate Program in Dentistry at the Federal University of Paraiba, Brazil, and part of JN's thesis submitted in partial fulfillment of honors requirements in Biomedical Engineering at the University of Arkansas.

Funding This study was partially supported by Nacional Council for Scientific and Technological Development (CNPq), Brazil, and by the Erosive Tooth Wear Research Program of the Indiana University School of Dentistry.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent For this type of study, formal consent is not required.

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