AND TOOTHBRUSH STIFFNESS ON THE DEVELOPMENT OF NON-CARIOUS CERVICAL LESIONS

By

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DEDICATION

This thesis is dedicated to my parents, my wife, and my son for their support, love, and patience which were my inspiration to success.

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INTRODUCTION

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Non-carious cervical lesion (NCCL) is defined as loss of dental hard tissue near the cement-enamel junction (CEJ) caused by non-bacteria related processes. Three factors - erosion, abfraction and abrasion - have been considered as the common causative factors of NCCLs (Grippo et al., 1991; Levitch et al., 1994; Attin et al., 1997; Khan et al., 1999; Palamara et al., 2001; Eisenburger et al., 2003).

The most common method to maintain good oral hygiene is toothbrushing (Wiegand and Schlueter, 2014), which is also considered to be a contributor to the development of dental abrasion (Addy and Hunter, 2003; Tellefsen et al., 2011; Wiegand and Schluter, 2014). Miller first noted the effects of toothpaste abrasivity on dental hard tissue in 1907 (Harte and Manly, 1975; Harte and Manly 1976). Such lesions can lead to dentin hypersensitivity (Bartlett et al, 2013), and also create areas for plaque retention, increasing the risk for caries development. In advanced stages, they can affect the dental structural integrity and pulpal vitality (Hollinger and Moore. 1979; Hong et al., 1988; Osborne et al., 1999).

Currently, varying levels of toothpaste abrasiveness and toothbrush stiffness are known to affect the dentin. Prevention of NCCLs is important, since they may lead to pain or loss of tooth form, function and esthetics, especially when advanced (pathological) stages are reached. In those circumstances, restorative therapy may be provided; however, placing restorations does not necessarily stop the progression of the NCCLs and may have financial implications.

Therefore, research in this area should focus on understanding the main mechanisms related to NCCLs and how they can be prevented or modified. Major challenges to understanding NCCLs are the myriad of toothbrushing parameters (toothpaste, the stiffness of toothbrush, frequency, force, the direction of force, technique) that must be studied using clinically relevant models, and the lack of adequate quantitative methods to evaluate the NCCL progression. Our proposed approach to overcome these challenges involves primarily the establishment of a reliable evaluation method, which could be used for the study of the toothbrushing parameters in vitro and later in vivo. Considering the dental anatomy in the cervical area and the amount of anticipated surface loss, based on clinical observations, we proposed to use non-contact profilometry and tridimensional subtraction analysis as a promising approach to investigate the development of NCCLs.

OBJECTIVE

The study aimed to investigate the influence of dentifrice abrasivity and toothbrush stiffness on the development of NCCLs in vitro, using tridimensional optical profilometry.

Null Hypotheses

- The abrasive level of the dentifrice has no influence on the initiation and progression of NCCLs;
- 2. The stiffness of the toothbrush has no impact on the initiation and progression of NCCLs;
- 3. The interaction between the abrasive level and toothbrush stiffness does not affect the initiation and progression of NCCLs.

Alternative Hypotheses

- 1. The abrasive level of the dentifrice has a significant influence on the initiation and progression of NCCLs;
- 2. The stiffness of the toothbrush has a significant impact on the initiation and progression of NCCLs;
- 3. The interaction between the abrasive level and toothbrush stiffness does affect the initiation and progression of NCCLs.

REVIEW OF THE LITERATURE

Non-carious cervical lesions (NCCLs) can be defined as the loss of dental hard tissue near the cemento-enamel junction without bacterial involvement. Abrasion, erosion, and abfraction have been mentioned as common etiological factors of NCCLs (Grippo et al., 1991; Levitch et al., 1994; Attin et al., 1997; Khan et al., 1999; Palamara et al., 2001; Eisenburger et al., 2003), either independently or in association (Barbour and Rees, 2006). These etiological factors differ from one another depending on the tooth structure loss process. Abrasion is the loss of tooth structure due to friction by materials such as toothbrushes or abrasives in toothpaste (Lee and Eakle, 1984; Barbour and Rees, 2006; Ceruti, 2006). In contrast, dental erosion is the loss of tooth structure driven by acids. The acid could be from either extrinsic sources, such as the diet or medications, or intrinsic sources, such as gastric acid (Lee and Eakle, 1984; Passon and Jones, 1986; Rees and Hammadeh, 2004). On the other hand, abfraction starts due to the weakening of the tooth structure in areas of concentrated stress as a result of cuspal flexure from heavy and repeated occlusal loading, which progresses to dental hard tissue loss (McCoy, 1982; Lee & Eakle, 1984; Grippo, 1991; Rees, 2006).

The dental profession has been aware of NCCLs for many years. However, the studies of their prevalence have shown conflicting and inconsistent findings. For instance, Shulman and Robison (1948) documented findings equal to only 2%, whereas Bergstorm and Eliasson (1988) reported prevalence as high as 90%. The studies were focused on different populations, and were conducted 40 years apart, which may reflect an entirely

different awareness and understanding of NCCLs. This may partially justify the difference in the results. Many studies showed a relationship between age and prevalence of NCCLs, which also could explain the disparity of the findings, since Shulman and Robinson (1948) examined young males and Bergström and Eliasson (1988) adults of 31 to 60 years of age.

Bartlett and Shah (2006) reported in their review that the prevalence of NCCLs can be as high as 85%. In a study of 83 adults, Zipkin et al. (1949) reported that the mandibular teeth were less likely to exhibit NCCLs than maxillary teeth, with no significant difference between the left and right sides of the mouth. Bergström and Eliasson (1988) found a correlation between age and both the prevalence and severity of NCCLs among 250 31- to 60-year-old patients. Corroborating these data, in a survey of 295 adults, Yan and Yang (2014) found that 72.5% of the participants had NCCLs, and the lesions were more common in the posterior (73.4%) and maxillary teeth (55.6%); the most NCCLs occurred in first premolars - over 32%. The authors also reported that the prevalence of NCCLs was higher in patients older than 40 years. Older individuals are expected to have more NCCLs because their teeth have been retained longer and are, therefore, more exposed to wear processes (abrasion, erosion, and abfraction) (Aw et al., 2002). In addition, older people tend to have more gingival recession and bone loss. The resultant exposed cementum and root surfaces are more easily abraded than the enamel (Piotrowski et al., 2001).

It has been known that toothpaste abrasivity is significantly correlated to toothbrush abrasion (De Menezes et al., 2004). Many factors, including chemical composition of the abrasive, concentration, abrasive particle size, diluents, and the

dilution rate of toothpaste can play a major role in toothpaste abrasivity (Franzo et al., 2010; Schemehorn et al., 2011). For instance, abrasive wear increases as the size and concentration of abrasive particles increase; this reveals a linear relationship between the size and concentration of abrasive particles with abrasive wear (Davis and Winter, 1980; De Boer et al., 1985; Joiner, 2010). Additionally, abrasive particles decrease subsequently as the dilution rate of toothpaste increases, which may lead to a tooth wear minimization (Turssi et al., 2010).

Relative Dentin Abrasivity (RDA) and Relative Enamel Abrasivity (REA) are the methods to describe the effect of abrasives in toothpaste on both dentin and enamel, respectively. Different studies have used various ways to examine the abrasivity of the toothpaste; these methods include microscopic analysis, surface profilometry, and the weight loss technique (Philpotts et al., 2005). The most frequently used method to determine RDA and REA is the radiotracer method (ISO 11609) (Harte and Manly, 1975; Wiegand et al., 2009; Voronets and Lussi, 2010). Several investigators have compared toothpastes of high and low abrasivity and examined which products cause more tooth surface loss. Several studies of eroded and sound dentin indicated that there is a significant relation between RDA and abrasive wear of dentin (Harte and Manly, 1975; Wiegand et al., 2009). For example, in-situ research conducted by Hooper et al. (2003) on 15 healthy volunteers found a positive correlation between RDA levels and dentin wear. In this study, each subject had one dentin and one enamel specimen that were held by an upper removable acrylic appliance and worn for a specific period of time while subjected to different treatment regimens. Another in vitro study (Philpotts et al., 2005) tested a different range of RDA and REA on 100 specimen blocks and found a positive

correlation between RDA value and dentin wear.

Toothpaste is delivered to the mouth by a toothbrush, which could modify its action (Wiegand et al., 2008; Tellefsen et al., 2011). Toothbrush filaments in cross section may be round, square, hexagonal, and other shapes, with different degrees of smoothness and roughness (Yankell et al., 2000). Several factors affect the stiffness of a brush, including the number of tufts, their diameter, modulus of elasticity of the bristle, and the number of bristles in each tuft hole (Rawls et al., 1990). The smaller the tuft hole, the fewer filaments it will contain, and therefore, the softer the toothbrush will be (2010 Sunstar Americas, Inc. http://www.saiftp.biz/saidsp/Bristles Demystified Brochure.pdf). Also, the length and diameter of the filament can be varied, having a direct effect on the stiffness and hardness of the toothbrush. The harder or stiffer toothbrushes are those with filaments of larger diameter (Van der Weijden et al., 2000), and with shorter bristle length (2010 Sunstar Americas, Inc. http://www.saiftp.biz/saidsp/Bristles Demystified Brochure.pdf). If the modulus of elasticity and the length are constants, the filament diameter is the only factor that affects stiffness (Heath and Wilson, 1971).

The dental plaque should be effectively removed by using a combination of toothbrush and toothpaste in order to maintain oral health and prevent dental caries and periodontal disease. However, increased toothbrushing frequency may lead to tooth wear (Akgül et al., 2003; Bhardwaj, 2014). The relationship between brushing abrasion and dental wear has been established some time ago (Davis and Winter, 1976). However, it has become more relevant recently due to increasing tooth retention rates among older adults and the growing emphasis on oral hygiene for oral disease prevention (Smith and Knight, 1984). Litonjua et al. (2004) reported that toothbrushing alone could induce

wedge-shaped NCCLs in vitro independently of other factors. These results were confirmed by Dzakovich and Oslak (2008), who reported that horizontal brushing with commercial toothpastes can cause significant NCCLs in vitro.

According to Dzakovich and Oslak (2008), there was no visual relationship between NCCL size and either toothbrush firmness or toothpaste abrasivity. However, this study relied on subjective visual examination and did not quantify the surface loss of the simulated NCCLs. A previous study demonstrated the importance of toothpaste abrasivity and toothbrush stiffness, especially for root dentin (Arrageg et al., 2015). Although NCCLs can involve both enamel and root dentin, some authors reported that dentin was more prone to abrasion than enamel (Davis and Winter, 1980); this finding has been verified in a review by Addy et al. (2002).

The effects of toothbrush stiffness on the abrasion process have been indicated by several studies. Dyer et al. (2000) investigated the effects of three types of toothbrushes (soft, medium, hard) on an acrylic substrate that had a hardness similar to that of dentin. Soft brushes caused more abrasion than hard ones when used with toothpaste. This tendency occurs because soft brushes are less rigid and flex more than harder brushes, transporting greater amounts of toothpaste to areas that could not be reached by the harder ones (Dyer et al., 2000). Wiegand et al. (2009) found a similar outcome. The researchers tested the effect of different toothbrushes' filament stiffness on 96 eroded dentin samples. They stated that the lower filament stiffness caused more dentin wear than the higher filament stiffness. Additionally, Teche et al. (2001) investigated the abrasion capacity of four soft toothbrush brands. They concluded that there is a significant difference in the flexibility and diameter of soft-bristle toothbrushes and also

that toothbrushes with softer bristles endorsed greater abrasion capacity.

In contrast, some authors believed in the opposite theory, that hard toothbrushes can cause more tooth surface loss compared to soft toothbrushes (Tellefsen et al., 2011). For instance, limited data comparing natural and artificial filaments showed that hard brushes cause more abrasion than soft brushes (Skinner and Takata, 1951; Harrington and Terry, 1964). In addition, Harte and Manly (1975) found that hard toothbrushes produced more abrasion on sound dentin than the softer toothbrushes. Moreover, the common idea held by many dentists that the soft toothbrush causes less abrasion than the hard one had been reinforced by Brandini et al. (2011).

To further cause difficulties, Addy and Hunter (2003) concluded that such characteristics such as filament stiffness, type, and filament end configuration could contribute to toothbrushing abrasion. In contrast, other researchers stated that abrasivity could not contribute to the severity of toothbrushing abrasion (Bjorn and Lindhe, 1966; Bergstro and Lavsted, 1979). Also, some authors found that degree of stiffness in a toothbrush is considered a secondary factor affecting abrasion (Dyer et al., 2000;Litonjua et al., 2005). Wiegand et al. (2008) found that the abrasion of sound enamel was caused primarily by the level of toothpaste abrasivity. Another study (Voronets et al., 2008) reported no significant difference between hard and soft toothbrushes in the development of toothbrush abrasion.

Tellefsen et al. (2011) assessed the relative abrasivity of different toothbrush types both quantitatively and qualitatively. The authors stated that brushing with water alone showed minor differences in toothbrush types and insignificant toothbrush abrasion. On the other hand, when toothpaste was introduced into the brushing process, the wear

increased and was noticeable.

Studies have established the relation between toothpaste and dental wear. However, based on the previous findings, it could be concluded that the contribution of either toothbrush or toothpaste to the increase of dental wear can be difficult to define (Dyer et al., 2000). Moreover, the interaction between both toothpaste and toothbrush may affect dental wear.

METHODS AND MATERIALS

STUDY DESIGN

Experimental Design

This study examined three experimental factors:

- 1. Toothpaste abrasivity, at four levels (Table I): high- (Z103), medium- (Z124) and low-abrasive (Z113), and a non-abrasive slurry used as a negative control.
- 2. Stiffness of toothbrush bristles, at three levels: soft, medium, and hard.
- 3. Toothbrushing strokes, at three levels: baseline (0), 35,000, and 65,000 strokes.

Specimens (n=24) were prepared from human premolars and brushed using slurries of varying abrasivity and toothbrushes with different stiffness. They were analyzed by optical profilometry after each brushing time. The response variable was dentin volumetric loss (mm³).

Specimen Preparation

A total of 288 extracted human upper first premolars, free of any dental caries restorations, stains, or enamel and root defects, were selected. The teeth were cleaned with a hand periodontal scaler and distributed into twelve groups (24 teeth/group) based on the similarity of their dimensions (mesio-distal and bucco-lingual) at the CEJ and anatomy.

Paired teeth were mounted on acrylic blocks (Fig 1-A, B), resulting in a total of twelve blocks for each group. The root surfaces were covered by a layer of acrylic resin (TRIAD denture base material) to simulate the contour of the gingiva leaving exposed a 2-mm area apical to the CEJ (Fig 1-C). After molding, contouring and exposing the area required, the acrylic was light polymerized for 5 minutes in a Triad curing machine (Triad 2000, Dentsply Sirona Inc).

Toothbrushing

Horizontal toothbrush technique along with filament stress concentration in the cervical area (CEJ) was mimicked on extracted upper first premolars. The teeth were selected and fixed in custom-made acrylic devices allowing their positioning in the automatic brushing machine. Reference areas apical and occlusal to the brushing surfaces were determined and protected from the brushing abrasion by fabrication of a protective custom tray. Briefly, 0.5-mm plastic tray sheets (Thermal Forming Material, Clear Splint Biocryl) were molded against each dental specimen using a vacuum machine (ECONO-VAC, Buffalo Dental Mfg); after that, the plastic tray was cut in the area of the CEJ, dividing the plastic tray into coronal and root parts, and leaving the CEJ and adjacent 2 mm root dentin surface exposed (Fig 2). Reference areas were used to determine tooth volume loss, in each of the studied times, by tridimensional image subtraction analysis (Proform software, Scantron).

The specimens were positioned in a V-8 toothbrushing machine (Sabri Dental Enterprises Inc.; Fig 3), with their long axes perpendicular to the long axes of the toothbrushes (Lactona, Dental Care Clinic). In addition, the plane of the toothbrush head

was positioned parallel to the plane of the specimen, so that the filaments of the toothbrush contacted the specimens in a perpendicular plane. A total of 144 toothbrushes were used, and each toothbrush was assigned to one specimen, throughout the 65,000 brushing double-strokes. A brushing load of 200g was used during the experiments. The width of the toothbrush head was greater than the width of the exposed tooth test area; therefore, during function, the toothbrush bristles did contact both the exposed tooth structure and the protective acrylic on either side (Fig 4).

Slurries were prepared using different abrasives by mixing dental silica abrasives in 5% carboxymethyl cellulose solution, according to the ISO11609 guidelines (Table I). A volume of 60 ml was used for each specimen. The reference areas were protected using the custom-made plastic trays, and specimens brushed for 35,000 and then 65,000 double-strokes.

After every 10,000 double-strokes, the slurry was manually stirred. After finishing each brushing period, the specimens were thoroughly rinsed in deionized water and impressions were taken.

Impression

Impressions of the specimens were made at baseline and after each brushing period. A total of three impressions were made for each group, using the aid of a petri dish. For the baseline impression, each block of four specimens was mounted in the lid of a petri dish using double-sided cellophane adhesive tape to secure the specimens in the bottom of the lid. An elastomeric impression material (Hydrophilic Vinyl Polysiloxane, Examix, GC America, Inc.) was injected onto the labial surface of the specimens to

fabricate an index or position guide to orient the samples for subsequent impressions. Once set, the index was removed. An indelible pen mark was placed on the petri dish to ensure that subsequent impressions were oriented in the same way. Using this method, the impressions of the specimens were made at similar angles and directions facilitating the alignment of scans for the subtraction analysis. The same impression material was loaded into the base of the petri dish, and the lid pressed against its base. The impression was removed from the petri dish, trimmed and scanned. For subsequent impressions, the specimens were placed into the previously fabricated index and seated onto the double-sided tape. Then, the impressions were made as previously described.

Optical Profilometry

An area of the impression (20 mm long (X) \times 25 mm wide (Y)) was scanned with an optical profilometer (Proscan 2000, Scantron; Fig 5). The sensor that was used was the 10 mm S65/10a (04.41.1665 -10 mm), at 300 Hz and with two repetitions (Fig 6). The step size was set at 0.2 mm for both X and Y directions.

After scanning all impressions, the scans were analyzed and prepared for subtraction analysis following eight pre-determined steps, as follows: 1. auto-leveling; 2. interpolation of points in the X and Y directions; 3. application of warpage filter (number 2); 4. loading of unwanted points and deletion; 5. inversion of the height of the scan (using flip height tool); 6. three-point levelling; 7. selection of 3 references areas (selecting "zoom to the highest point", and deleting that point); 8. saving the modified scan. Proform software was used for superimposition of scans and subtraction analysis.

Sample Size Calculation

Based on prior studies, the within-group standard deviation was estimated to be 1.1 mm³. With a sample size of 21 teeth per toothpaste-toothbrush combination, the study had 80% power to detect a 1.0 mm³ difference between any two toothpaste-toothbrush combinations, assuming two-sided tests, each conducted at a 5% significance level. Due to the design of the brushing machine (fits 8 specimens at a time), the study used 24 teeth per toothpaste-toothbrush combination.

Statistical Methods

Summary statistics (mean, standard deviation, standard error, range) of the volumetric loss was calculated for each toothpaste-toothbrush combination at each brushing stroke tested. The effects of toothpaste abrasivity, toothbrush stiffness, and toothbrushing strokes on volumetric loss were analyzed using mixed-model analysis of variance (ANOVA). The ANOVA included fixed effect terms for the three experimental factors and all two-way and three-way interactions among the factors.

Toothbrushing stroke was repeated within each sample with an unspecified variance/covariance structure to account for different variances and correlations.

A random effect was also included to account for possible non-independence within the mounted pair of teeth. Pair-wise comparisons were performed using the Fisher's Protected Least Significant differences method to control the significance level at 5%. The distribution of the volumetric loss data was examined and a transformation of the data or a nonparametric test will be used if necessary to satisfy model assumptions.

RESULTS

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A mixed-model ANOVA was used to analyze volumetric loss, with fixed effects for toothpaste abrasivity ("slurry"), toothbrush stiffness ("toothbrush"), brushing strokes ("strokes"), and their interactions, a random effect for the right-left pairing within specimens, and a repeated effect for brushing strokes within the specimens. Due to non-normality, a log (base 10) transformation was performed on the volumetric loss data prior to analysis. Pair-wise comparisons utilized Fisher's Protected Least Significant Differences method. Overall, the effects of toothpaste abrasivity, toothbrush stiffness, brushing strokes, and their interactions were significant.

After 35,000 brushing strokes, there were no differences among toothbrushes, when used with the non-abrasive (control) and low-abrasive slurries. When brushing with the medium- and high-abrasive slurries, the soft brush caused significantly less tooth wear than medium and hard toothbrushes (p<0.05, Table II).

All toothbrushes produced significantly greater tooth wear when used with the high abrasive slurry, in comparison to the medium and low abrasive slurries. Medium slurry caused more tooth wear than the low slurry; low slurry caused more tooth wear than the control (Table II).

After 65,000 brushing strokes, there were no differences among toothbrushes, when used with the non- (control) and low-abrasive slurries. When brushing with the medium-abrasive slurry, the hard toothbrush caused more tooth wear than the soft toothbrush; and neither of them differed from the medium toothbrush. When combined

with the high abrasive slurry, the soft toothbrush caused significantly less tooth wear than the medium and hard toothbrushes (Table III).

All toothbrushes produced significantly greater tooth wear when used with the high-abrasive slurry, in comparison to the medium-and low-abrasive slurries. Medium-abrasive slurry caused more tooth wear than the low; low-abrasive slurry caused more tooth wear than the control (Table III).

All combinations of toothbrushes (soft, medium, hard) and abrasive slurries (low, medium, high) produced significantly greater tooth wear when brushing at 65,000 stokes than 35,000 strokes (Figs 5, 6, 7).

FIGURES AND TABLES

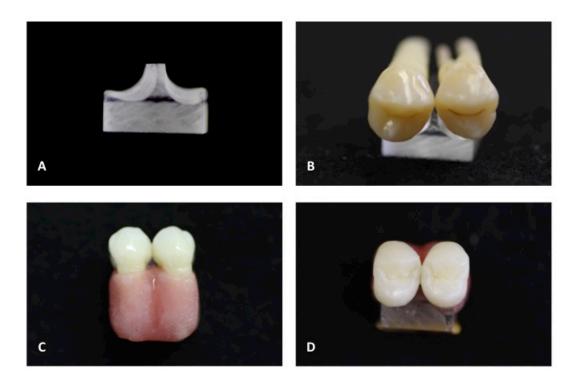


FIGURE 1. Specimen Preparation: Acrylic block prepared (A). Teeth mounted on acrylic block (B). Acrylic resin sheet adapted over the root portion to mimic the contour of the gingiva and expose 2 mm of root dentin surface near the CEJ (C). Occlusal view of the pair of teeth, with similar dimensions (D).



FIGURE 2. Specimens with protected and exposed areas. The plastic tray is cut in the area of CEJ, exposing the experimental surface (CEJ and the adjacent 2 mm root dentin surface), and protecting the reference surfaces.



FIGURE 3. Photograph of the brushing machine.



FIGURE 4. Simulation of the bristles in contact with the specimen during the toothbrushing process.

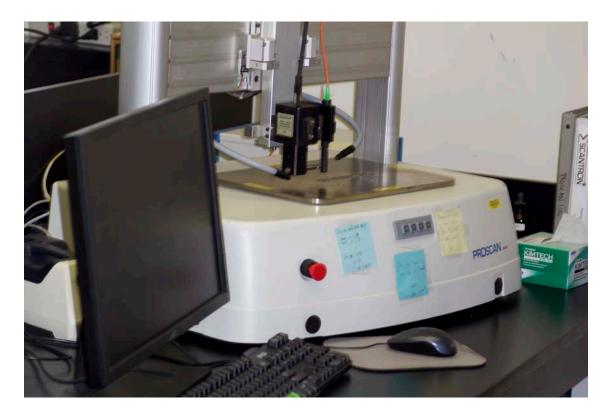


FIGURE 5. A photograph shows the optical profilometer used in the project.



FIGURE 6. A photograph shows the sensor used in the project.

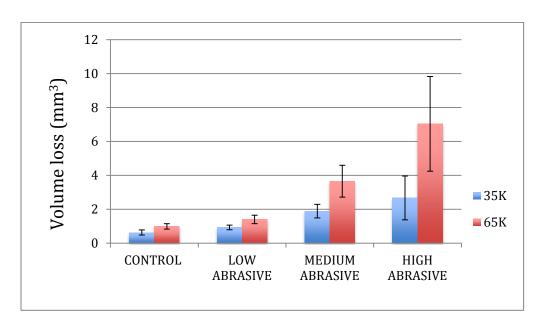


FIGURE 7. Mean (standard-deviation) tooth wear (in mm³) at 35K and 65K strokes, when using soft toothbrush with each abrasive level (control, low, medium, high). 65K showed significantly higher values for all comparisons (p<0.05).

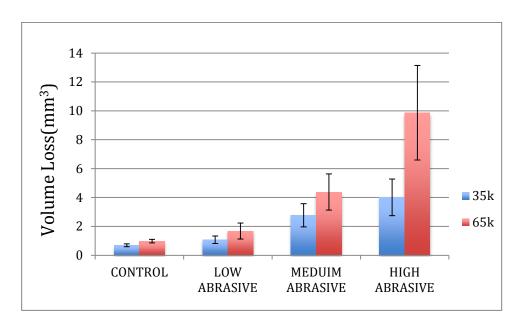


FIGURE 8. Mean (standard-deviation) of tooth wear (in mm³) at 35K and 65K strokes, when using medium toothbrush with each abrasive level (control, low, medium, high). 65K showed significantly higher values for all comparisons (p<0.05).

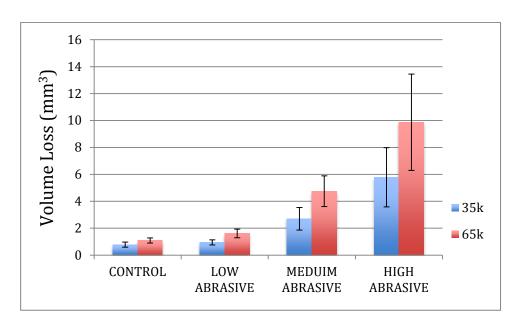


FIGURE 9. Mean (standard-deviation) of tooth wear (in mm³) at 35K and 65K strokes, when using hard toothbrush with each abrasive level (control, low, medium, high). 65K showed significantly higher values for all comparisons (p<0.05).

Table I Abrasive slurries composition.

Slurry	Silicas*	RDA**	Load	Amount	0.5% CMC sol.	
			(%)	(g)	(g)	
Low	Zeodent 113	69.24 (2.62)	5	3	57	
Medium	Zeodent 124	146.90 (3.52)	10	6	54	
High	Zeodent 103	208.03 (9.39)	15	9	51	
Control					60	

^{*} Manufactured by J.M. Huber Corporation.
** Mean (standard error).

Table II

Mean (standard-deviation) tooth wear (in mm³) of each toothbrush (soft, medium, hard) and abrasive level (control, low, medium, high) at 35K strokes. Significant interaction was observed (p < 0.001).

Toothbrush	Control		Low abrasive		Medium abrasive		High abrasive	
			Z113		Z124		Z103	
Soft	0.63 (0.15)	a	0.93 (0.14)	a	1.89 (0.40)	a	2.67 (1.29)	a
	A		В		C		D	
Medium	0.69 (0.10)	a	1.08 (0.26)	a	2.77 (0.80)	b	4.01 (1.27)	b
	À		В		C		D	
Hard	0.78 (0.19)	a	0.95 (0.19)	a	2.70 (0.84)	b	5.78 (2.20)	b
	A		В		C		D	

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

Table III

Mean (standard-deviation) tooth wear (in mm³) of each toothbrush (soft, medium, hard) and abrasive level (control, low, medium, high) at 65K strokes. Significant interaction was observed (p < 0.001).

Toothbrush	Control		Low abrasive		Medium abrasive		High abrasive	
			Z113		Z124		Z103	
Soft	0.99 (0.16)	a	1.40 (0.25)	a	3.66 (0.94)	a	7.04 (2.79)	a
	A		В		C		D	
Medium	0.98 (0.12)	a	1.68 (0.55)	a	4.38 (1.25)	ab	9.87 (3.27)	b
	A		В		C		D	
Hard	1.09 (0.19)	a	1.61 (0.32)	a	4.75 (1.14)	b	11.80 (3.58)	b
	A		В		C		D	

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

DISCUSSION

In vitro experimental model parameters

The experimental model used in this study simulated the development of NCCLs due to toothbrushing abrasion. Specimens were selected and prepared, aiming for the concentration of brushing forces in the cervical area, similar to what was done previously by Dzakovich and Oslak (2008). For such, the gingiva was simulated by adding a layer of acrylic resin to the root surfaces, up to 2 mm from the CEJ. Reference areas apical and occlusal to the test surface (CEJ and adjacent 2 mm root dentin) were protected from the brushing abrasion by the using a protective custom tray. The exposed root surfaces were brushed in a custom-made brushing machine. Standard toothbrush pressure of 200 g was used, which is in line with the average brushing force of 1.6 ± 0.3 N, or equivalent to 163 to 200 g (Wiegand et al., 2013). This value does not necessarily represent the pressure at the testing dental surface, as it is likely to be influenced by the irregular morphology of the teeth/gingiva or experimental set-up (including the protective plastic sheet). However, and more importantly, this applied pressure has shown in previous studies (Sabrah et al., unpublished) and also in a pilot for this study to be adequate to simulate the development of NCCLs. This protocol simulated a horizontal brushing technique with average force, which would mostly affect the exposed root surfaces on the buccal surface. Toothbrushing was done for a total of 35,000 and 65,000 brushing stroke cycles. These numbers roughly represent three and a half years and six and a half years, respectively; considering 5,000 cycles to represent a 6-month period of individual brushing (Kanter et al., 1982).

These intervals were chosen to allow the simulated NCCLs to develop, in order to be able to test the effects of toothbrush stiffness and slurry abrasivity. We speculated that the force was distributed onto the test surface at the beginning of brushing, but concentrated to specific areas, once the lesion started to appear. This could favor the faster progression of the lesion. Noteworthy is that even with a relatively fast progression, there was no plateau effect on lesion growth, during the studied periods.

Toothbrush stiffness (soft, medium, hard) is not necessarily constant among the brands of toothbrushes (Harte and Manly, 1976). Regarding this fact, and aiming to reduce the source of variation, we decided to use only one brand of toothbrush with three different stiffness levels (soft, medium, hard). All toothbrushes had nylon bristles with rounded end.

Standard slurries of three abrasive levels were used in this study, by mixing dental silica abrasives in 5% carboxymethyl cellulose solution, according to the ISO11609 guidelines, to simulate low, medium, and high levels of toothpaste abrasivity. The rationale for testing different abrasive levels was that commercial toothpastes may present a wide range of abrasivity, which may be associated to their specific claims. For instance, dentin hypersensitivity toothpastes tend to be less abrasive, while whitening/anti-staining formulations tend to be more abrasive (Schemehorn et al., 2011).

The tooth wear measurements were performed indirectly, by taking impressions of the simulated NCCLs and evaluating them by 3D optical profilometry. High fidelity elastomeric impression material (Hydrophilic Vinyl Polysiloxane, Examix, GC America, Inc.) was used and impressions were taken at baseline and after each brushing period. This procedure presents three advantages over the direct scanning of the specimen

surfaces. First, it helps to prevent the dehydration, and related dimensional changes, that could occur to the specimens during the scanning procedure (approximately 15 min). Second, it has been suggested that the color and transparency of dental hard tissue might affect the result of non-contact profilometry (Rodriguez et al., 2008). Third, it helped the logistics of the study, as brushing simulation did not have to wait for all the specimens to be scanned after baseline and 35k time-points, as the impressions registering the lesion information had been taken and stored for the analyses.

Non-contact surface profilometry was used in the study because it measures the surface topography without the risk of damaging the test specimen, as compared to the contact surface profilometry (Heurich et al., 2010). However, we acknowledge that this is of greater relevance only in erosive tooth wear studies, where dental surfaces are fragile, and may have little impact in our study.

Abrasive slurry effect

Brushing with water indicated tooth loss, with a suggested increase of the mean values at 65k, suggesting not only formation but also progression of the lesion. However, the possibility of the error of the method during the subtraction analysis cannot be completely ruled out. There is a degree of subjectivity on this analysis due to the manual superimposition of the two tridimensional images, which makes the detection and measurement of the lesion volume less accurate, at lower levels.

In this study, regardless of the type of the toothbrush, more surface loss was achieved by the high-abrasive slurry (Z103) than the medium-abrasive slurry (Z124), also the medium-abrasive slurry cause more surface loss than the low- abrasive slurry (Z113),

Furthermore, the low-abrasive generate more surface loss than the control group, indicating that even the least abrasive toothpaste can increase the risk for toothbrushing abrasion. The slurries were different from each other by their type of abrasive and also concentration, where high-, medium- and low-abrasive slurries presented 15, 10 and 5% loads of abrasive, respectively. As result, the testing slurries had different abrasivity mean values (standard-error), as measured by the radioactive dentin abrasivity (RDA) test, with high: 208 (9), medium: 146 (4) and low: 69 (3), representing different types of toothpastes, as previously indicated. The results observed in the present study corroborate earlier studies showing that higher RDA-value of the toothpaste slurry resulted in greater surface loss (Dyer et al., 2000; Hooper et al., 2003; Wiegand et al., 2009). Overall, significantly greater tooth loss was observed at 65,000 strokes compared to 35,000, regardless of toothbrush stiffness and slurry abrasivity. This was not surprising, as this is a cumulative, time-dependent process (Addy and Hunter, 2003).

The results for the comparison (ranking) of the effects of slurry abrasivity and toothbrush stiffness did not vary between the two time points studied, except that there was no difference between soft and medium toothbrushes when used with medium slurry, at 65,000 strokes. We speculate that this may have happened because of the excessive depth of the lesions, which may have led to exposure of the pulp chambers/root canal, possibly incurring an error in the tooth loss measurements. However, it should be born in mind that pulp exposure is dictated by not only the depth of the lesion, but also other variables, such as size of the pulp chamber/root canal, calcification, and age.

Toothbrush stiffness effect

The current study found that toothbrush stiffness was irrelevant when brushing with water or low-abrasive slurry. However, greater surface tooth loss was observed for the medium and hard toothbrushes when used with the medium- and high-abrasive slurries. These results contradict the previous studies reported by Dyer et al. (2000), who postulated that softer brushes could be more detrimental to a tooth as they were more flexible than a hard toothbrush and had the ability to carry more abrasive/slurry to a larger area during the tooth-brushing motion.

When the modulus of elasticity and the length are constant, the filament diameter will be the only factor that affects the stiffness: the wider diameter is stiffer than the one of narrow diameter (Heath and Wilson, 1971). In our study, we found that dentin wear increased along with the increased diameter of toothbrushes. From that information we can say that the current study also contradicts the study of Wiegand et al. (2009). The authors observed that dentin wear decreased along with the increased diameter (stiffer) of toothbrushes (Wiegand et al., 2009). The explanation for these contrasting results may be the type of substrate and also experimental design. In Wiegand et al. (2009), specimens of eroded bovine dentin were used. While they (cut and polished specimens) can be acceptable for some laboratory tests, they do not take the anatomy of the teeth (gingival and teeth contours) into consideration. In our model, we used human teeth and tried an actual simulation of the tooth-brushing process, in order to be more clinically relevant. Also, the findings by Dyer et al. (2000) were based on theoretical calculations, which may not necessarily translate into the actual situation clinically.

Several studies showed similar results to the present study. Harte and Manly (1976) found that a hard toothbrush caused more abrasion to the tooth surface than a soft toothbrush. Furthermore, Brandini et al. (2011) reported that the firmness of the toothbrushes correlated with the clinical presence of NCCLs. In that study, 58 patients were examined, (15 men and 43 women; mean age, 23.6 ± 1.8 years and 22.3 ± 2.4 years, respectively) and NCCLs were found in 53.5% of them. Significantly more NCCLs were observed when subjects brushed their teeth with toothbrushes having firm bristles (medium and hard).

Although the present study showed no differences between medium and hard toothbrushes, there was a numerical trend (non-significant) suggesting that hard toothbrushes could cause more dental loss, especially when used with a highly abrasive slurry. Such situations should be further investigated. The previous investigation by Arrageg et al. (2015) showed that when high-abrasive slurry was used, a hard toothbrush caused more tooth loss than the medium toothbrush in vitro, but this may have been due to the test on eroded surfaces, which was not simulated in the current study.

Clinically, our results suggest that the combination between types of toothpaste and toothbrush may be important, especially for patients at higher risk for the development of NCCLs. If the patient prefers medium- and high-abrasive toothpastes, a soft toothbrush should be recommended. On the other hand, if the patient prefers medium or hard toothbrushes, low-abrasive toothpaste is advised. However, it should be born in mind that other clinically relevant factors not simulated in this study (dental plaque, staining removal, brushing technique, frequency, force, etc), should also be considered.

SUMMARY AND CONCLUSION

Within the limitation of the present study, the following conclusions can be drawn:

- 1. Toothbrush bristle stiffness was irrelevant when brushing with low-abrasive (or with no) toothpaste.
- 2. Toothbrush bristle stiffness became a factor when brushing with medium- and high-abrasive toothpastes; in such cases, the medium and hard toothbrush increased tooth wear.
- 3. The combination between types of toothpaste and toothbrush may be important clinically, especially for patients at higher risk for the development of NCCLs.

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ABSTRACT

INFLUENCE OF DENTIFRICE ABRASIVITY AND TOOTHBRUSH STIFFNESS ON THE DEVELOPMENT OF NON-CARIOUS CERVICAL LESIONS

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Background: Non-carious cervical lesions (NCCLs) can be defined as the loss of dental hard tissue near the cemento-enamel junction without bacterial involvement.

Abrasion, erosion and abfraction have been mentioned as common etiological factors of NCCLs. Abrasion is the loss of tooth structure due to friction by materials such as toothbrushes or abrasives in toothpaste. In contrast, dental erosion is the loss of tooth structure driven by acids. Abfraction, on the other hand, starts due to the weakening of the tooth structure in areas of concentrated stress as a result of cuspal flexure from heavy and repeated occlusal loading, which progresses to dental hard-tissue loss.

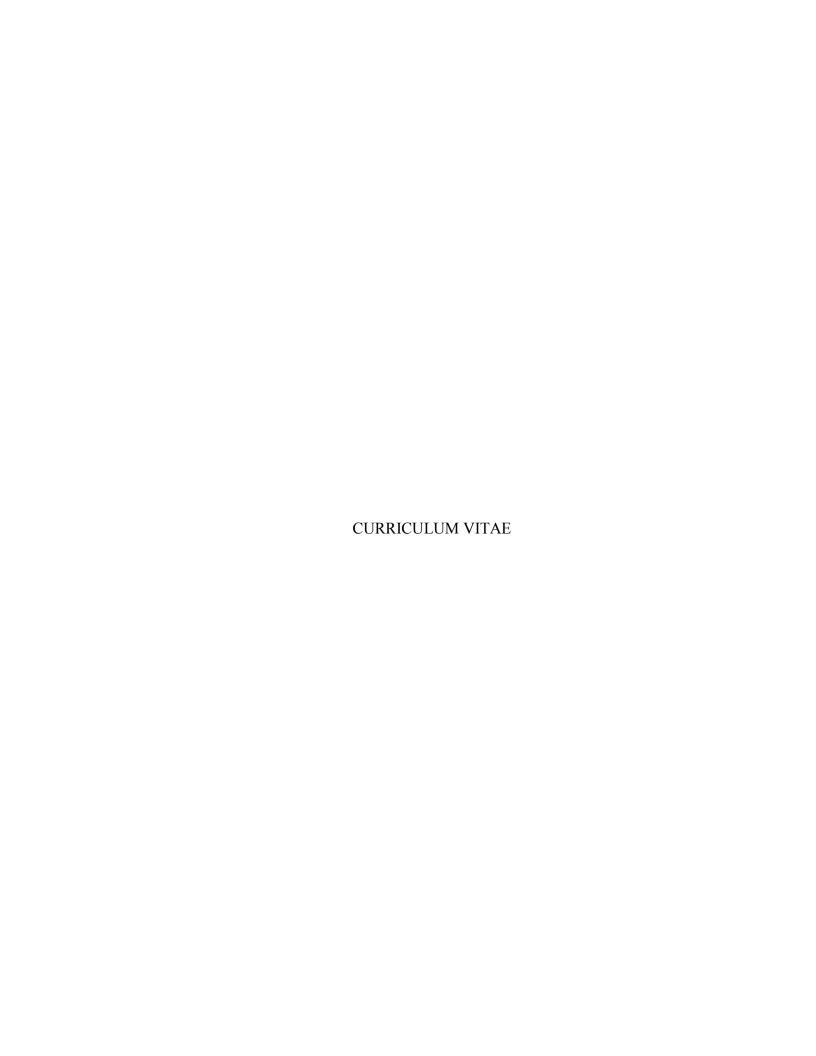
Purpose: The present study focused on the abrasion aspect of NCCLs. Specifically, it aimed to investigate the influence of dentifrice abrasivity and toothbrush stiffness on the development of NCCLs in vitro

Hypothesis: NCCL development is affected by both the abrasive level of the dentifrice and the stiffness of the toothbrush, as well as their interaction.

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Materials and Methods: A total of 288 extracted human upper first premolars, free of any dental caries and root defects, were selected. The teeth were be cleaned with a hand periodontal scaler and randomly assigned into twelve groups (total of 24 teeth/group). Specimens were brushed in an automated toothbrushing machine, using simulated toothpaste slurries of varying abrasivity and toothbrushes of varying stiffness.

This study examined three experimental factors: 1. Toothpaste abrasivity, at four levels: high, medium, low, and non-abrasive slurry (as negative control); 2. Toothbrush stiffness, at three levels: soft, medium, and hard; 3. Toothbrushing cycles at three levels: baseline, 35k, and 65k strokes. Specimens were analyzed by optical profilometry at baseline and after each brushing level. The response variable was the dentin volumetric loss, in mm³. All toothbrushes caused significantly higher tooth wear when associated to the high abrasive slurry, compared to medium- and low-abrasive slurries. Medium-caused more tooth wear than low-abrasive slurry, which in turn led to more tooth wear than the control. Hard and medium toothbrushes were not significantly different, but both caused significantly higher volumetric loss than Soft toothbrushes. There were no differences among toothbrushes, when used with the non-abrasive (control) and low-abrasive slurries. Overall, 35k strokes resulted in significantly less tooth volumetric loss than 65k.



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