

TITLE

3D-Image Analysis of the Impact of Toothpaste Abrasivity on the Progression of Simulated Non-Carious Cervical Lesions

SHORT TITLE

Impact of toothpaste on NCCL progression

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This is the author's manuscript of the article published in final edited form as:

Sabrah, A. H., Turssi, C. P., Lippert, F., Eckert, G. J., Kelly, A. B., & Hara, A. T. (2018). 3D-Image analysis of the impact of toothpaste abrasivity on the progression of simulated non-cariou cervical lesions. *Journal of Dentistry*. <https://doi.org/10.1016/j.jdent.2018.03.012>

ABSTRACT

Objectives: To investigate the effect of toothpaste abrasive level on the progression of non-carious cervical lesions (NCCLs) using 3D-image subtraction. **Methods:** Upper first premolars were allocated into seven groups (n=16) of toothpaste/abrasive slurries: A-Zeodent113/5%, B-Zeodent124/10%, C-Zeodent103/15%, D-Sensodyne Pronamel, E-Crest Cavity-Protection, F-Crest Pro-Health-Whitening, and G-Deionized water (DIW). Teeth were mounted on acrylic blocks, and their root surfaces covered with acrylic resin, except for 2-mm near the cemento-enamel junction that was exposed to toothbrushing. Specimens were brushed with the slurries for 5,000-, 15,000-, 35,000- and 65,000-strokes. Impressions were taken at baseline and after each brushing time, and then scanned by a 3D optical profilometer. Dentine volume loss was calculated by image subtraction software and subjected to mixed-model ANOVA and multiple comparison tests ($\alpha=0.05$). **Results:** No significant differences among slurries were observed at 5,000 and 15,000. At 35,000, F showed higher loss than all other groups except C, which did not differ from the others. At 65,000, F ($4.19\pm 3.29 \text{ mm}^3$) showed the highest loss, followed by C ($2.33\pm 1.47 \text{ mm}^3$), which differed from all the other groups except B ($1.85\pm 0.91 \text{ mm}^3$). Groups B, A ($1.35\pm 0.65 \text{ mm}^3$), D ($1.17\pm 0.48 \text{ mm}^3$), E ($1.40\pm 0.68 \text{ mm}^3$) and G ($1.12\pm 0.73 \text{ mm}^3$) did not differ from each other. Groups F and C showed significant increase of volume loss starting at 35,000, while B, A, D and E only at 65,000; no increase loss was observed for G. **Conclusions:** 3D-image subtraction was able to quantify and differentiate tooth loss, but only at advanced stages. The progression of NCCLs was more evident and faster for highly abrasive slurries.

Clinical significance: Upon root dentin exposure, brushing with lower abrasive dentifrices is advisable to reduce the risk for NCCLs development.

KEYWORDS

Non carious cervical lesion, toothpaste, abrasivity, non-contact profilometry

INTRODUCTION

Non-carious cervical lesions (NCCLs) have long been described as highly prevalent [1-5] but their etiological and physiopathological mechanisms remain unclear [2,6]. In advanced stages, they result in functional and aesthetic problems, dentine hypersensitivity and often require extensive restorative work. Therefore, the management of NCCLs should focus on the early identification of these lesions and adoption of preventive measures.

NCCLs initiate and progress due to the interplay of different wear processes [3,5,7-11], including abrasion (abrasive wear), erosion (corrosive wear) and abfraction (fatigue wear), which may act independently or in association. Abrasion results from either the contact of the tooth with harder asperities or the entrainment of harder particulates on its surface; while erosion develops by the contact of dental surfaces with non-bacterial acids and/or chelating agents [12-13]. The existence of abfraction remains controversial and systematic reviews cannot confirm or reject an association between occlusal stress and NCCLs [14, 15].

Toothbrushing abrasion is probably the most relevant abrasive wear mechanisms affecting the cervical area, with a clearly established relationship [16]. However, it has become more relevant recently due to the increasing tooth retention rates [17], growing emphasis on oral hygiene procedures including toothbrushing for oral diseases prevention, and widespread availability of abrasive whitening toothpastes. Laboratory simulations have reported that toothbrushing can induce wedge-shaped NCCLs independently of other factors [18]. Relevant aspects of toothbrushing include: toothpaste (abrasivity, composition), filament stiffness (soft, medium, firm), and behavioral aspects (brushing movement, force, frequency, duration) [19]. A meta-

analysis showed the association between NCCLs and frequency, method of toothbrushing and toothbrush stiffness [9].

Although more abrasive toothpastes can hasten dental abrasion [20], especially of exposed cervical dentine, no studies have yet clearly determined their impact on the initiation and progression of NCCLs over time. Clinical trials, while ideal, suffer from the lack of control of many variables to isolate abrasivity and brushing time effects. In addition, the majority of trials rely on subjective qualitative methods for the evaluation of NCCL progression. In this respect, the quantitative analysis of tridimensional images acquired by non-contact profilometry from NCCLs seems to be a promising evaluation method, in both the laboratory and clinical setting.

The aims of this *in vitro* study were to investigate the suitability of a subtraction analysis of profilometric 3D-images to measure the progression of NCCLs; and to use this method to investigate the influence of toothpaste abrasivity on the progression of NCCLs. The null hypotheses tested in this paper were: (1) the use of 3D-subtraction analysis could not detect and monitor the progression of simulated NCCLs over time; (2) the abrasive level of toothpastes did not affect the initiation and progression of NCCLs.

MATERIALS AND METHODS

Experimental design

The experiment followed a factorial 7×4 design with two factors: 1. slurries, at 7 levels, prepared from either abrasives: higher (Zeodent 103/15%), medium (Zeodent 124/10%) and lower (Zeodent 113/5%) or marketed toothpastes: lower (Sensodyne Pronamel), medium (Crest Cavity Protection) and higher (Crest Pro-Health-Whitening) abrasive (Table 1); deionized water (DIW)

was used as negative control; 2. Brushing time, at 4 levels: 5,000, 15,000, 35,000 and 65,000 strokes. The main response variable was dentine volume loss, in mm^3 . Secondary outcomes were lesion shape classification (%) and lesion angle (degrees).

Specimen preparation

A total of 112 out of 200 upper 1st premolars were selected with no restorations, stains or any type of enamel and root defects. The use of human teeth was reviewed and approved by the local Institutional Review Board under the number # NS0911-07. Teeth were cleaned with a periodontal scaler and allocated into seven groups each with 16 teeth of similar anatomy and dimensions (mesio-distal 9 ± 0.5 mm and bucco-lingual 11 ± 0.5 mm) at the cemento-enamel junction (CEJ). Teeth were mounted on acrylic blocks in which each block carried two teeth, resulting in a total of eight pairs for each group. Pairing was done in order to better simulate the horizontal toothbrushing technique with concentration of filament stress in the cervical area. Root portions were covered by a light-polymerizing acrylic resin sheet (Triad, VLC material, Dentsply Int., York, PA, USA), with the exception of the root surface area 2-mm near the CEJ (Figure 1). After contouring and exposing the root surface area with the aid of a scalpel, the specimen set was light-cured for 5 min.

Reference areas apical and occlusal to the brushing surfaces were determined and protected from the brushing abrasion by fabrication of a protective bleaching tray. Briefly, 1-mm thick plastic tray sheets were molded against each pair of teeth using a bleaching tray vacuum machine (ECONO-VAC, Buffalo Dental Mfg Co, Syosset, NY, USA). After that, a window was cut in the plastic tray material in the area of the CEJ and 2 mm of the root surface above it, leaving the reference areas protected by the plastic tray. Reference areas were used to aid in the positioning of the images for the 3D image subtraction analysis.

Impression

Impressions of the specimens were taken at baseline and after each brushing period (5,000, 15,000, 35,000, 65,000 strokes), with the aid of a petri dish. The eight specimen pairs for each group were mounted on the dish and the impression material (Hydrophilic Vinyl Polysiloxan, Examix NDS Injection Type; GC America, Alsip, IL, USA) loaded on the lid and positioned against the specimens. This guided method allowed the impressions to be taken at similar angles and directions, facilitating the alignment of scans for the subtraction analysis.

Toothbrushing

The specimens were positioned in a V-8 toothbrushing machine, with their long-axis perpendicular to the long-axis of the toothbrushes (Oral B-40, Procter and Gamble, Cincinnati, OH, USA). A brushing load of 200 g was used during the experiment. Slurries were prepared using the different abrasives or toothpastes (Table 1). A volume of 60 ml was used for each specimen block. The reference areas were protected using plastic trays and specimens brushed for 5,000, 15,000, 35,000, and 65,000 double strokes. After finishing each brushing period, the specimens were thoroughly rinsed in deionized water and impressions were taken as described above.

Optical profilometry

An area 20-mm long (X) × 25-mm wide (Y) of each impression was scanned with an optical profilometer (Proscan 2000, Scantron, Taunton, UK). The sensor used was the 10-mm S65/10a (04.41.1665 -10 mm), at 300 Hz and with 100 repetitions in X axis direction and 125 repetitions in Y axis direction. The step size was set at 0.2 mm for both X and Y directions. Each scan had

the reference points automatically identified in the highest location of the protected areas (one in each of the two paired crowns and one in the acrylic area). Using a dedicated software (Proform, Scantron, Taunton, UK), the reference points guided the superimposition of the scans (each brushing period vs. baseline), allowing the natural curvature of the specimen to be considered in the evaluation. For the subtraction analysis, an area of 3×6 mm was selected in each tooth, covering the exposed dentine area (lesion) as well as the adjacent references (occlusal and apical). Dentine volume loss after each brushing time was calculated by a trained examiner (AS), previously checked for intra and inter-examiner agreement.

For agreement, three trained examiners (AS, AH and AK) evaluated the dentine loss of six specimens (2 per group) from 3 different groups (C, F, and G) over different brushing strokes (5,000; 15,000; 35,000 and 65,000 strokes). Each examiner performed this evaluation three independent times, in random sequence and blind conditions to calculate the intra- and inter-examiner agreement. In addition, the within- and between examiner absolute errors were also determined.

Determination of lesion shape

After each brushing cycle, each specimen was photographed using Nikon SMZ 1500 (Nikon, Tokyo, Japan) and its associated software. Visual assessment of the lesion shape was done after the last brushing cycle by a single, blinded examiner, who classified the lesions into flat, cupped and wedged shape. The frequencies of each shape were then recorded for the different abrasive/toothpastes groups.

Determination of lesion angle

The internal angle between the occlusal and apical walls of the NCCLs was calculated using the two-dimensional function of dedicated software (Proscan, Scantron, Taunton, UK). After determining the deepest part of the lesion on the tooth long-axis direction, the two inclines were drawn (following the lesion walls) at a distance of approximately 1.5 mm from the identified deepest point. The angle between the inclines was measured by the software.

Statistical analysis

Intra-examiner and inter-examiner agreement were assessed using intraclass correlation coefficients. The within- and between examiner absolute errors were calculated based on the average lesion size that was 0.94 mm³.

Volume loss and lesion angle data were examined for normality using Shapiro-Wilk tests.

Volume loss was summarized (mean and standard deviation) for each level of group (A, B, C, D, E, F, and G) and brushing stroke (5,000, 15,000, 35,000 and 65,000) and mixed-model ANOVA was used to evaluate the effects of group, brushing stroke, and their interaction. Angle calculations were summarized (mean and standard deviation) for each level of group (A, B, C, D, E, F, and G) and mixed-model ANOVA was used to evaluate the effects of group. The absolute and relative frequencies of lesion shape were summarized. Pearson correlations were used to investigate associations between slurry abrasivity level, dentine volume loss and lesion angle. Statistical significance was set at 5%. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

The intra-class correlation coefficients showed that the intra-examiner agreements were 0.99 (AS), 0.98 (AH) and 0.92 (AK), while the inter-examiner agreement was 0.98. Overall within examiner error was $\pm 0.22 \text{ mm}^3$ (AH: $\pm 0.15 \text{ mm}^3$, AK: $\pm 0.33 \text{ mm}^3$, and AS: $\pm 0.15 \text{ mm}^3$).

Between examiner error was $\pm 0.09 \text{ mm}^3$. And the combined between and within examiner error was $\pm 0.24 \text{ mm}^3$.

Table 2 shows the summary statistics (mean and standard deviation) for each groups (A, B, C, D, E, F, and G) and brushing stroke (5,000, 15,000, 35,000, and 65,000). ANOVA showed that the interaction between group and brushing strokes ($p < 0.001$) had significant impact on volume loss. Dentine volume losses at 5,000, and 15,000 were not significant for all tested groups.

Significant difference in volume loss started at 35,000 brushing strokes where volume loss of group F (Crest Pro-Health Whitening) was significantly higher than groups A (Zeodent 113), D (Sensodyne Pronamel), E (Crest Cavity Protection), and G (DIW). After 65,000 brushing strokes, group F (Crest Pro-Health Whitening) volume loss was significantly higher than all other groups. Group C (Zeodent 103) volume loss was significantly higher than groups A (Zeodent 113), D (Sensodyne Pronamel), E (Crest Cavity Protection), and G (DIW). Groups A (Zeodent 113), B (Zeodent 124), D (Sensodyne Pronamel), E (Crest Cavity Protection), and G (DIW) were not significantly different from each other.

When testing the brushing stroke effect, the dentine volume loss of group A (Zeodent 113) was significantly higher at 65,000 than at 5,000. The loss of group B (Zeodent 124) was significantly higher at 65,000 compared with other brushing strokes. For groups C (Zeodent 103) and F (Crest

Pro-Health Whitening) the loss was significantly higher at 65,000 than other brushing strokes, with 35,000 being significantly higher than 5,000 and 15,000. For group D (Sensodyne Pronamel), dentine loss was significantly higher at 65,000 compared to 15,000. The loss of group E (Crest Cavity Protection) was significantly higher at 65,000 than both 5,000 and 15,000. Finally, no change was observed on the dentine loss of group G (DIW) over the tested brushing strokes.

The frequencies of different shapes of the lesions and calculated lesion angles are summarized in Table 3. The angles for the higher abrasive toothpaste (Crest Pro-Health Whitening) resulted in acute angles compared to less abrasive toothpastes that led to obtuse angles.

Table 4 summarizes the results from Pearson correlations and shows that slurry abrasivity level was strongly positive correlated with dentin volume loss after 35,000 and 65,000 brushing strokes. At the same time points a strong negative correlation was seen between slurry abrasivity level and lesion angle.

DISCUSSION

The present study simulated the cumulative nature of tooth wear due to toothbrushing abrasion. In a simplified theoretical exercise, and considering that adequate plaque removal may require toothbrushing for about 2 min or 20 s per sextant [21], each tooth surface (buccal, lingual and occlusal) would need approximately 6 s of toothbrushing. This equates to 15 about brushing strokes per surface, per day. As result, a year would represent a total of 5,475 strokes.

Extrapolating, the number of brushing strokes of 5,000, 15,000, 35,000, and 65,000 performed in

this study would represent approximately 1, 3, 7 and 13 years, respectively, which might be reasonably in line with the time expected for NCCLs to develop clinically.

To the authors' knowledge, no controlled studies with quantitative outcomes have focused on how the abrasive level of toothpastes affects the initiation and progression of NCCLs to date. Previous reports on the effect of toothpaste abrasivity and toothbrush stiffness progression on NCCLs were limited to qualitative descriptions [22]. The subtraction analysis of profilometric 3D-images used in this study allows for quantitative measurements [10]. Based on the intra- and inter-examiner agreement and on the within- and between examiner error and on the capacity to differentiate the abrasive level of the slurries (discussed later), 3D-subtraction analysis can be considered a suitable tool to measure the progression of NCCLs, thereby leading to the rejection of the first null hypothesis. However, for early-stage lesions, the results may not be straightforward, as surface loss may be below the method's detection threshold. The volume loss values observed after 5,000 brushing strokes most likely include errors resulting from quantification limits of the method. This became apparent in the DIW group, where loss values were reported, although no further increases in dentine volume loss were noted with the increasing number of brushing strokes. A potential explanation could be the low resolution of the scan measurements. A relatively high step-size (0.2 mm) was used in the present study to cover the area of interest in a reasonable amount of time; however, this could probably influence the ability of the profilometer to detect smaller changes. Another artificial aspect of this study was the use of slurries at a fixed dilution throughout the toothbrushing procedure, as the slurry gradually dilutes in the presence of saliva [23].

The lack of lesion progression in specimens brushed with DIW seems to suggest that toothpaste carries more relevance for the progression of the NCCLs compared to the toothbrush. This

observation corroborates previous reports showing that NCCLs developed only when brushing was performed with toothpastes, but not with water [22]. The toothbrushes used presented soft stiffness. Approximately a 200 g load was applied on the toothbrush head. Although filament stiffness likely follows toothpaste abrasivity in terms of relevance in the abrasion process for dentine [24], the controversial findings in the literature with some authors finding more abrasion with increased bristle stiffness [25, 26], whereas others the opposite [27, 28], call for further studies.

The test of three different standard slurries (Zeodent 113, Zeodent 124, and Zeodent 103) determined the effect of abrasives on the NCCLs progression. At 35,000 and 65,000 brushing strokes there was a trend toward increased dentine volume loss with increasing slurry abrasivity. This is in line with the strong positive correlation found between slurry abrasivity and dentine volume loss after 35,000 and 65,000 brushing strokes.

The effect of slurry abrasivity and the rejection of the second null hypothesis becomes clear when comparing the volume loss for each tested group as a function of brushing strokes. Worth mentioning is that for Zeodent 103 and Crest Pro-Health Whitening (most abrasive) dentine volume loss increased exponentially with brushing strokes. This finding may be explained by the fact that as dentine wears away, a softer and therefore less resistant dentine is exposed, rapidly increasing its volume loss.

Both DIW and Sensodyne Pronamel caused flat lesions on all specimens. On the other hand, the higher abrasives/toothpastes caused more wedge-shaped lesions. Compared to visual assessment of the lesion shape, the use of non-contact profilometry enabled us to quantify the lesion angle, providing a better estimate of the lesion shape. A previous report showed no relationship between the shape of the formed lesion and the abrasivity of the toothpaste when evaluated

visually [22]. However, when using non-contact profilometry the higher abrasive toothpaste (Crest Pro-Health Whitening) caused significantly more acute angles (mean: 61.8°) compared to the lower abrasive toothpastes (Sensodyne Pronamel, Zeodent 113, Crest Cavity Protection and Zeodent 124), which in turn forms more obtuse (means ranging from 139.1° to 107.1°) angle lesions. This clearly illustrates that non-contact profilometry angle measurements can significantly differentiate the groups according to the abrasive level of the toothpastes/abrasives. However, the qualitative visual assessment of the lesion shape could not predict such distinction [21].

CONCLUSION

3D-image subtraction could quantify and differentiate dentine volume loss, but only at simulated advanced stages. The use of 3D- image subtraction showed potential to properly monitor the long-term progression of NCCLs that developed faster and more markedly, due to brushing with higher abrasive slurries. Higher abrasive slurries produced lesions with acute angles which correlated well with the toothpaste abrasivity.

DECLARATION OF INTEREST

The authors of this paper have no interest to declare.

ACKNOWLEDGEMENTS

This project was supported by the Erosive Tooth Wear Research Program, of the Indiana University School of Dentistry, Indianapolis, IN, USA.

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Figure 1. Mounted teeth on acrylic block with their root portions covered with acrylic resin, except for 2-mm near the cemento-enamel junction.

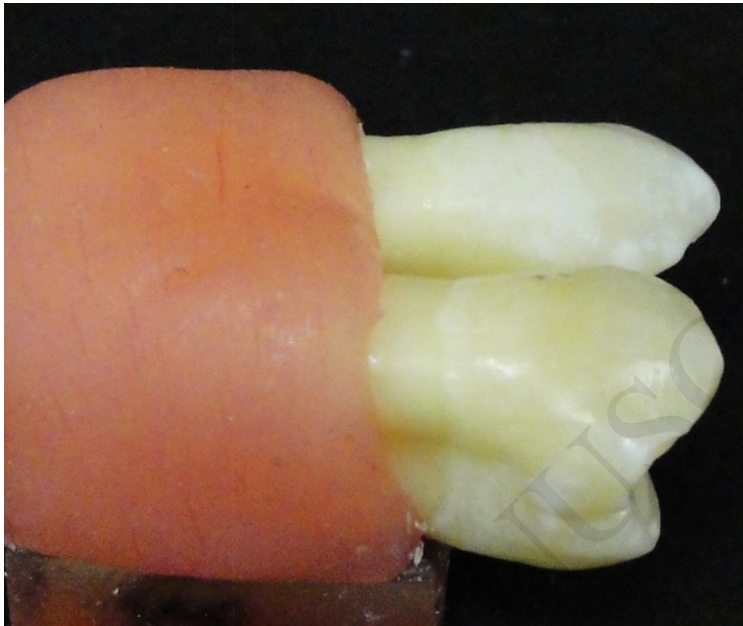


Table 1. Abrasive and toothpaste slurries composition and abrasivity*.

Abrasive / Toothpaste	Abrasive load (%)	Amount (g)	0.5% CMC (g)	DI-water (g)	RDA (SE)*	Manufacturer
A. Zeodent 113 (low)	5	3	57	—	69.2 (2.6)	Huber Engineered Materials, Havre de Grace, MD, USA
B. Zeodent 124 (mid)	10	6	54	—	146.9 (3.5)	Huber Engineered Materials, Havre de Grace, MD, USA
C. Zeodent 103 (high)	15	9	51	—	208.0 (9.4)	Huber Engineered Materials, Havre de Grace, MD, USA
D. Sensodyne Pronamel (low)	—	25	—	40	30.1 (1.1)	GlaxoSmithKline Consumer Healthcare, Brentford, UK
E. Crest Cavity Protection (mid)	—	25	—	40	98.6 (2.9)	Procter & Gamble Co., Cincinnati, OH, USA
F. Crest Pro-Health Whitening (high)	—	25	—	40	220.0 (6.8)	Procter & Gamble Co., Cincinnati, OH, USA
G. Deionized water (control)	—	—	—	60	—	—

* *Radioactive Dentin Abrasivity mean (standard-error) using ISO 11609 methodology.*

Table 2. Means (standard deviation) of dentine volume loss (mm³) by abrasive/toothpaste, at different brushing strokes.

Abrasive / Toothpaste	Dentine volume loss mean (mm ³)			
	5,000	15,000	35,000	65,000
A. Zeodent 113 (low)	0.82 (0.35) Aa	0.97(0.47) Aab	1.07 (0.41) Bab	1.35 (0.65) Cb
B. Zeodent 124 (mid)	0.98 (0.34) Aa	0.81 (0.33) Aa	1.18 (0.38) ABa	1.85 (0.91) BCb
C. Zeodent 103 (high)	0.72 (0.25) Aa	0.80 (0.29) Aa	1.31 (0.57) ABb	2.33 (1.47) Bc
D. Sensodyne Pronamel (low)	0.79 (0.47) Aab	0.71 (0.34) Aa	0.97 (0.39) Bab	1.17 (0.48) Cb
E. Crest Cavity Protection (mid)	0.80 (0.32) Aa	0.83 (0.39) Aa	1.03 (0.50) Bab	1.40 (0.68) Cb
F. Crest Pro-Health Whitening (high)	1.25 (1.32) Aa	1.20 (1.07) Aa	1.93 (1.38) Ab	4.19 (3.29) Ac
G. Deionized water (control)	0.88 (0.51) Aa	0.83 (0.43) Aa	1.02 (0.72)Ba	1.12 (0.73) Ca

Means followed by the same uppercase letters indicate no difference between groups within each column. Means followed by the same lowercase letters did not differ from each other within each row.

Table 3. Frequency (%) of the different shapes of the lesions in each group and mean lesion angle (standard deviation), at 65,000 strokes.

Abrasive/Toothpastes	Flat	Cup	Wedge	Lesion angle
A. Zeodent 113 (low)	4 (25.0%)	5 (31.3%)	7 (43.8%)	131.1 (25.5) A
B. Zeodent 124 (mid)	0 (0.0%)	3 (18.8%)	13 (81.3%)	107.1 (25.1) B
C. Zeodent 103 (high)	0 (0.0%)	6 (37.5%)	10 (62.5%)	81.6 (43.0) C
D. Sensodyne Pronamel (low)	16 (100.0%)	0 (0.0%)	0 (0.0%)	139.1 (18.2) A
E. Crest Cavity Protection (mid)	7 (43.8%)	2 (12.5%)	7 (43.8%)	125.2 (24.0) AB
F. Crest Pro-Health Whitening (high)	1 (6.3%)	5 (31.3%)	10 (62.5%)	61.8 (29.7) C
G. Deionized water (control)	16 (100.0%)	0 (0.0%)	0 (0.0%)	142.5 (16.3) A

Means followed by the same uppercase letters indicate no difference between groups in terms of lesion angle.

Table 4. Correlation between abrasivity level (RDA), volume loss and lesion angle according to the number of brushing strokes.

Brushing strokes	RDA × volume loss		RDA × lesion angle	
	p value	r	p value	r
5,000	0.176	-0.58	0.303	0.51
15,000	0.139	-0.62	0.293	0.52
35,000	0.004	-0.91	0.048	0.82
65,000	0.002	-0.94	0.037	0.84

r = correlation coefficient.