Enamel microcracks in terms of orthodontic treatment: A novel method for their detection and evaluation

Irma DUMBRYTE¹, Laura LINKEVICIENE², Tomas LINKEVICIUS² and Mangirdas MALINAUSKAS³

¹ Vilnius Research Group, Polocko 21/Zvirgzdyno 1, Vilnius LT-01205, Lithuania
² Institute of Odontology, Faculty of Medicine, Vilnius University, Zalgirio 115, Vilnius LT-08217, Lithuania
³ Laser Research Center, Vilnius University, Saulėtekio 10, Vilnius LT-10223, Lithuania
Corresponding author, Irma DUMBRYTE; E-mail: i.dumbryte@gmail.com

The study aimed at introducing current available techniques for enamel microcracks (EMCs) detection, and presenting a method for direct quantitative analysis of an individual EMC. Measurements of the detailed EMCs characteristics (location, length, and width) were taken from the reconstructed images of the buccal tooth surface (teeth extracted from two age groups of patients) employing a scanning electron microscopy (SEM) and our derived formulas before and after ceramic brackets removal. Measured parameters of EMCs for younger age group were 2.41 µm (width), 3.68 mm (length) before and 2.73 µm, 3.90 mm after debonding; for older — 4.03 µm, 4.35 mm before and 4.80 µm, 4.37 mm after brackets removal. Following debonding EMCs increased for both groups, however the changes in width and length were statistically insignificant. Regardless of the age group, proposed method enabled precise detection of the same EMC before and after debonding, and quantitative examination of its characteristics.

Keywords: Crack, Damage, Enamel, Orthodontic debonding, Scanning electron microscopy

INTRODUCTION

For highly skilled orthodontists and rapidly developing technologies, almost all patients can be rewarded with a smile, an aspiration for a lifetime. Many more questions concern the possible undesirable changes in the enamel structure during treatment with brackets, especially following the debonding procedure. Various bracket removal techniques and enamel clean-up methods have been evaluated and compared looking for the most appropriate and least-damaging tooth structure¹-⁴. However, it has been recognized that a certain degree of enamel damage is unavoidable consequence of orthodontic treatment⁵.

More and more attention, especially among patients, is drawn to enamel microcracks (EMCs) as one of the form of enamel damage after debonding. EMCs, quite often visible by the naked eye, may jeopardize the integrity of the enamel, cause stain and plaque accumulation on the rough fractured surface, thus increasing susceptibility to carious lesions and compromising the appearance of the teeth⁶-⁸. Studies have shown that the bracket removal procedure is related with the EMCs increase and formation of new EMCs⁹-¹⁰. Various methods, such as transillumination, staining, ultrasound, or optical coherence tomography (OCT) have been described in the literature for EMCs detection¹¹-¹⁷. Some of these techniques (e.g., transillumination and staining) can be applied directly intraorally for EMCs visual examination and diagnosis¹⁴. However, it is known that certain changes of EMCs characteristics occur during force application procedures in the course of orthodontic treatment. The tendency of development greater extent enamel damage during brackets removal requires a detailed quantitative analysis of EMCs. At the moment no method is invented and applied for direct measurement of EMCs parameters (length, width, depth) intraorally. Thus, precise examination of enamel damage under laboratory conditions remains the most important source of information about the changes of EMCs characteristics during orthodontic treatment.

There are several laboratory techniques (scanning electron microscopy (SEM), stereomicroscopy, confocal optical profilometry (COP), three dimensional (3D) scanning methods) that can be employed to measure volumetric enamel loss, actual depth of enamel removed, or perform spot or line measurements of EMCs parameters¹¹,¹²,¹₅-²¹. The most often methods used and described in the literature, their advantages and disadvantages in terms of quantitative EMCs characteristics examination are presented in Table 1.¹⁵-¹⁷,²²-²⁴. Although SEM technique is routinely utilized for subjective observation of surfaces (e.g., surface roughness evaluation, detection of EMCs or other tooth structure irregularities) following orthodontic debonding, it has certain advantages over stereomicroscopy, COP and 3D scanning methods, such as OCT or ultrasound, in terms of EMCs evaluation (Table 1). In this study it was presented as a technique for direct precise measurements of an individual EMC with micrometer resolution.

Therefore, the objective of this in vitro study was to present a method for quantitative evaluation of EMCs employing SEM before and after brackets removal. Additional aim was to determine the versatility of this technique by applying the same analysis for the teeth from various age groups (younger and older age groups possessing enamel with different mechanical properties).
### Table 1  Comparison of methods for enamel microcracks (EMCs) characteristics evaluation

| Method; device                                      | Working principle | Enamel microcracks (EMCs) characteristics evaluated | Advantages                                                                                                                                                                                                                                                                                                                                 | Disadvantages                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|-----------------------------------------------------|-------------------|-----------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Stereomicroscopy; stereomicroscope (equipped with a camera) | Optical microscopy | Number Direction Length Location                     | Direct evaluation of EMCs. No necessity for specific sample preparation. Non-destructive technique.                                                                                                                                                                                                                                                                                                                                                       | Lower resolution compared to scanning electron microscopy (SEM), useful magnification only up to 1,000–2,000 times. Due to limited magnification no possibility to measure width parameter. Lateral characterization of a tooth surface (2D), no possibility to measure depth of EMCs. Allow only a few measurements per tooth surface. Difficult to analyze non-flat surfaces. Time consuming procedure. In vitro measurements. |
| Scanning electron microscopy (SEM); scanning electron microscope | Electron microscopy | Number Direction Length Location Width              | Direct evaluation and measurement of EMCs. Higher resolution (better than 1 nanometer) and ability of a higher magnification (up to 2 million times) compared to stereomicroscopy; visualization of structures that would normally be not visible by optical microscopy. Possibility to view the three dimensional (3D) external shape of an object. Non-destructive, highly sensitive technique.                                                                                                                                                                                                                                           | Requirements for sample preparation (not obligatory to coat the teeth with a conductive layer for EMCs evaluation). Lateral characterization of a surface (2D), no possibility to measure depth of EMCs. Allow only a few measurements per tooth surface. Difficult to analyze non-flat surfaces. Time consuming procedure. In vitro measurements. |
| Confocal optical profilometry (COP); confocal optical profilometer | Optical microscopy | Number Direction Length Location Width              | Direct evaluation and measurement of EMCs. Lateral and axial characterization (3D) of a surface. No necessity for specific sample preparation. Non-destructive, highly sensitive technique.                                                                                                                                                                                                                     | Allow only a few measurements per tooth surface. More sensitive to non-flat surfaces than SEM. Time consuming procedure. In vitro measurements.                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Optical coherence tomography (OCT); optical coherence tomograph | Low-coherence interferometry | Number Direction Length Location Enamel loss (volumetric) Depth | Real-time image. High spatial resolution (spatial axial resolution of a few micrometers). Allow accurate and reproducible measurements of many points (can measure up to 50,000 points) of tooth surfaces and perform volumetric calculations of the total loss of substance (3D imaging). Non-destructive, non-radiative technique. Time efficient procedure.                                                                                                                                                                                                                                 | Indirect measurement (through replication procedure) because tooth surface causes scattering of the laser beam and consequent loss of resolution. Limited penetration depth and scanning range. In vivo and in vitro measurements.                                                                                                                                                                                                                                                                                                                                                   |
| Ultrasound                                          | High frequency sound waves | Number Direction Length Location Enamel loss (volumetric) Depth | Real-time image. High resolution. Accurate method. Non-destructive, non-radiative technique. Time efficient procedure.                                                                                                                                                                                                                                                                                                                                  | Sample preparation (selection of appropriate coupling media for teeth enamel). Sensitive technique to non-flat surfaces. In vivo and in vitro measurements.                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
MATERIALS AND METHODS

One hundred twenty recently extracted maxillary premolars were included in the final study. The teeth were extracted for orthodontic purposes from two groups of patients: younger age group (age range, 18–34 years), and older age group (age range, 35–54 years)\textsuperscript{25}, and were used with the patients’ informed consent. Primary teeth selection criteria were as follows: intact buccal enamel with no white spots and signs of dental fluorosis; no pre-treatment with chemical agents (such as H\textsubscript{2}O\textsubscript{2}); no previous orthodontic, endodontic or restorative treatment; secondary teeth selection criteria: buccal enamel surface with EMCs or without them.

Following extraction, all the teeth were prepared in accordance with the guidelines of the International Organization for Standardization\textsuperscript{26}. The teeth were decontaminated in 0.5% chloramine T solution and then stored in distilled water that was changed weekly before preparation and testing.

The sample size was calculated using the sample size calculator\textsuperscript{27}, by which a sample size of 103 was required to detect differences with a 5% confidence interval (CI) and 95% confidence level (CL; population size 140). The total number of teeth included in the final study was higher than the number estimated.

The research was conducted in accordance with the protocol presented in Fig. 1. The buccal enamel surfaces of all the teeth included in the study (both from the younger and older age group) were examined employing SEM (TM-1000, Hitachi, Tokyo, Japan)\textsuperscript{9,12,13}. The SEM was operated at 15 kV, at $\leq 5\times 10^{-2}$ Pa (electron gun vacuum) and at $\sim 30–50$ Pa (specimen chamber vacuum). The teeth were not coated with a conductive layer prior to SEM evaluation. The initial examination of EMCs was performed at $\times 50–100$.

Evaluation of the buccal tooth surface and further detailed analysis of EMCs is shown in Fig. 2. The SEM micrographs of the buccal enamel surfaces of all the teeth were taken. To reconstruct images of some larger crowns, montages (stitching together of multiple images) of the SEM micrographs were made. From these micrographs vertical height ($h$) of every tooth’s crown was measured. For detailed analysis of EMCs, the buccal tooth surface was divided in three zones of equal height: first zone — cervical third, second zone — middle third, third zone — occlusal third\textsuperscript{9,11,12,28}. Following initial examination using SEM, the teeth from the younger and older age groups were divided into four subgroups of 15: subgroup 1, teeth having EMCs; subgroup 2, teeth without EMCs; subgroup 3, teeth showing EMCs, served as a control, and subgroup 4, EMCs free teeth, served as a control. The presence of EMC was the main criteria for grouping teeth. The teeth having EMCs were randomly assigned to subgroup 1 or subgroup 3, the teeth without EMCs — to subgroup 2 or subgroup 4. Control subgroups were composed in order to study the effect of dehydration on existing EMCs or formation of new ones.

Using a digitally sketched ruler, every zone was divided into 10 measurement areas (MAs); a total of 30 MAs of each tooth was obtained (Fig. 2). With a help of our derived formula, a measurement step ($x$, the distance between two MAs) was calculated. One EMC of every tooth was analyzed. If several EMCs were visible, one, the longest, was chosen and examined in detail. The width of the longest EMC was measured in each zone where it was located (10 MAs of the width could be

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**Fig. 1** Graphic representation of the study protocol.
The buccal enamel surface divided in 3 zones of equal height

Fig. 2 Evaluation of the buccal enamel surface employing SEM.

A measurement step \((x, \text{ the distance between two measurement areas [MAs]) and length } (l)\) of EMC were quantified utilizing formulas. For \(l\) analysis, the number \((n)\) of MAs, that is, the distance between the first and the last MA in which an EMC was located, was calculated.

registered in every zone). The length of the longest EMC was calculated (Fig. 2).

The location (cervical, middle, and occlusal third), length, and width of the longest EMC for subgroups 1 and 2 from both age groups were evaluated before and after the removal of brackets. The width of the longest EMC was examined in the same segment before and after debonding in spite of the changes in its length.

In subgroups 3 and 4 (controls), the teeth were subjected to the same analysis but not bonded. All the teeth from control subgroups were assessed twice by SEM, as were the other specimens after the same time and means of storage. All the evaluation was accomplished by the same examiner.

The teeth were bonded with maxillary premolar ceramic brackets (Clarity, 3M Unitek, Monrovia, CA, USA) according to the standardized requirements for the bonding procedure: etching with 34.5% phosphoric acid gel (Vococid, Voco, Cuxhaven, Germany) for 30 s, rinsing with water for 20 s, drying with air for 10 s, applying the primer (Contex Primer, Dentaurum, Ispringen, Germany) and curing it with light for 10 s. Resin adhesive (Transbond XT, 3M Unitek) was applied on the bracket base, the bracket was firmly positioned on the enamel surface, and the excess adhesive was removed from the margins of the bracket with a dental probe. Light-cure adhesive was polymerized for 20 s (10 s on each proximal surface) using a halogen light (Mini LED, Satelee, Cambridgeshire, UK). After bonding, all the teeth were placed in distilled water at 37°C and stored for 24 h prior to further testing. The brackets were debonded utilizing Debonding instrument (3M Unitek) on the basis of the manufacturers' recommendations. All visible residual adhesive was carefully removed using a slow-speed handpiece and a carbide-finishing bur under normal clinical conditions. Following debonding, the enamel surface was reevaluated with SEM as presented previously.

Measurement error was tested and parameters were presented in the earlier published study12).

Statistical analysis

Statistical analysis was carried out using the Statistical Package SPSS 17.0 (SPSS, Chicago, IL, USA). The mean, standard deviation (SD), maximum, and minimum were calculated for each variable. A paired samples \(t\) test was applied to evaluate differences between length and width measurements before and after brackets removal. An independent samples \(t\) test was performed to compare the mean overall width and length between two unrelated groups on the same continuous, dependent variable (younger and older age groups, teeth with and without EMCs). For graphical representation, error bars were utilized in which 95% CI was shown. In case of overlapping CI, there was no statistical difference between the groups. In other cases, with no overlapping CI, the statistical significance of the differences was evident with 95% probability. Significance level was set to \(p\leq0.05\).

RESULTS

Assessment of the teeth for new EMCs employing SEM was performed repeatedly three times every second
day by the same examiner. Following repeated teeth evaluations, no discrepancies between results were noticed.

The mean width and length values of EMCs for subgroup 1 from younger and older age groups before and after brackets removal are given in Table 2. Obtained direct measurements enabled comparisons between two groups: EMCs from the older age group showed higher width ($p<0.05$; Fig. 3) and length ($p>0.05$) values; wider EMCs were noticed in all three zones before and after debonding: the first zone (cervical third; $p>0.05$), the second zone (middle third; $p<0.05$), and the third zone (occlusal third; $p<0.05$). Width and length values increased for both groups following removal ceramic brackets ($p>0.05$) The greatest width increase was observed in the third zone (occlusal third) both for younger (0.66 µm; $p>0.05$) and older (1.93 µm; $p>0.05$) age groups.

In subgroup 2 new EMCs were recorded in 3 of

![Fig. 3 Mean overall width of EMCs with 95% CI for younger and older age groups before and after debonding ceramic brackets.](image_url)

| Table 2 | Width and length of enamel microcracks (EMCs) for subgroup 1 from younger and older age groups before bonding and after the removal of ceramic brackets* |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Width of enamel microcracks (EMCs, µm) for younger age group |                |                |                |                |                |                |                |                |                |                |                |                |
|                | Before bonding (n=15) |                |                | After removal (n=15) |                |                |                |                |                |                |                |                |                |                |
|                | Mean | SD | Max | Min | Mean | SD | Max | Min | RC (%) | p | Mean | SD | Max | Min | RC (%) | p |
| First zone    | 2.69 | 1.60 | 7.83 | 0.42 | 2.83 | 1.86 | 9.84 | 0.85 | 5.20 | NS | 2.83 | 1.86 | 9.84 | 0.85 | 5.20 | NS |
| Second zone   | 2.52 | 1.54 | 8.11 | 0.28 | 2.81 | 1.49 | 7.02 | 0.71 | 11.51 | NS | 2.81 | 1.49 | 7.02 | 0.71 | 11.51 | NS |
| Third zone    | 1.64 | 1.19 | 6.20 | 0.45 | 2.30 | 1.24 | 6.42 | 0.56 | 40.24 | NS | 2.30 | 1.24 | 6.42 | 0.56 | 40.24 | NS |
| Overall width | 2.41 | 1.53 | 8.11 | 0.28 | 2.73 | 1.57 | 9.84 | 0.56 | 13.28 | NS | 2.73 | 1.57 | 9.84 | 0.56 | 13.28 | NS |

|                | Length of enamel microcracks (EMCs, mm) for younger age group |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
|                | Before bonding (n=15) |                |                | After removal (n=15) |                |                |                |                |                |                |                |                |                |                |                |
|                | Mean | SD | Max | Min | Mean | SD | Max | Min | RC (%) | p | Mean | SD | Max | Min | RC (%) | p |
| Overall length | 3.68 | 2.14 | 9.17 | 0.90 | 3.90 | 2.35 | 9.17 | 0.90 | 5.98 | NS | 3.90 | 2.35 | 9.17 | 0.90 | 5.98 | NS |

|                | Width of enamel microcracks (EMCs, µm) for older age group |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
|                | Before bonding (n=15) |                |                | After removal (n=15) |                |                |                |                |                |                |                |                |                |                |                |
|                | Mean | SD | Max | Min | Mean | SD | Max | Min | RC (%) | p | Mean | SD | Max | Min | RC (%) | p |
| First zone    | 3.30 | 3.45 | 27.35 | 0.37 | 4.25 | 3.73 | 25.86 | 0.64 | 28.79 | NS | 4.25 | 3.73 | 25.86 | 0.64 | 28.79 | NS |
| Second zone   | 4.58 | 5.68 | 33.02 | 0.37 | 4.58 | 5.02 | 35.94 | 0.73 | 0 | NS | 4.58 | 5.02 | 35.94 | 0.73 | 0 | NS |
| Third zone    | 3.84 | 5.00 | 20.66 | 0.37 | 5.77 | 6.56 | 31.00 | 0.55 | 50.26 | NS | 5.77 | 6.56 | 31.00 | 0.55 | 50.26 | NS |
| Overall width | 4.03 | 4.98 | 33.02 | 0.37 | 4.80 | 5.19 | 35.94 | 0.55 | 19.11 | NS | 4.80 | 5.19 | 35.94 | 0.55 | 19.11 | NS |

|                | Length of enamel microcracks (EMCs, mm) for older age group |                |                |                |                |                |                |                |                |                |                |                |                |                |                |
|                | Before bonding (n=15) |                |                | After removal (n=15) |                |                |                |                |                |                |                |                |                |                |                |
|                | Mean | SD | Max | Min | Mean | SD | Max | Min | RC (%) | p | Mean | SD | Max | Min | RC (%) | p |
| Overall length | 4.35 | 2.29 | 8.70 | 1.15 | 4.37 | 2.37 | 9.00 | 0.92 | 0.46 | NS | 4.37 | 2.37 | 9.00 | 0.92 | 0.46 | NS |

*Max, maximum; Min, minimum; SD, standard deviation; RC, relative change.

NS indicates non-significant.
Table 3 Width and length of enamel microcracks (EMCs) for subgroup 2 from younger and older age groups after the removal of ceramic brackets*

| Width of enamel microcracks (EMCs, µm) after the removal of ceramic brackets | Younger age group (n=3) | Older age group (n=4) |
|---|---|---|
| First zone | Mean | SD | Max | Min | Mean | SD | Max | Min |
| Second zone | 2.47 | 1.17 | 4.32 | 1.01 | 1.23 | 0.78 | 2.79 | 0.44 |
| Third zone | —** | — | — | — | 3.26 | 4.07 | 11.42 | 0.37 |
| Overall width | 2.40 | 1.08 | 5.21 | 0.67 | 1.96 | 2.28 | 11.42 | 0.37 |

| Length of enamel microcracks (EMCs, mm) after the removal of ceramic brackets | Younger age group (n=3) | Older age group (n=4) |
|---|---|---|
| Overall length | Mean | SD | Max | Min | Mean | SD | Max | Min |
| Overall length | 1.35 | 0.58 | 1.88 | 0.73 | 2.23 | 1.53 | 4.18 | 0.72 |

* Max, maximum; Min, minimum; SD, standard deviation.
** Absence of EMCs in third zone after bracket removal for younger age group.

15 (20%) teeth for younger and 4 of 15 (26.67%) teeth for older age groups. The mean width and length values of these new EMCs are presented in Table 3. Newly formed EMCs possessed lower length (p>0.05) compared with the characteristics of EMCs for subgroup 1 from both groups. Width of new EMCs followed the same pattern, however significant result was found in the older age group (Fig. 4).

Descriptive statistics of the width and length values of EMCs for subgroup 3 are shown in Table 4. Differences in width and length values for both age groups were quite small and nonsignificant. New EMCs were not recorded for subgroup 4 from younger and older age groups.

**DISCUSSION**

The aim of our study was to introduce current available techniques for EMCs detection and present a method for direct quantitative analysis of an individual EMC employing SEM. Additional objective was to determine the versatility of this technique by applying the same analysis for the teeth from younger and older age groups possessing enamel with different mechanical properties.

Although an in vitro study design was chosen, before the investigation the opportunities to perform an in vivo examination of EMCs utilizing for example, a fiber-optic microscope were also assessed. It is possible to detect the EMC intraorally with such equipment, however at the moment, measurements of the quantitative EMC characteristics ensuring micrometer resolution required for characterization of an individual EMC cannot be made directly clinically. Alternatively, replication of the buccal tooth surface is another technique combining in vivo and in vitro measurement, e.g., OCT requires replication procedure because tooth surface causes scattering of the laser beam and consequent loss of resolution21). However, using of indirect measurements is always introducing additional errors. Furthermore, in all cases, the standardization of the experiment is crucial for the precise evaluation (detection of the same measurement sites) of the EMCs before and after debonding in order to make a comparative study. On the other hand, in laboratory (in vitro) placing a marker on the buccal tooth surface (or using guiding anatomical landmarks) can be employed as a simple and reliable method for relocating EMCs. Vice versa, the detection of the same place of the EMC intraorally after two years of treatment with brackets is technically restricted. Thus, the results of an
Table 4  Width and length of enamel microcracks (EMCs) for subgroup 3 from younger and older age groups*

|                     | Initial measurement (n=15) | Final measurement (n=15) |
|---------------------|-----------------------------|--------------------------|
|                     | Mean | SD  | Max  | Min  | Mean | SD  | Max  | Min  | RC (%) | p     |
| First zone          | 3.25 | 3.24| 14.88| 0.45 | 3.36 | 2.81| 14.53| 0.71 | 3.38   | NS    |
| Second zone         | 2.34 | 1.61| 9.24 | 0.35 | 2.40 | 1.53| 8.35 | 0.49 | 2.56   | NS    |
| Third zone          | 1.87 | 1.50| 5.99 | 0.35 | 1.85 | 1.26| 5.71 | 0.49 | -1.07  | NS    |
| Overall width       | 2.53 | 2.28| 14.88| 0.35 | 2.59 | 2.05| 14.53| 0.49 | 2.37   | NS    |

|                     | Initial measurement (n=15) | Final measurement (n=15) |
|---------------------|-----------------------------|--------------------------|
|                     | Mean | SD  | Max  | Min  | Mean | SD  | Max  | Min  | RC (%) | p     |
| Overall length      | 3.61 | 1.98| 7.64 | 0.76 | 3.65 | 1.95| 7.64 | 0.76 | 1.11   | NS    |

|                     | Initial measurement (n=15) | Final measurement (n=15) |
|---------------------|-----------------------------|--------------------------|
|                     | Mean | SD  | Max  | Min  | Mean | SD  | Max  | Min  | RC (%) | p     |
| First zone          | 2.23 | 1.48| 9.88 | 0.46 | 2.19 | 1.04| 6.63 | 0.62 | -1.79  | NS    |
| Second zone         | 3.11 | 2.54| 9.26 | 0.31 | 3.21 | 2.70| 9.89 | 0.31 | 3.22   | NS    |
| Third zone          | 2.69 | 1.92| 7.56 | 0.29 | 3.15 | 2.29| 9.11 | 0.25 | 17.10  | NS    |
| Overall width       | 2.73 | 2.14| 9.88 | 0.29 | 2.88 | 2.26| 9.89 | 0.25 | 5.49   | NS    |

|                     | Initial measurement (n=15) | Final measurement (n=15) |
|---------------------|-----------------------------|--------------------------|
|                     | Mean | SD  | Max  | Min  | Mean | SD  | Max  | Min  | RC (%) | p     |
| Overall length      | 3.01 | 1.54| 5.04 | 0.87 | 3.04 | 1.57| 5.04 | 0.58 | 1.00   | NS    |

*Max, maximum; Min, minimum; SD, standard deviation; RC, relative change.
NS indicates non-significant.

The *in vitro* study will always serve as a control for future clinical trials on the enamel damage evaluation.

Presented data of this investigation showed that it was possible to measure width and length parameters (mean, maximum, and minimum values) of the EMCs from both age groups employing SEM. The latter device possesses higher resolution and ability of a greater magnification compared to optical microscopes (e.g., stereomicroscope), and is less sensitive to non-flat surfaces than confocal optical profilometer. Obtained findings indicated that the teeth having EMCs from the older age group possessed higher width and length values compared with the younger one. Aging and the related changes in the mechanical properties of human enamel (increase in hardness, elastic modulus, brittleness, and decrease in fracture toughness with age) might explain the aforementioned differences. Further analysis revealed that following debonding width and length of all EMCs increased. Changes for the younger age group were 0.32 µm in the width and 0.22 mm in the length parameters; for the older age group —0.77 µm in the width and 0.02 mm in the length values. The tendency of EMCs to increase during bracket removal can be supported by the literature.

Applied technique enabled us to evaluate the morphology of the EMC in the cervical, middle, and occlusal third, and locate the same measurement site before and following debonding. During the examination of the width parameter in every zone the highest increase was noticed in the occlusal third, both for younger (0.66 µm) and older (1.93 µm) age groups. The increase in the enamel brittleness with distance from the dentin-enamel junction to the occlusal surface might be the reason for greater EMCs increase in the third zone (occlusal third) following debonding. However, it is important to note that due to the higher bond strength *in vitro* than *in vivo* (because of the oral humidity, etc.), the obtained increase in EMCs may be greater than in a clinical situation.

SEM can be utilized for new EMCs detection by
comparing the reconstructed images of the buccal tooth surface before and following bracket removal. Analyzing the teeth without EMCs before bonding procedure, new EMCs were recorded in 3 of 15 (20%) teeth for younger and 4 of 15 (26.67%) teeth for older age groups after debonding. Thus, the majority of teeth did not possess new EMCs. The formation of EMCs during the debonding procedure depends on the bond strength required to remove the bracket and the linear tensile strength of enamel\textsuperscript{32}. While minimum bond strength of 5.9–7.8 MPa was found to be adequate in daily clinical practice, the mean bond strength for the different enamel conditioning, adhesive and bracket combinations might range from 3.9 to 18.6 MPa\textsuperscript{32}. It has been shown that the mean linear tensile strength of enamel is 14.5 MPa\textsuperscript{32}. Thus, when the debonding force exceeds the mean linear tensile strength of enamel, new EMCs appear, the changes of EMCs characteristics (width, length parameters) or even fracture of enamel surface take place. Still, there is no one common agreement in the literature regarding the effect of debonding on the enamel, and the results presented vary from no enamel damage after bracket removal to 20–35% of the teeth with new EMCs following debonding ceramic brackets\textsuperscript{1, 5, 39, 40}. Measured width and length parameters of new EMCs were lower compared to the corresponding values of the teeth with EMCs before bonding. The latter difference suggests that not the bracket removal procedure but the enamel morphology and its structural changes play greater role in the enamel damage formation.

**CONCLUSION**

The proposed method, combining SEM and our derived formulas, enabled precise detection of the same EMC before and after orthodontic debonding, and quantitative examination of its characteristics (length and width). This technique showed to be versatile and could be applied for all the teeth both from younger and older age groups having enamel with different mechanical and optical properties.

**REFERENCES**

1) Ryf S, Flury S, Palaniappan S, Lussi A, van Meerbeek A, Zimmerli B. Enamel loss and adhesive remnants following bracket removal and various clean-up procedures in vitro. Eur J Orthod 2012; 34: 25-32.
2) Alessandri Bonetti G, Zanarini M, Incerti Parenti S, Lattuca M, Marchionni S, Gatto MR. Evaluation of enamel surfaces after bracket debonding: an in-vivo study with scanning electron microscopy. Am J Orthod Dentofacial Orthop 2011; 140: 696-702.
3) Pont HB, Özcan M, Bagis B, Ren Y. Loss of surface enamel after bracket debonding: an in vivo and ex vivo evaluation. Am J Orthod Dentofacial Orthop 2010; 138: 387-397; discussion 387-389.
4) Faria-Júnior EM, Guiraldo RD, Berger SB, Correr AB, Correr-Sobrinho L, Contreras EF, Lopes MB. In-vivo evaluation of the surface roughness and morphology of enamel after bracket removal and polishing by different techniques. Am J Orthod Dentofacial Orthop 2015; 147: 324-329.
5) Arhun N, Arman A. Effects of orthodontic mechanics on tooth enamel: a review. Semin Orthod 2007; 13: 281-291.
6) Sorel O, El Alam R, Chagnneau F, Cathelineau G. Comparison of bond strength between simple foil mesh and laser-structured base retention brackets. Am J Orthod Dentofacial Orthop 2002; 122: 260-266.
7) Zachrisson BU, Buyukkılıç T. Bonding in orthodontics. In: Graber TM, Vanarsdall RL, Vig KWL, editors. Orthodontics: current principles and techniques. St Louis: Elsevier-Mosby; 2005. p. 612-619.
8) Chen CS, Hsu ML, Chang KD, Kuang SH, Chen PT, Gung YW. Failure analysis: enamel fracture after debonding orthodontic brackets. Angle Orthod 2008; 78: 1071-1077.
9) Dumbyte I, Linkoviciene L, Malinauskas M, Linkovicius T, Peciuliene V, Tikusiš K. Evaluation of enamel micro-cracks characteristics after removal of metal brackets in adult patients. Eur J Orthod 2013; 35: 317-322.
10) Kitahara-Cêia FM, Mucha JN, Marques dos Santos PA. Assessment of enamel damage after removal of ceramic brackets. Am J Orthod Dentofacial Orthop 2008; 134: 548-555.
11) Shahabi M, Heravi F, Mohkber N, Karamad R, Bishara SE. Effects on shear bond strength and the enamel surface with an enamel bonding agent. Am J Orthod Dentofacial Orthop 2010; 137: 375-378.
12) Dumbyte I, Jonavicius T, Linkoviciene L, Linkovicius T, Peciuliene V, Malinauskas M. The prognostic value of visually assessing enamel microcracks: Do debonding and adhesive removal contribute to their increase? Angle Orthod 2016; 86: 437-447.
13) Dumbyte I, Jonavicius T, Linkoviciene L, Linkovicius T, Peciuliene V, Malinauskas M. Enamel cracks evaluation—a method to predict tooth surface damage during debonding. Dent Mater J 2015; 34: 828-834.
14) Clark DJ, Sheets CG, Paquette JM. Definitive diagnosis of early enamel and dentin cracks based on microscopic evaluation. J Esthet Restor Dent 2003; 15: 391-401.
15) Culjat MO, Singh RS, Brown ER, Neurgoanhar RR, Yoon DC, White SN. Ultrasound crack detection in a simulated human tooth. Dentomaxillofac Radiol 2005; 34: 80-85.
16) Hsieh YS, Ho YC, Lee SY, Chuang CC, Tsai JC, Lin KE, Sun CW. Dental optical coherence tomography. Sensors (Basel) 2013; 13: 8928-8949.
17) Leao Filho JC, Braz AK, de Araujo RE, Tanaka OM, Pirton MM. Enamel quality after debonding: evaluation by optical coherence tomography. Braz Dent J 2015; 26: 384-389.
18) Heravi F, Rashed R, Raziee L. The effects of bracket removal on enamel. Aust Orthod J 2008; 24: 110-115.
19) Habibi M, Nik TH, Hoooshmand T. Comparison of debonding characteristics of metal and ceramic orthodontic brackets to enamel: an in vitro study. Am J Orthod Dentofacial Orthop 2005; 132: 675-679.
20) Ahraari F, Heravi F, Fekrazad R, Farzanezgan F, Nakhaei S. Does ultra-pulse CO\textsubscript{2} laser reduce the risk of enamel damage during debonding of ceramic brackets? Lasers Med Sci 2012; 27: 567-574.
21) Al Shamsi Al, Cunningham JL, Lamey PJ, Lynch E. Three-dimensional measurement of residual adhesive and enamel loss on teeth after debonding of orthodontic brackets: an in vitro study. Am J Orthod Dentofacial Orthop 2005; 2013; 31: 301. e9-15.
22) MicrobeHunter Microscopy Magazine. URL: [http://www.microbehunter.com/electron-microscopes-vs-optical-light-microscopes/](http://www.microbehunter.com/electron-microscopes-vs-optical-light-microscopes/). Accessed July 2016.
23) Yamada MK, Uo M, Ohkawa S, Akasaka T, Wataru F. Non-contact surface morphology analysis of CO\textsubscript{2} laser-irradiated teeth by scanning electron microscope and confocal laser scanning microscope. Mater T 2004; 45: 1033-1040.
24) Imai K, Shimada Y, Sadr A, Suni Y, Tagami J. Noninvasive
cross-sectional visualization of enamel cracks by optical coherence tomography in vitro. J Endod 2012; 38: 1269-1274.
25) Park S, Wang DH, Zhang D, Romberg E, Arola D. Mechanical properties of human enamel as a function of age and location in the tooth. J Mater Sci Mater Med 2008; 19: 2317-2324.
26) International Organization for Standardization. Dental materials: testing of adhesion to tooth structure. ISO/TS 11405; 2003.
27) Sample size calculator. URL: ‘http://www.calculator.net/sample-size-calculator.html’. Accessed July 2016.
28) Bishara SE, Ostby AW, Laffoon J, Warren JJ. Enamel cracks and ceramic brackets failure during debonding in vitro. Angle Orthod 2008; 78: 1078-1083.
29) Park S, Quinn JB, Romberg E, Arola D. On the brittleness of enamel and selected dental materials. Dent Mater 2008; 24: 1477-1485.
30) Zheng Q, Xu H, Song F, Zhang L, Zhou X, Shao Y, Huang D. Spatial distribution of the human enamel fracture toughness with aging. J Mech Behav Biomed Mater 2013; 26: 148-154.
31) Pickett KL, Sadowsky PL, Jacobson A, Lacefield W. Orthodontic in vivo bond strength: comparison with in vitro results. Angle Orthod 2001; 71: 141-148.
32) Jena AK, Duggal R, Mehrotra AK. Physical properties and clinical characteristics of ceramic brackets: a comprehensive review. Trends Biomater Artif Organs 2007; 20: 123-138.