Microscopic evaluation of the enamel surface after debonding procedures: An *ex vivo* study using scanning electron microscopy

Sara Bernardi, Maria A. Continenza, Guido Macchiarelli

Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy

Corresponding author: Sara Bernardi, Department of Life, Health and Environmental Sciences, University of L'Aquila, Via Vetoio, Coppito 2, 67100, L'Aquila, Italy. Tel. +39.0862.433344.
E-mail: sara.bernardi@ univaq.it

Key words: Dental debonding; scanning electron microscopy; enamel surface.

Contributions: SB, designed the study, interpreted the data, drafted the article and gave the final approval of the version to be published; MAC, designed the study revised critically the article and gave the final approval of the version to be published; GM, designed the study, revised critically the article and gave the final approval of the version to be published.

Conflict of interest: The authors have no conflict of interest to disclose.

SUMMARY

The bracket placement on the dental surface includes the enamel conditioning to improve the adhesive bonding at enamel-adhesive and at adhesive-bracket interfaces. The final stage of the orthodontic therapy, after the bracket removal, is the debonding procedure. This stage aims to polish enamel surface to prevent the plaque accumulation and future enamel injures. The aim of this study is to evaluate and compare the enamel surface underwent to four different debonding procedures on two groups of extracted teeth, by using of the Scanning Electron Microscopy. The teeth were extracted at the Dental Clinic of University of L'Aquila for periodontal reasons and donated for research with the patients' consent. On one teeth group (group A) the enamel polishing techniques was performed with the aid of a magnifying loupe. On the other group (group B) the polishing was performed with naked eyes. The four techniques used included the use of i) Multiblade burr/soft-polisher tip Komet; ii) Multiblade burr/Blade; iii) Disks Sof-lex; and iv) Enhance/Pogo. The images were analysed by the Image J software and qualitative and quantitative considerations referring to Adhesive Index Residual and Enamel Damage Index were made. The quantitative and qualitative results showed the most conservative technique was the tungsten carbide bur followed by the polishing using the soft-polisher tip, under the aid of the magnification system. From the above considerations, it can be concluded the use of a magnifying loupe aids in significant way during the debonding procedures for the enamel surfaces' preservation.

Received for publication: 14 November 2017. Revision received: 16 December 2017. Accepted for publication: 22 December 2017.

©Copyright S. Bernardi *et al.*, 2018
Licensee PAGEPress, Italy
microscopie 2018; 29:7190
doi:10.4081/microscopie.2018.7190

This article is distributed under the terms of the Creative Commons Attribution Noncommercial License (by-nc 4.0) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

Introduction

Modern orthodontic treatments with fixed appliance, included in their protocol the need to follow the rules of adhesive dentistry (Buonocore, 1955) for brackets application on the vestibular surface of the teeth crowns. These include the etching of the enamel tissue and the application of adhesive cement for the brackets application. The etching is performed with a 37% orthophosporic acid to solve the enamel crystals. This stage allows to high the surface tensile strength and lead to the penetration of the bonding material into the etched enamel. When the brackets are removed, the debonding procedure declares the end of the orthodontic treatments. The debracketing procedure in case of metal brackets by means of orthodontic pliers acts at the level of the bracket-adhesive interface, causing the failure of the bond and leaving residuals on the enamel surfaces (Øgaard and Fjeld, 2010).

In case of ceramic brackets, which often are required by patients for aesthetic reasons, the debracketing is riskier since the forces act at enamel-adhesive interface (Øgaard and Fjeld, 2010). Therefore, the risks of irreparable damages at level of the enamel crystals in these cases is very high. So, the debonding final stage is crucial since it should lead to lead the enamel surface to a condition as much as possible similar to the pre-treatment status. Therefore, the debonding or cleaning procedure to remove the adhesive is fundamental to avoid late side effects such as white spot lesions or decays. So far, there is no standard protocol for the debonding procedure and there is no tool able to remove the adhesive material without damaging the enamel surface (Ulusoy, 2009). Conventional diamond burs can scratch the enamel due to their shape and sharpness. In addition, they can cause deep gouges on the enamel surface (Zarrinnia *et al*., 1995). One step and multi-step finishing system are available on the market, with a preference in literature of the multi-step finishing system (Sigilião *et al*., 2015). The aim of this study was to assess and evaluate the structure enamel surfaces treated with different debonding methods, by means of the Scanning Electron Microscopy (SEM).

Materials and Methods

Sample material

Twenty-four single-rooted extracted teeth were used for the evaluation. The teeth were extracted at the Dental Clinic of University of L'Aquila for periodontal reasons and donated for research with the patients consent. The teeth were with no signs of caries or macroscopical cracks or fracture on the coronal portion. The teeth were divided in two groups, A and B. In the group A the debonding procedures were performed with the aid of a magnifying loupe and in the group B without the aid of a magnification system. The use of residual dental material for the study was approved by the Institutional Review Board.

Enamel conditioning procedures

The vestibular surface of the crown was used for the samples preparation. The enamel conditioning procedure included the following steps according to the three steps bonding system, that provides high bond strengths (De Munck *et al*., 2005):

1. Mechanical polishing of the vestibular enamel surface for 10 s.
2. Washing with air-water dental syringe for 20 s.
3. Etching with 36% orthophosphoric acid (De Trey conditioner; Dentsply Sirona, Millford, DE, USA) for 20 s, followed by washing with water for 20 s.
4. Adhesive (Ormco Corp., Glendora, CA, USA) application on enamel surface and on the internal surface of brackets (Victory; 3M UniteK, Monrovia, CA, USA) by means of a dental micro brush.
5. Application of the composite resin for the brackets bonding on the treated enamel surface and brackets application.
6. Removal of the excessive resin on the brackets base by means of a dental probe.
7. Light curing for 40 s (20 s on the mesial and 20 s on the distal side).

Debonding procedures

The bracket remained on the vestibular surface of the tooth for 24 h, as described before (Sigilião *et al*., 2015), to allow the full polymerization of the resin. The brackets were removed with the appropriate orthodonthic pliers, trying to break the adhesive-enamel bond, in order to leave some adhesive on the enamel surface. After these stage, the teeth of group A and group B underwent to the following debonding procedure:

i) Removal of the resin by means of a multiblade burr followed by a soft-polisher tip Komet (Komet, Milan, Italy) using a high-speed hand piece with water cooling (n=3).
ii) Removal of the resin by means of a multiblade burr using a high-speed hand piece with water cooling and followed by a surgical blade (n=3).
iii) Removal of the resin by means of disks Sof-lex (3M ESPE, Seefeld, Germany) using a low-speed hand piece with air-cooling (n=3).
iv) Removal of the resin by means of Enhance Pogo system (Dentsply Sirona) using a low-speed hand piece with air-cooling (n=3).

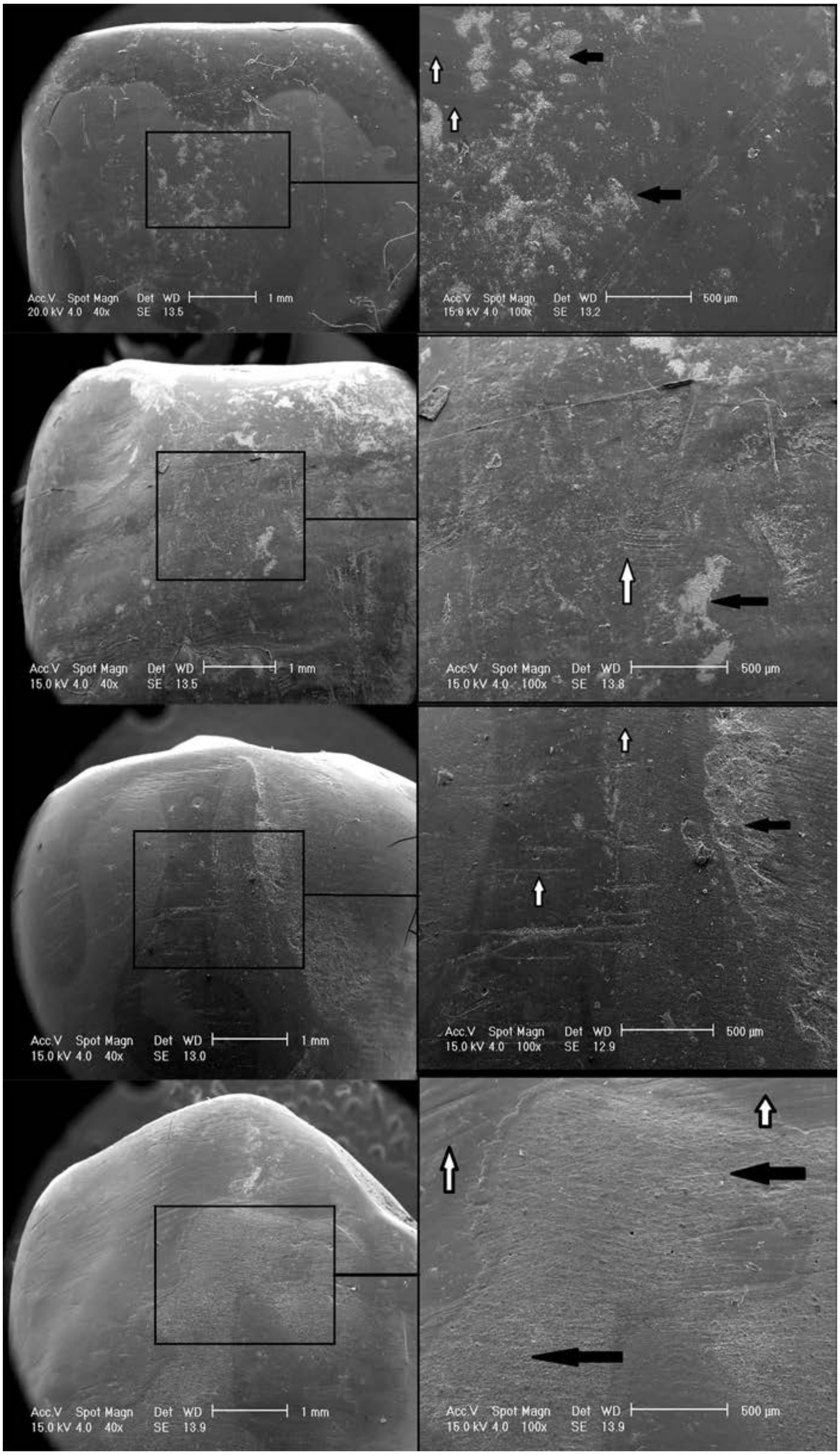

Figure 1. Qualitative observation of the Group A samples. The black arrows indicate the adhesive residuals and the white arrows the scratches and the grooves.

The debonding procedures were chosen basing on the protocols most used by orthodontists (Brauchli *et al.*, 2011).

SEM analysis

The teeth were stored in PBS solution and prepared for the SEM observation as described before (D'Attilio *et al.*, 2005). Briefly, the teeth were dried with high-pressure air, coated with 300 Å layer of gold and palladium, mounted on an aluminium stub and observed at the SEM (Philips XL30CP, The Netherlands). The observations were performed at 15 kV, at a working distance ranging from 12.5 to 13.5 mm, capturing images at 40x and 100 x magnification. The images were analysed by the Image J software, using the thresholding process (Schindelin *et al.*, 2012; Eliceiri *et al.*, 2012; Abramoff *et al.*, 2004), and qualitative and quantitative considerations referring to Adhesive Residual Index (ARI) and a modified Enamel Damage Index (EDI) (Gracco *et al.*, 2015) were made. The ARI included 4 scores: 0, indicating 0% adhesive remained on the tooth; 1, indicating less than 50% of the adhesive remained on the tooth; 2, indicating that 50% of the adhesive remained on the tooth; and 3, indicating that 100% of the adhesive remained on the tooth. The modified EDI include 4 score: 0, smooth surface without scratches, and perikymata might be visible; 1, acceptable surface, with fine scattered scratches (1-10% of the surface); 2, rough surface with numerous coarse scratches or slight grooves visible (11-50% of the surface); and grade 3, surface with coarse scratches, wide grooves and enamel damage visible without the aid of a magnification system (>50% of the surface).

Results

Qualitative observation

The SEM observations allowed to identify both the adhesive residuals and the enamel damages. So, after a qualitative recognition to distinguish the adhesive residuals from the enamel damages (Figures 1 and 2) the segmentation was performed to proceed to the quantitative image analysis (Figure 3).

Quantitative image analysis - Group A

Comparing the four used debonding procedures, the one achieving a good compromise between the adhesive removal and the enamel damages was the use of a multi-step technique involving firstly the use of a multiblade burr followed by the use of a soft-polisher tip (Komet).

As showed in Table 1, it was the only procedure where the ARI was rated at 1 and the EDI was rated 1.

Quantitative image analysis - Group B

In this group as well, the multi-step technique involving firstly the use of a multiblade burr followed by the use of a soft-polisher tip (Komet) showed lower ARI and EDI values compared to the others, especially in the EDI values (Table 2).

Comparison between Group A and Group B

Looking at the values reached in the two groups, and if we compare them, the group A appears to have lower ARI and EDI values than the group B, in all of the four used techniques (Table 3).

Discussion

The ideal finishing technique after the debracketing should not remove an excessive amount of enamel tissue and smooth the surface (Ulusoy, 2009). Indeed, a not conservative approach to the debonding procedure can lead to an irreversible damage of the enamel crystals such as deep cracks, increasing of tooth sensitivity and unaesthetic aspects in case of anterior teeth (Rodríguez-Chávez *et al.*, 2017). Despite these considerations, no standard protocol is available, nor systematic reviews nor meta-analysis which can guide the dental practitioner in the daily work routine. Some practitioners use diamonds burs to low the times at the chair, but regardless the experience and the careful use of the rotative instruments, the enamel crystals can be involved in the mechanical removal process. Some studies report and advise

Table 1. Indices and corresponding percentages of the adhesive residuals and of the enamel damages of Group A. The sub group 1A is the one resulting with the low EDI score.

Group A	Technique used	Adhesive residual index (ARI)	Enamel damage index
1A	Multiblade burr/soft-polisher tip Komet	1 (8%)	1 (7%)
2A	Multiblade burr/Blade	1 (6%)	2 (15%)
3A	Disks Sof-lex	1 (3%)	2 (30%)
4A	Enhance / Pogo	2 (45%)	2 (20%)

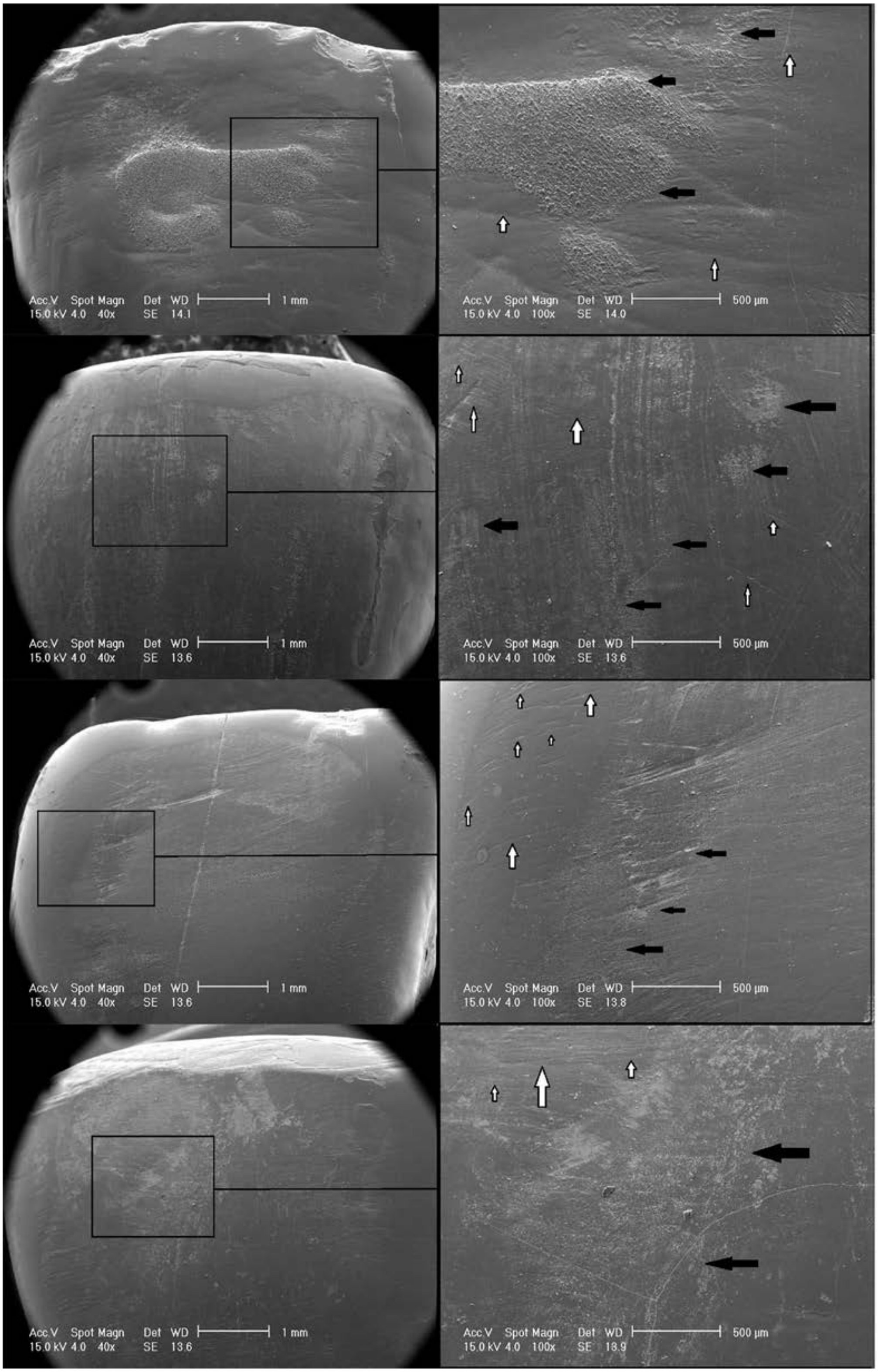

Figure 2. Qualitative observation of the Group B samples. The black arrows indicate the adhesive residuals and the white arrows the scratches and the grooves.

the use of tungsten carbide burs, followed by a finishing system. Zachrisson and Artun (1979) suggested the use of low speed tungsten carbide burs, and Campbell (1995) recommended to add water cooling at high speed. A study by Alessandri Bonetti *et al*. (2011) reported no clinically relevant damage to the enamel using a 12-bladed tungsten carbide bur followed by finishing with graded medium, fine, and superfine Sof-Lex discs. Ulusoy (2009) recommended the use of Enanched Pogo System after the cleaning with tungsten carbide bur to low the time on the chair. In addition, this study reported that the use of Sof-Lex discs decreased the surface roughness, but left enamel abrasion. Øgaard and Fjeld (2010) in their review specified the use of carbide finishing bur with larger wedge angle and oblique ground chamfer conventional carbide burs is more conservative than conventional carbide bur, due to the shape of the blade. In addition, they underlined the fact that even though the use of a low-speed handpiece leaves more adhesive residual than the use of highspeed turbine or ultrasonic scaler tips, in the latter the enamel loss is much higher (Øgaard and Fjeld, 2010). Sigilião *et al.* (2015) in their study, focusing very much on the surface roughness, reported how literature strongly recommends the sequential use of multiple tools for polishing than one-step procedures to effectively smooth the surface. Regarding the use of a magnification system, Baumann *et al.* (2011) recommended their use in the debonding procedure. Indeed, their results were very promising and the EDI and ARI resulted lower when the dental loupes were used.

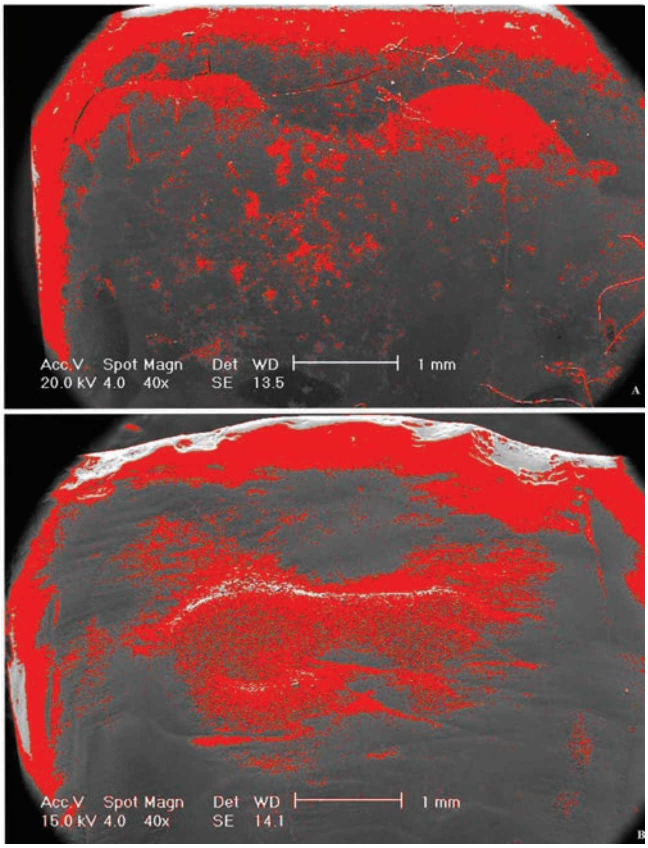

Figure 3. Segmentation of representative samples from group A (a) and group B (b).

Table 2. Indices and corresponding percentages of the adhesive residuals and of the enamel damages of Group B. The sub group 1B is the one resulting with the lower EDI score.

Group A	Technique used	Adhesive residual index (ARI)	Enamel damage index
1B	Multiblade burr/soft-polisher tip Komet	2 (35%)	2 (15%)
2B	Multiblade burr/Blade	2 (15%)	2 (25%)
3B	Disks Sof-lex	1 (4%)	3 (55%)
4B	Enhance / Pogo	2 (40%)	2 (30%)

Table 3. Comparison of the scores of the ARI and EDI in Group A and Group B. The Group B scores are mostly higher than those of Group A.

Technique used	Adhesive residual index (ARI) Group A	Adhesive residual index (ARI) Group B	Enamel damage index Group A	Enamel damage index Group B
Multiblade burr/soft-polisher tip Komet	1	2	1	2
Multiblade burr/Blade	1	2	2	2
Disks Sof-lex	1	1	2	3
Enhance / Pogo	2	2	2	2

Due to all of these controversies and to this open discussion, the enamel surface after the debonding procedures has been investigated through different system. Profilmeter, atomic force microscopy, environmental scanning electron microscopy and energy dispersive X-ray spectroscopy were used for these type of study, due to the quantitative data provided by these instruments (Sigilião *et al*., 2015; Rodríguez-Chávez *et al*., 2017). However, scanning electron microscopy revealed to be a good investigation tool for the observation of adhesive residual and enamel damages (Gracco et al., 2015). Indeed, the Related index scores are assigned through the observation by expert microscopists or professionals of the samples. Image quantitative analysis is a good support to withdraw quantitative data from histological and electron microscopy images, within the limit of these techniques (Schindelin *et al*., 2012; Vyas *et al*., 2016). Bijelić and colleagues in 2017 used an open source software to segmentate histological images to quantify the growth plate and trabecular bone in a mice model. The FIJI thresholding is considered one of the best segmentation algorithm in the crack image analysis (Mohan and Poobal, 2017), and therefore very suitable for the analysis of damages on enamel surface.

In our study this type of analysis, which represents the originality of the present work, allowed us to quantitatively confirm the qualitative observations from the operators. Indeed, the true treated area was measured and therefore it was possible to calculate the percentage area of adhesive residuals and enamel damages. Overall the results are in agreement with the protocols provided by literature so far: the use of a multiblade burr followed by a finishing system and with the aid of a magnification system significantly reduce the enamel damages and effectively removes the adhesive residuals from the surfaces.

References

Abramoff MD, Magalhaes PJ, Ram SJ. Image processing with ImageJ. Biophotonics Intern 2004;11:36-42.

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.

Baumann DF, Brauchli L, Van Waes H. The influence of dental loupes on the quality of adhesive removal in orthodontic debonding [Einfluss von Lupenbrillen auf die Qualität der Adhäsiv- Restentfernung bei orthodontischem Debonding]. J Orofac Orthop 2011;72:125-32.

Bijelić N, Belovari T, Stolnik D, Lovrić I, Baus Lončar M. Histomorphometric parameters of the growth plate and trabecular bone in wild-type and trefoil factor family 3 (Tff3)-deficient mice analyzed by free and open-source image processing software. Microsc Microanal 2017;23:818-25.

Brauchli LM, Baumgartner EM, Ball J, Wichelhaus A. Roughness of enamel surfaces after different bonding and debonding procedures: an in vitro study. J Orofac Orthop 2011;72:61-7.

Buonocore MG. A Simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res 1955;34:849-53.

Campbell PM. Enamel surfaces after orthodontic bracket debonding. Angle Orthod 1995;65:103-10.

D'Attilio M, Traini T, Di Iorio D, Varvara G, Festa F, Tecco S. Shear bond strength, bond failure, and scanning electron microscopy analysis of a new flowable composite for orthodontic use. Angle Orthod 2005;75:410-5.

De Munck JD, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res 2005;84:118-32.

Eliceiri KW, Berthold MR, Goldberg IG, Ibáñez L, Manjunath BS, Martone ME, et al. Biological imaging software tools. Nat Methods 2012;9:697-710.

Gracco A, Lattuca M, Marchionni S, Siciliani G, Alessandri Bonetti G. SEM-Evaluation of enamel surfaces after orthodontic debonding: A 6 and 12-month follow-up in vivo study. Scanning 2015;37:322-26.

Mohan A, Poobal S. Crack detection using image processing: A critical review and analysis. Alexandria Eng J 2017 (in press).

Øgaard B, Fjeld M. The enamel surface and bonding in orthodontics. Semin Orthod 2010;16:37–48. doi: 10.1053/j.sodo.2009.12.003

Rodríguez-Chávez JA, Arenas-Alatorre J, Belio-Reyes IA. Comparative study of dental enamel loss after debonding braces by analytical scanning electron microscopy (SEM). Microsc Res Tech 2017;80:680-6.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T et al. Fiji: an open-source platform for biological-image analysis. Nat Methods 2012;9:676-82.

Sigilião LCF, Marquezan M, Elias CN, Ruellas AC, Sant'Anna EF. Efficiency of different protocols for enamel clean-up after bracket debonding: an in vitro study. Dental Press J Orthod 2015;20:78-85.

Ulusoy Ç. Comparison of finishing and polishing systems. J Appl Oral Sci 2009;17:209-15.

Vyas N, Sammons RL, Addison O, Dehghani H, Walmsley AD. A quantitative method to measure biofilm removal

efficiency from complex biomaterial surfaces using SEM and image analysis. Sci Rep 2016;6:32694.
Zachrisson BU, Årthun J. Enamel surface appearance after various debonding techniques. Am J Orthod 1979;75:121-37.
Zarrinnia K, Eid NM, Kehoe MJ. The effect of different debonding techniques on the enamel surface: An in vitro qualitative study. Am J Orthod Dentofac Orthop 1995;108:284-93.