NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS
SCHOOL OF HEALTH SCIENCES
DEPARTMENT OF DENTISTRY
POST-GRADUATE PROGRAM “ORTHODONTICS”
SPECIALIZATION IN ORTHODONTICS

A comparison of two different in-vivo stripping methods on enamel surface

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ATHENS 2022

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Κλινική μελέτη αξιολόγησης της επιφανειακής αδαμαντίνης μετά από αφαίρεση αδαμαντίνης όμορων επιφανειών με δύο διαφορετικές μεθόδους.

Νίκη Κατσίγιαλου

ΑΘΗΝΑ 2022

Επιβλέπων Καθηγητής για την εκπόνηση της Μεταπτυχιακής Διπλωματικής Εργασίας κ. Ιωσήφ Σηφακάκης

Τριμελής Επιτροπή για την Αξιολόγηση της Μεταπτυχιακής Διπλωματικής Εργασίας:

1. Επίκ. Καθηγητής Σηφακάκης Ιωσήφ
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3. Καθηγητής Ζηνέλης Σπύρος

Ευχαριστίες

Θα ήθελα να ευχαριστήσω από καρδιάς τον Επίκουρο Καθηγητή κ. Ιωσήφ Σηφακάκη για τη βοήθεια του στη διαμόρφωση του θέματος της διπλωματικής μου εργασίας και για την καθοδήγηση του σε κάθε επιμέρους τμήμα του ερευνητικού αυτού έργου. Χωρίς τη βοήθεια του δε θα ήταν δυνατή η συγγραφή της.

Τον ευχαριστώ θερμά επίσης για την επιστημονική του καθοδήγηση καθ' όλη τη διάρκεια του μεταπτυχιακού μου προγράμματος. Οι συμβουλές και τα σχόλια του ήταν καθοριστικά για την ανάπτυξη ορθής κλινικής ορθοδοντικής σκέψης. Τον ευχαριστώ ιδιαιτέρως όμως για την εμπιστοσύνη αλλά κυρίως τη στήριξη του όλα αυτά τα χρόνια η οποία ήταν καθοριστική για την επιτυχή ολοκλήρωση του προγράμματος αυτού.

Ειλικρινείς ευχαριστίες θα ήθελα να εκφράσω και στον Καθηγητή και Διευθυντή του Εργαστηρίου Ορθοδοντικής κ. Χαλαζωνίτη Δημήτριο, τον Αναπληρωτή Καθηγητή κ. Τσολάκη Απόστολο καθώς και στα υπόλοιπα μέλη ΔΕΠ για την καθοδήγηση τους και τις γνώσεις που μου μεταλαμπάδευσαν καθ' όλη τη διάρκεια της φοίτησης μου στο μεταπτυχιακό αυτό πρόγραμμα.

Σε αυτό το κομμάτι δε θα μπορούσα να παραλείψω και να μην ευχαριστήσω επίσης τους επιστημονικούς συνεργάτες του προγράμματος. Θα ήθελα να αναφερθώ ονομαστικά και να ευχαριστήσω θερμά κάθε έναν από αυτούς γιατί η συμπαράσταση τους ήταν αναγκαία και καθοριστική σε κάθε βήμα μου στο μεταπτυχιακό. Ευχαριστώ θερμά λοιπόν, τον κ. Δαμανάκη Γεώργιο, κ. Κατσαβριά Γεώργιο, κ. Σανούδο Ματθαίο, την κ. Νασίκα Μαρία, τον κ. Κωνσταντώνη Δημήτριο, κ. Αγγελόπουλο Γεράσιμο καθώς και τον κ. Γιαγτζή Σπύρο.

Τελειώνοντας θα ήθελα να αναφέρω και να ευχαριστήσω ξεχωριστά δύο άτομα που ενώ δεν ανήκουν στο διδακτικό και επιστημονικό προσωπικό του τμήματος, οι υπηρεσίες τους είναι απολύτως απαραίτητες για την ομαλή λειτουργία του. Ευχαριστώ από καρδιάς τη γραμματέα μας κ. Παπαδοπούλου Μαρία και την βοηθό μας στην κλινική κ. Τσουτσάνη Σταυρούλα για την προθυμία τους και τη βοήθεια τους σε όλη τη διάρκεια του μεταπτυχιακού μου.

Περιεχόμενα/Contents

Περίληψη

Εισαγωγή

Η αφαίρεση οδοντικής ουσίας από τις όμορες επιφάνειες των δοντιών χρησιμοποιείται συχνά στην Ορθοδοντική. Συνήθως επιλέγεται όταν απαιτείται ελάχιστος επιπλέον χώρος για τη διόρθωση του συνωστισμού ή αλλαγή της μορφολογίας των όμορων επιφανειών κάποιων δοντιών. Σκοπός της παρούσας ex-vivo κλινικής μελέτης ήταν να αξιολογήσει δύο διαφορετικές μεθόδους αφαίρεσης οδοντικής ουσίας όμορων επιφανειών όσον αφορά στις πιθανές αλλαγές στην αδρότητα και τη στοιχειακή δομή της αδαμαντίνης ύστερα από ένα ικανό διάστημα παραμονής τους στη στοματικό περιβάλλον.

Μέθοδοι

39 Υγιείς προγόμφιοι υποβλήθηκαν σε αφαίρεση οδοντικής ουσίας από τις όμορες επιφάνειες τους τουλάχιστον 6 μήνες πριν εξαχθούν για ορθοδοντικούς λόγους. Τα συστήματα που χρησιμοποιήθηκαν για την αφαίρεση της οδοντικής ουσίας ήταν τα εξής: α) το σύστημα Ortho-Strips με χρήση ειδικής χειρολαβής και β) το χειροκίνητο σύστημα IntensivProxoStrip. Κάθε ένα περιλάμβανε 4 διαφορετικές ταινίες για αφαίρεση οδοντικής ουσίας, φινίρισμα και λείανση. Στις άπω επιφάνειες των δοντιών δεν έγινε αφαίρεση οδοντικής ουσίας οπότε χρησιμοποιήθηκαν ως επιφάνειες ελέγχου. Οι θεραπευμένες και μη θεραπευμένες επιφάνειες αξιολογήθηκαν με οπτικό προφιλόμετροκαι Raman και SEM/EDXαναλύσεις. Τα δεδομένα αναλύθηκαν με περιγραφική στατιστική και γενικευμένα γραμμικά μοντέλα στο άλφα=5%.

Αποτελέσματα

Και οι δύο μέθοδοι μείωσαν σημαντικά την κυματοειδή επιφάνεια της άθικτης αδαμαντίνης ($P<0.001$), με το χειροκίνητο σύστημα να προκαλεί μικρότερη μείωση απ' ότι το μηχανοκίνητο ($P\leq0.001$). Από την άλλη πλευρά, η αδρότητα της αδαμαντίνης αυξήθηκε σημαντικά ($P<0.001$)και με τις δυο μεθόδους, χωρίς όμως να παρατηρείται διαφορά στην αύξηση της ανάμεσα στα δύο συστήματα. Οι αναλύσεις EDXκαι Ramanδεν υπέδειξαν καμία αλλαγή στη στοιχειακή δομή της αδαμαντίνης ύστερα από παραμονή των δοντιών στο στοματικό περιβάλλον για τουλάχιστον 6 μήνες.

Συμπεράσματα

Ύστερα από την αφαίρεση οδοντικής ουσίας από τις όμορες επιφάνειες των προγομφίων και με τα δύο συστήματα που χρησιμοποιήθηκαν στην παρούσα μελέτη, η επιφάνεια της αδαμαντίνης παρατηρήθηκε περισσότερο επιπεδωμένη και με μεγαλύτερη αδρότητα. Στη στοιχειακή της δομή πριν και μετά την αφαίρεση της οδοντικής ουσίας και αφού τα δόντια παρέμειναν για αρκετό καιρό στο στοματικό περιβάλλον, δεν παρατηρήθηκε κάποια αλλαγή.

Abstract

Introduction

Inter-Proximal enamel Reduction (IPR) is routinely used in orthodontics in order to generate small to moderate amounts of space within the dental arch. Aim of this ex vivo study was to evaluate the effect of two different in-vivo IPR systems on the enamel surface's waviness, roughness, and elemental composition after an extended period of intraoral exposure.

Materials &Methods

39 healthy premolars from 15 orthodontic extraction cases, at least 6 months before their extraction, were subjected in-vivo to IPR on their mesial side with two different methods: (a) instrumented method with the Ortho-Strips system (on handpiece) and (b) manually with the IntensivProxoStrip (strips)—each with 4 different grits for contouring, finishing and polishing. The distal side of each premolar served as its own internal control. Treated and untreated tooth surfaces were evaluated by optical profilometry, Raman and SEM/EDX analyses. Data was analyzed with descriptive statistics and generalized linear models at alpha=5%.

Results

Both IPR methods significantly reduced waviness of the enamel surface ($P<0.001$), with manual IPR leading to smaller waviness reductions than the instrumented IPR ($P\leq0.001$). On the other side, both IPR methods led to a significant increase in enamel surface roughness ($P<0.001$), with no significant differences between IPR methods. EDX and Raman analyses did not demonstrate any alterations on elemental composition of enamel after at least 6 months of intraoral exposure.

Conclusions

Both stripping systems led to a flatter but rougher enamel surface. Similar elemental composition was demonstrated between intact and stripped enamel surface of both experimental groups after an extended period of intraoral stay.

Introduction

Interproximal enamel reduction (IPR) or interproximal stripping or grinding was first proposed by Ballard in the 1940s as an option to manage tooth mass discrepancy. However, it is nowadays routinely used in orthodontics as a means to reduce or re-contour the interproximal enamel surfaces of permanent teeth (1). IPR might be indicated in cases with mild or moderate crowding as well as towards the elimination of an existing Bolton Index discrepancy (1-4). Further indications pertain to dental esthetics and include changes in tooth shape, normalization of gingival contours, and elimination of black gingival triangles (5). Furthermore, some researchers indicate that post-treatment retention and stability might also be enhanced by IPR of the mandibular anterior segment (6-8).

Reports in the literature suggest that up to half of the enamel thickness might be safely removed without considerable side-effects (9). This would translate to about 0.3 mm for the upper incisors, 0.2 mm for the lower incisors, or 0.6 mm for the posterior teeth (10), whereas other reports indicate that the maximum that can be safely gained by IPR for posterior and anterior teeth might be 7.0 mm and 2.5 mm, respectively (9,11).

Even though IPR in controlled amount can be regarded safe as far as serious adverse effects are concerned, grinding the enamel surface makes it rougher (12-14). Scratches and furrows remain on the enamel surface after IPR, sometimes even after the tooth surface has been carefully polished (15). Potential adverse effects on the enamel grinding during striping have been a controversial issue, with some reports of increased plaque accumulation and a consequent elevated caries risk of caries at sites of IPR (16). Nevertheless, in the literature no conclusive association has been found between the degree of enamel roughness and caries development (17,18). Furthermore, concerns have been raised over the qualitative alterations of the enamel surface after IPR. Energy-Dispersive X-ray Spectroscopy (EDS) is an analytical technique used in in vitro research protocols and can prove useful in analyzing the elemental composition or chemical characterization of such tooth surfaces (19,20).

The degree of enamel roughness after IPR, however, also depends on the applied IPR technique / protocol. A number of special instruments and devices, be it automatic / mechanical or translatory / rotating, have been introduced over the recent decades for IPR (4,14,21). A recent systematic review could not draw robust conclusions regarding the enamel roughness after various striping methods – mostly to the limited material available (18). Therefore, the aim of this ex vivo study was to evaluate two different IPR systems regarding possible alterations of the enamel surface in terms of waviness, roughness, and elemental composition after an extended period of intraoral stay. The null hypothesis was that for both striping methods there would be no difference in waviness, roughness, and elemental composition of the stripped or non-stripped enamel surfaces after an extended period of intraoral stay.

Materials &Methods

The study was conducted according to the guidelines of the declaration of Helsinki and approved by the Ethics Committee of the School of Dentistry, National and Kapodistrian University of Athens, Greece (382/9.11.2018). Informed consent was obtained from all participants or their legal guardians. IPR was performed in vivo on 39 healthy premolars intended for extraction for orthodontic reasons according to the treatment plan. The teeth evaluated in the present study were collected from 15 orthodontic extraction cases. Inclusion criteria included absence of tooth cracks / hypoplasia / caries / fillings, or exposure to chemical agents (i.e., bleaching) at the first premolars. Exclusion criteria included (i) teeth with white spots or fluorosis, (ii) patients with any systemic disease or use of any drugs that could affect salivary composition / flow, and (iii) patients who had previously undergone orthodontic treatment. Prior to initiation of this study, all patients received radiographic caries assessment in the first premolars by posterior bitewing radiographs using the long-cone technique.

An a priori sample size calculation was performed with a 1:1 allocation in two different IPR groups. Based on a previous study on handpiece-based IPR followed by polishing (14), a mean post-treatment roughness of 0.4 (Standard Deviation [SD] of 0.1) was taken as basis, and a 25% deterioration in roughness was hypothesized for the manual IPR with strips (assuming a common SD). It was found that a sample of 17 tooth surfaces (since the other half of the tooth would be used as control) would be needed to identify a potentially existing difference with an independent-samples t-test with alpha=0.05 and beta=0.20 (power of 80%). This was rounded up to 20 tooth surfaces per group to account for potential losses due to artefacts.

All patients were given proper oral hygiene instructions and monitored for 2 weeks prior to administering IPR. They were instructed by the orthodontist to brush their teeth for 3 minutes using a fluoride-containing toothpaste (1450 ppm) provided for daily use throughout the study. They were told not to use any other oral agents, including oral irrigators or antimicrobial mouth rinses.

Intraoral IPR was performed on the mesial surface of all premolars. The distal surface of each tooth acted as its own internal control to account for tooth-specific irregularities. Before the stripping procedure, precautions to minimize risk of damage to adjacent teeth were taken. In all subjects, elastic separators were placed on the mesial and distal contacts of the first premolars for 3 days, while a metal separator was used during stripping / polishing to improve access to the interproximal surfaces.

Two different IPR methods were evaluated in this study, both from the same company (Intensiv Dental SA, Montagnola, Switzerland): a) Ortho-Strips system, attached on an Intensiv Swingle Ortho contra-angle handpiece. These strips were one-sided flexible diamond-coated strips of four different grit sizes (60 μm and 40 μm for contouring, 25 μm for finishing, and 15 μm for polishing). b) IntensivProxoStrip, used by hand. These are unique, diamond-coated metal strips with grips of 60 μm, 40 μm, 15 μm and 8 μm. IPR was performed according to the manufacturer's instructions under water cooling by one operator. New devices / strips were used for each stripping session. The stripping auxiliaries were applied at consecutive sessions of 10 seconds each. In each patient, the contralateral premolars were treated with different methods. The two groups included 19 and 20 teeth respectively, since one tooth was retained due to changes in the treatment plan.

Premolars were extracted with minimal invasive surgery after exposure to oral conditions for at least 6 months. The extracted teeth were cleaned under tap water and stored in distilled water with 0.5% sodium azide in a refrigerator at 4°C.

The treated (mesial) and untreated (distal) region of each tooth were investigated by a three-dimensional (3D) optical profilometer (Wyko NT1110, Veeco, Tucson, AZ, USA) operated under the following conditions: vertical scanning mode, Mirau lens (20×2 FOW), 30 μm vertical scan length, 231 μm × 304 μm acquisition window (20.3× magnification) and 0.1 nm (z-axis), and 0.2 μm (x- and y-axes) resolution. In order to discriminate the roughness from waviness component a Gaussian regression filter was applied two times. In the case of waviness, a long wavelength cutoff (L=0.08) was used while in the case of roughness a short wavelength filter was employed (L=0.025). Figure 1 presents the two-dimensional (2D), 3D, line profile and selected roughness parameters of raw data (first row), waviness (second row) and roughness (third row). For both waviness and roughness, the Vision 64 version 5.7 software (Bruker Corporation, Tucson, AZ) was used to record three amplitude parameters: (i) Sa (average roughness): The arithmetic average of the absolute values of the measured height deviations from the mean surface taken within the evaluation area; (ii) Sq (root mean square-RMS roughness): The root mean square average of the measured height deviations from the mean surface taken within the evaluation area; (iii) Sz (height S-Parameter): The ten-point height over the complete 3D surface.

One Raman spectrum was acquired from the distal and medial regions of three teeth from each group. The teeth were placed on the stage of a microscope (LEICA BME, Leica Microsystems Ltd, Heerbrugg, Switzerland) and the regions of interest were determined employing optical lens at x40 nominal magnification. A special device (MicroViewer-785, Raman Microscope Adaptor) was used to attach the Raman probe to the microscope. Spectra were acquired with an EZ Raman-I, high-sensitivity portable Raman analyzer equipped with a Laser (Soliton, Laser Und Messtechnik, Gliching, Germany) operating at 480 mW output power, 785 nm emitted wavelength and a nominal resolution of 4.5–6.5 cm^{-1}. Baseline correction and smoothening (Fast Fourier Transform filtering) were applied to all spectra. All spectra were peak fitted with Peak-Fit software (v4.12, SeaSolve Software Inc., Framingham, MA, USA) employing Pearson IV amplitude curve fitting (standard width per spectrum).

The surface morphology and elemental composition of all specimens were studied with a Scanning Electron Microscope (SEM) (Quanta 200, FEI, Hillsboro, OR) and by x-ray energy-dispersive microanalysis (EDX) employing a spectrometer (Quantax, Bruker, Berlin, Germany) attached to the SEM equipped with a slew-window silicon drift detector (X Flash 6|10, Bruker) under high vacuum, 15 kV accelerating voltage, 108 μA beam current, area scan mode (250 μm × 220 μm), 200 s acquisition time and 1~2% dead time.

Statistical analysis

Initially, normal distribution for all variables was checked through visual inspection and formally with the Shapiro-Wilk test. Descriptive statistics were calculated, including means and SDs for all experimental and control groups. Differences between experimental-control side within each group were assessed with Generalized Linear Models accounting for within-tooth clustering with robust standard errors. Differences between the two IPR methods were likewise tested with GLMs, after again adjusting for the control-side values of each tooth. All analyses were run in Stata 14.0 (Stata Corp, College Station, TX, USA) with a two-sided alpha of 5% and an openly provided dataset (doi: 10.5281/zenodo.6577836).

Results

In total, 39 premolars (19 in the instrumented and 20 in the manual group) were extracted post-IPR after a minimum 6 months of intraoral use (mean 7.46 months; SD 0.92 months).

Optical profilometry

Representative 3D-profilometric images of the treated and control surfaces are illustrated in Figure 2. The untreated surfaces exhibited high peaks slopes and shallow valleys, whereas the treated specimens demonstrated peaks followed by deep valleys a typical pattern of surface grinding. The results of the 3D-roughness parameters are summarized in Tables 1 and 2. As far as waviness is concerned, both methods statistically significantly reduced waviness compared to the un-stripped (control) sides of each tooth ($P<0.001$ in all instances; Table 1). Also, for all three waviness amplitudes, manual IPR reduced affected significantly less the enamel surface (-55.5 to -58.6%) compared to tooth sides treated with instrumented IPR with a handpiece (-76.2 to -77.0%; $P<0.001$ or $P=0.001$). As far as roughness is concerned (Table 2), again both IPR methods significantly increased enamel roughness (28.3 to 79.1%; $P<0.05$ in all instances), but with no significant differences between instrumented (handpiece) and manual IPR method ($P>0.05$ in all instances).

Raman spectroscopy

Figure 3 and 4 show representative spectra from control and treated tooth surface from both groups tested. Both groups showed identical spectra between the regions tested. The characteristic peaks at 777, 896 and 1077 (cm^{-1}) stand for $CO^{2-}{}_3$ vibrations, 1054 for $PO+CO^{2-}{}_3$ and the main peak at 960 for $PO^{3-}{}_4$.

SEM/EDX analysis

Figures 5 and 6 depict representative backscattered electron images from the groups tested. Both images show surface scratches i.e. parallel lines and shallow grooves. The X-ray EDS spectrum of a surface treated with Ortho-Strips system is depicted on Figure 7 and Table 3. The results indicated that high variability existed between different specimens, but the majority of tooth enamel consisted of Ca (>60%), followed by P (>30%), and with smaller traces of Na or Cl. Stripping with either method didn’t seem to have a considerable influence on enamel composition.

Discussion

The results of the present study indicate that stripping affects the enamel surface waviness and roughness, but does not alter significantly the molecular structure according to Raman and EDX analysis. Thus, the null hypothesis was only partially rejected.

Enamel proximal reduction is inevitably a common procedure in orthodontics especially for generating small to moderate amount of space in cases of arch length discrepancy. Surface roughness after stripping has been the subject of numerous experimental and theoretical investigations since surface irregularities may theoretically promote plaque adherence and induce iatrogenic damage, such as increased temperature sensitivity of the recontoured teeth, dental caries, periodontal tissue breakdown, gingival recession, and excessive root proximity (22-25). Some studies have shown that IPR may produce residual furrows and grooves and increase the susceptibility of proximal enamel to demineralization (16,26,27).

On the other hand, several studies have demonstrated that IPR followed by meticulous polishing leads to tooth surfaces that are equally smooth or even smoother than intact enamel (13,14, 28). The roughness parameters of the 3D optical profilometer evaluated in the present study are commonly used in several stripping protocols. The results of the current study indicated that significant differences were observed between the treated and untreated enamel surfaces, with the former being flatter, but also rougher. This is in agreement with results of an in-vitro stripping protocol, which showed increased roughness after IPR with the Ortho-Strips system at almost all the evaluated roughness parameters (15). However, the results of the present study are not necessarily generalizable to all methods of instrumented IPR, since several studies point out that Ortho-Strips leads to smoother enamel in comparison to several other stripping systems (12,14,29). This is somehow expected since this system uses oscillating files following a coarse-to-fine sequence in selecting grits and ensures both polishing and finishing of the enamel surface. This is also true for the second (manual) IPR system evaluated in the present investigation.

A major advantage of the present study is that stripping was performed in-vivo on teeth to be extracted for orthodontic reasons. These teeth exhibited physiologic mobility and the pressure was partially absorbed by displacement of the periodontal ligament. On the other hand, stripping protocols in previous research protocols applied on extracted teeth that have been embedded in substrates might present different stiffness. As a result, the amount of enamel tissue ground may differ between studies according to their design. Stripping duration may also affect enamel roughness and is often partially or not at all reported in published studies. In the present study new devices were used for each stripping session, since a recent study demonstrated a significant reduction in the surface roughness of the strips after in-vitro stripping in all IPR systems that were tested (30).

Stripping raises concerns not only for the morphological characteristics of the new enamel surface but also for the qualitative features of this new enamel layer, in the short and long term. This is another advantage of the present study since the stripping surfaces were evaluated after an extended period of intraoral stay by Raman spectroscopy and EDX analysis. The former is a spectroscopic technique typically used to determine vibrational modes of molecules and it can provide a structural fingerprint by which molecules can be identified on the surface being examined. In the present study, no qualitative differences were observed in the elemental structure between the treated and untreated enamel surface and both groups showed identical spectra between the regions tested. So far, there are no reports evaluating differences in the elemental structure of the enamel after striping by Raman spectroscopy and this technique has only previously been employed to assess alterations of the enamel surface before and after bonding of orthodontic attachments(31).

Analyses of the enamel's elemental composition through the EDX analysis did not demonstrate any considerable alterations due to stripping and similar results were found for both treated and untreated surfaces (Table 3). The remineralizing effect of saliva might have contributed to the improvement of the surface state through the precipitation of ions (19,20), since all teeth remained exposed in the intraoral environment for at least 6 months. This is in agreement with the results of a recent study evaluating stripped enamel with EDX, after 30 days of exposure to either saliva or casein phosphopeptide amorphous calcium phosphate with sodium fluoride, and showing no significant differences in the content of calcium or phosphate (19). Another study evaluated a limited number of teeth after in-vivo stripping with Ortho Strips with dispersive energy spectroscopy and reported a small decrease in the percentage of Ca and P in teeth extracted right after stripping (32). However, the levels of these elements were elevated among stripped teeth extracted after 4-month period of oral exposure (32). A further study evaluated the microhardness of stripped enamel surfaces—since this property is directly related to the enamel's mineral content. None of the 5 different stripping systems tested had a considerable influence on the enamel microhardness of permanent teeth (33). Finally, another experimental protocol used optical emission spectrometry to evaluate in-vitro IPR on teeth extracted for periodontal reasons and found that increased demineralization was reported after a 15 days pH-cycling model (34).

In the present study, SEM images were further taken to provide a visualization of the surfaces under examination. SEM analysis is a qualitative evaluation of the enamel surface in contrast to the quantitative results of the profilometric analysis. The main drawbacks of this method are the assessor's subjectivity and the fact that not all grooves and every rough point can be measured (13,21). Among the specimens of the present study, the waves of perikymata disappeared after stripping and parallel lines or shallow grooves were found on the surface of treated enamel. A recent SEM study showed that striping with the instrumented Ortho-Strips system left more regular and less rough enamel surface conditions in comparison with classic manually-used diamond abrasive strips (12).

Even though the present study provides considerable evidence on enamel properties after a realistic in-vivo IPR protocol, it also has some limitations. Stripping in the clinical setting is always conducted by a clinician and cannot be completely standardized. Hand stripping with metal strips is inevitably conducted in a less standardized manner in comparison with instrumented (handpiece) systems, and variability in the applied grinding pressure and the position or the direction of the strip can be expected. In the present study, all clinical procedures were performed by one experienced operator and the duration of stripping was kept constant for both groups. However, the different methodologies used in the literature may render direct comparisons to other studies challenging. Moreover, the real surface geometry is so complicated that a finite number of parameters cannot provide a full description and this is one of the reasons for introducing new parameters for surface evaluation. This is also the reason why in this study, several widely-accepted methods were used to provide an overview of the enamel surface condition.

Conclusions

Stripping with either an instrumented handpiece-based or a manual strip-based system led to a flatter but rougher enamel surface, with parallel lines and shallow grooves found on the surface. However, similar elemental composition was demonstrated between intact and stripped enamel surface of both experimental groups after an extended period of exposure in the intraoral cavity.

Conflict of interest

None to declare.

Acknowledgments
We thank the company Intensiv (Montagnola, Switzerland) and Memodent (Athens, Greece) for providing the stripping systems; both companies were not otherwise involved in the experimental process, data acquisition, or interpretation.

Funding
No funding was received for this work.

Data availability

The data underlying this article are available in Zenodo at http://doi.org/10.5281/zenodo.6577836.

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Tables

Table 1. Mean values and standard deviations in parentheses along with p values for waviness and roughness components of teeth treated with the stripping device on handpiece (n=19).

	Waviness component			Roughness component		
	Control	Treated	P value	Control	Treated	P value
Sa	1.89(0.66)	0.39(0.14)	P<0.001	0.22(0.04)	0.28(0.03)	P<0.001
Sq	2.38(0.82)	0.49(0.18)	P<0.001	0.30(0.06)	0.37(0.05)	P<0.001
Sz	13.24(4.31)	2.68(1.03)	P<0.001	8.85(3.19)	11.97(5.85)	P = 0.026

Table 2. Mean values and standard deviations in parentheses or median and 25/75% percentiles in brackets along with p values for waviness and roughness components of teeth treated with hand stripping manually (n=20).

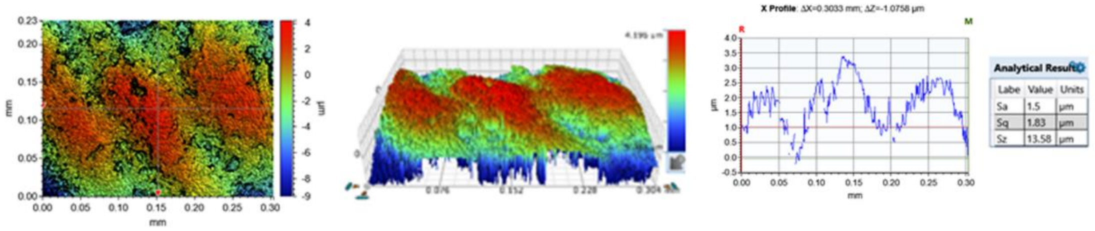
	Waviness component			Roughness component		
	Control	Treated	P value	Control	Treated	P value
Sa	2.05(1.01)	0.69(0.22)	P<0.001	0.23(0.06)	0.28(0.05)	P=0.003
Sq	2.75(1.22)	0.84(0.29)	P<0.001	0.27[0.24-0.35]	0.37[0.32-0.42]	P=0.001
Sz	13.13(5.63)	4.39(2.00)	P<0.001	8.10(4.09)	11.31(3.84)	P=0.037

Table 3. Quantitative results (mean, SD) in atomic percentage after EDS analysis for the control and the treated surfaces.

	Na	**P**	**Cl**	**Ca**	**Ca/P**
ProxoStrip (manually)	1,25 (0,86)	31,98 (3,65)	0,99 (0,43)	65,76 (4,79)	2,08 (0,39)
Control	0,91 (0,18)	32,75 (0,62)	1,16 (0,18)	65,17 (0,65)	1,99 (0,05)
Ortho-Strips (on handpiece)	1,60 (0,43)	33,90 (0,71)	1,15 (0,15)	63,33 (0,99)	1,86 (0,06)
Control	2,34 (0,51)	35,32 (0,63)	1,01 (0,24)	60,98 (1,13)	1,72 (0,06)

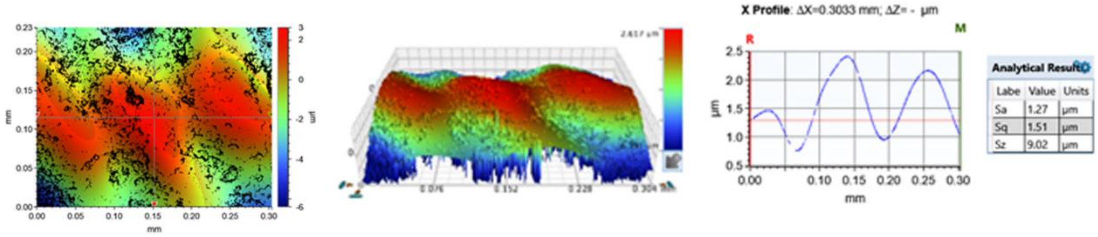
Figures

Raw data



Labe	Value	Units
Sa	1.5	µm
Sq	1.83	µm
Sz	13.58	µm

Waviness (Gaussian regression filter (Long wavelength, cut off L=0.08mm))



Labe	Value	Units
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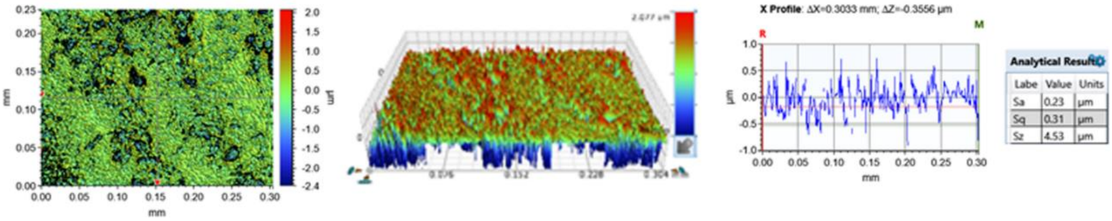
Sq	1.51	µm
Sz	9.02	µm

Roughness (Gaussian regression filter (Short wavelength, cut off L=0.025mm))



Labe	Value	Units
Sa	0.23	µm
Sq	0.31	µm
Sz	4.53	µm

Figure 1. 2D image (first column), 3D image (second column), line scan at a horizontal line (third column) and surface parameters values (fourth column). Vertical bars in second column indicate individual amplitude range per image (20.3× times magnification, 231×304 µm^2 imaged area)

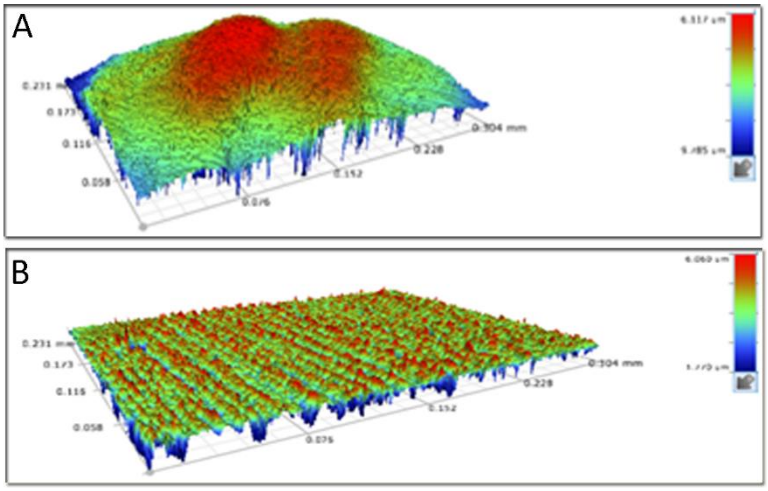


Figure 2. Representative 3D profilometric images from the surfaces of control (A) and treated (B) groups. All images have the same orientation with the long axis of the tooth parallel to the long axis of the images. Vertical bars indicate individual amplitude range per image (20.3× times magnification, 231×304 μm^2 imaged area).

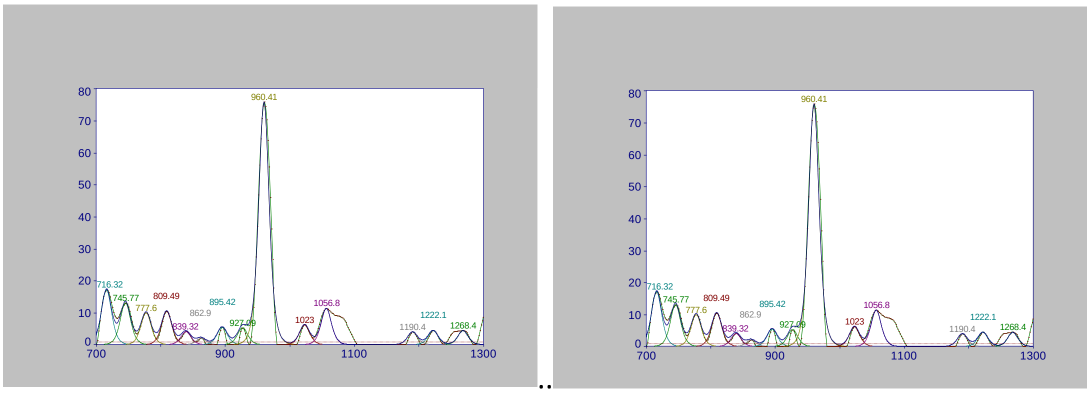


Figure 3. Raman spectra from the control (left) and treated region (right) of the same teeth from the ProxoStrip group (manual stripping).

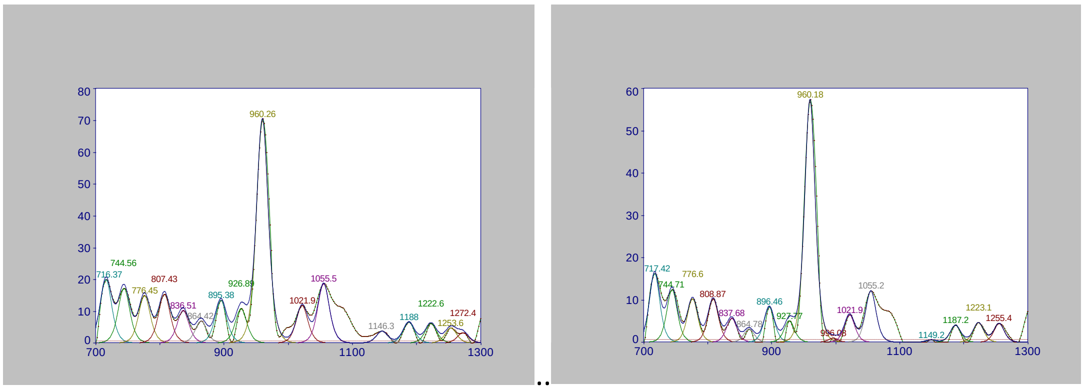


Figure 4. Raman spectra from the control (left) and treated region (right) of the same teeth from the Ortho-Strips group (on handpiece).

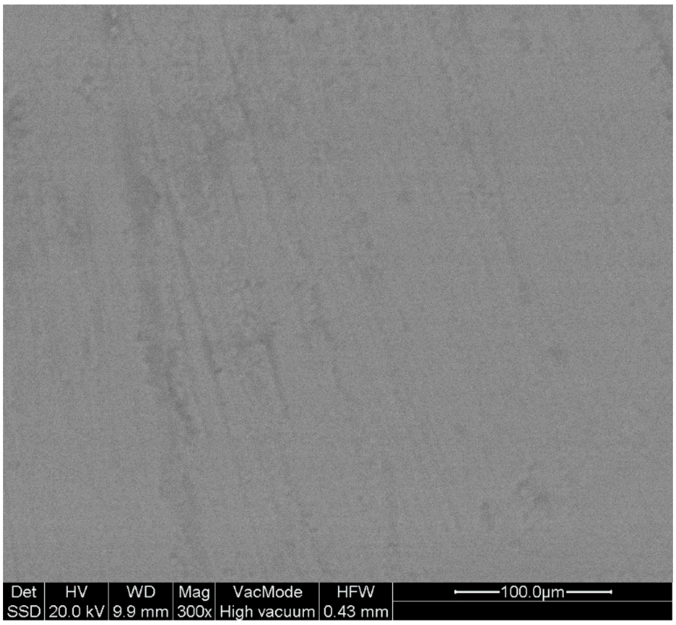


Figure 5. Representative backscattered electron images (BE) from a surface treated with ProxoStrip (manual stripping). Parallel lines and shallow grooves and were found on the surface of treated enamel by SEM analysis. Nominal magnification: 300 ×.

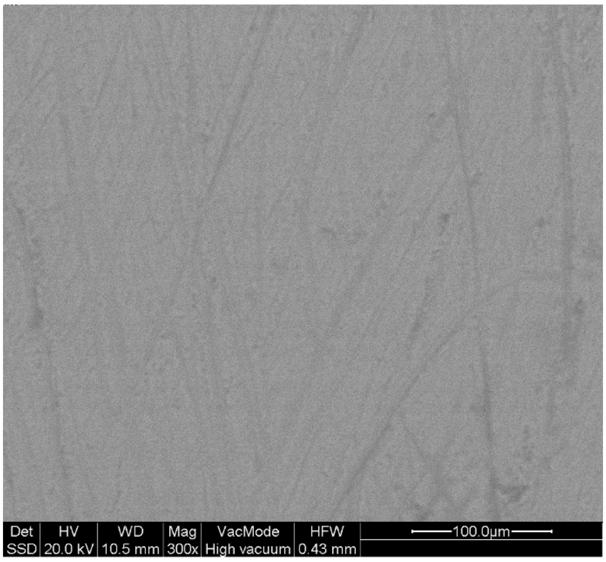


Figure 6. Representative backscattered electron images (BE) from a surface treated with Ortho-Strips system (on handpiece). Nominal magnification: 300 ×.

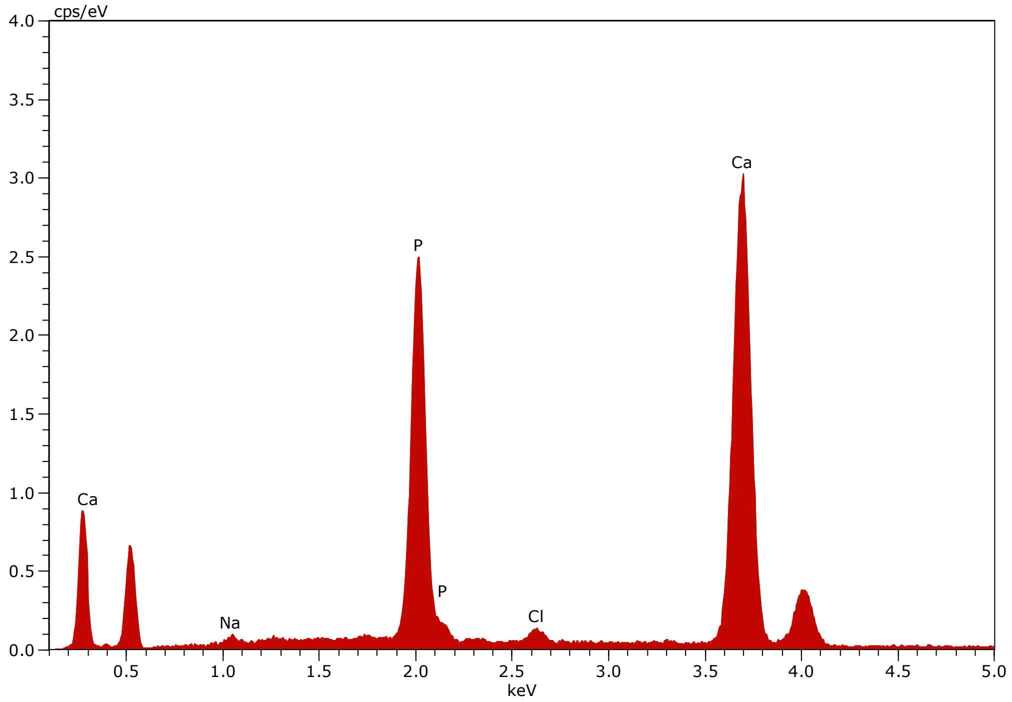
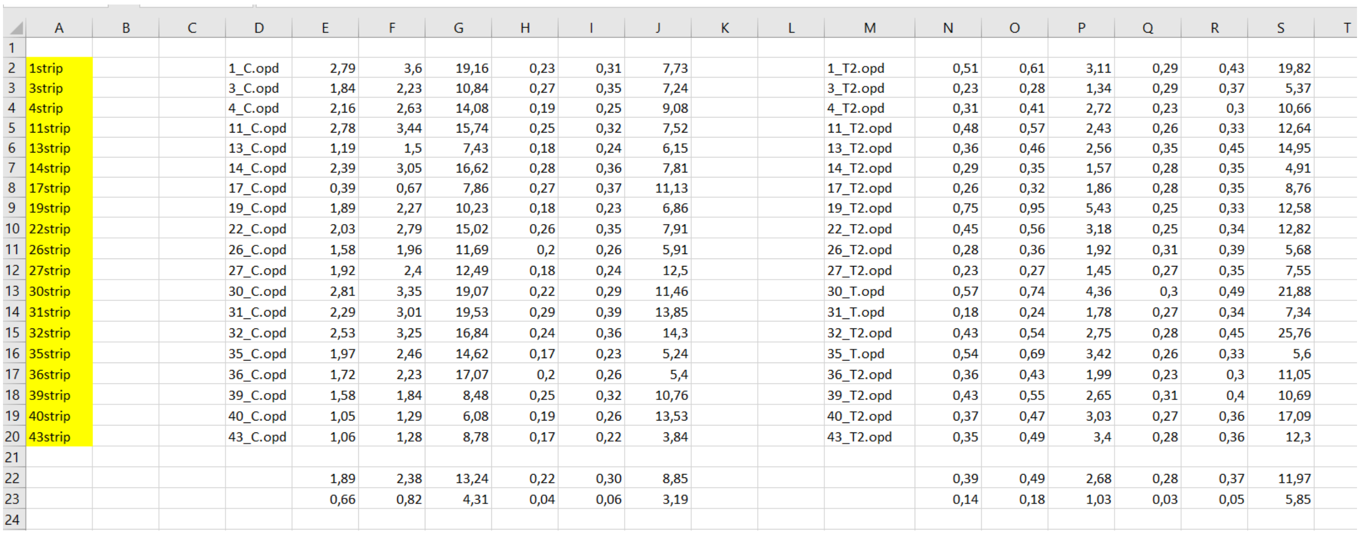
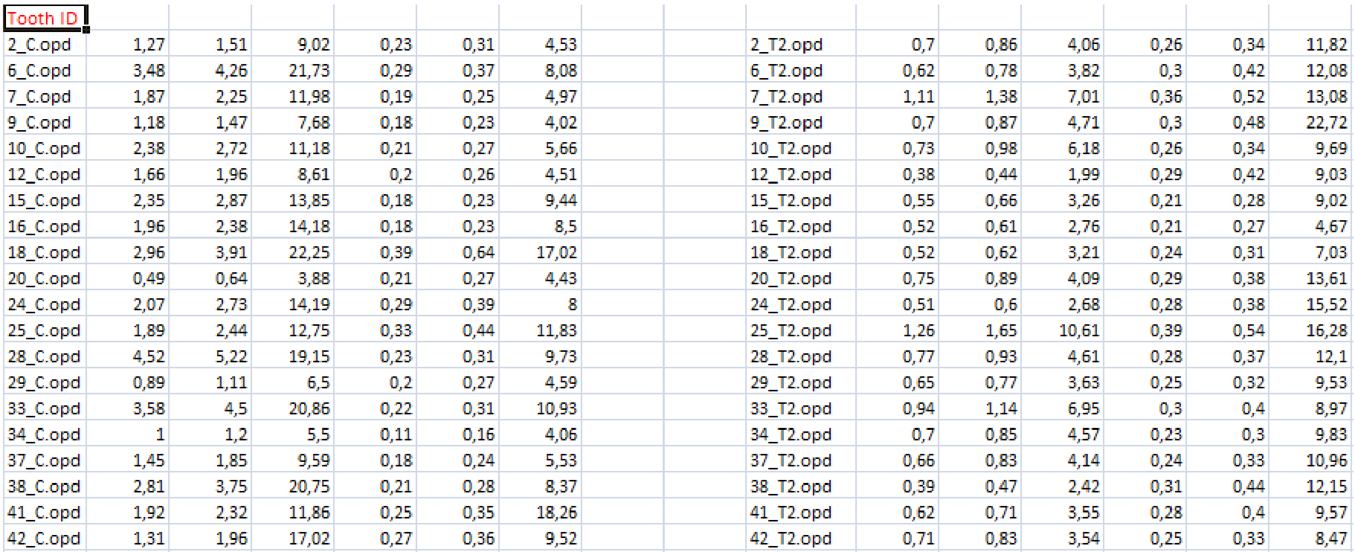


Figure 7. EDX spectrum for element identification of the surfaces treated with Ortho-Strips system.

Appendix



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1																				
2	1strip			1_C.opd	2,79	3,6	19,16	0,23	0,31	7,73			1_T2.opd	0,51	0,61	3,11	0,29	0,43	19,82	
3	3strip			3_C.opd	1,84	2,23	10,84	0,27	0,35	7,24			3_T2.opd	0,23	0,28	1,34	0,29	0,37	5,37	
4	4strip			4_C.opd	2,16	2,63	14,08	0,19	0,25	9,08			4_T2.opd	0,31	0,41	2,72	0,23	0,3	10,66	
5	11strip			11_C.opd	2,78	3,44	15,74	0,25	0,32	7,52			11_T2.opd	0,48	0,57	2,43	0,26	0,33	12,64	
6	13strip			13_C.opd	1,19	1,5	7,43	0,18	0,24	6,15			13_T2.opd	0,36	0,46	2,56	0,35	0,45	14,95	
7	14strip			14_C.opd	2,39	3,05	16,62	0,28	0,36	7,81			14_T2.opd	0,29	0,35	1,57	0,28	0,35	4,91	
8	17strip			17_C.opd	0,39	0,67	7,86	0,27	0,37	11,13			17_T2.opd	0,26	0,32	1,86	0,28	0,35	8,76	
9	19strip			19_C.opd	1,89	2,27	10,23	0,18	0,23	6,86			19_T2.opd	0,75	0,95	5,43	0,25	0,33	12,58	
10	22strip			22_C.opd	2,03	2,79	15,02	0,26	0,35	7,91			22_T2.opd	0,45	0,56	3,18	0,25	0,34	12,82	
11	26strip			26_C.opd	1,58	1,96	11,69	0,2	0,26	5,91			26_T2.opd	0,28	0,36	1,92	0,31	0,39	5,68	
12	27strip			27_C.opd	1,92	2,4	12,49	0,18	0,24	12,5			27_T2.opd	0,23	0,27	1,45	0,27	0,35	7,55	
13	30strip			30_C.opd	2,81	3,35	19,07	0,22	0,29	11,46			30_T.opd	0,57	0,74	4,36	0,3	0,49	21,88	
14	31strip			31_C.opd	2,29	3,01	19,53	0,29	0,39	13,85			31_T.opd	0,18	0,24	1,78	0,27	0,34	7,34	
15	32strip			32_C.opd	2,53	3,25	16,84	0,24	0,36	14,3			32_T2.opd	0,43	0,54	2,75	0,28	0,45	25,76	
16	35strip			35_C.opd	1,97	2,46	14,62	0,17	0,23	5,24			35_T.opd	0,54	0,69	3,42	0,26	0,33	5,6	
17	36strip			36_C.opd	1,72	2,23	17,07	0,2	0,26	5,4			36_T2.opd	0,36	0,43	1,99	0,23	0,3	11,05	
18	39strip			39_C.opd	1,58	1,84	8,48	0,25	0,32	10,76			39_T2.opd	0,43	0,55	2,65	0,31	0,4	10,69	
19	40strip			40_C.opd	1,05	1,29	6,08	0,19	0,26	13,53			40_T2.opd	0,37	0,47	3,03	0,27	0,36	17,09	
20	43strip			43_C.opd	1,06	1,28	8,78	0,17	0,22	3,84			43_T2.opd	0,35	0,49	3,4	0,28	0,36	12,3	
21																				
22					1,89	2,38	13,24	0,22	0,30	8,85				0,39	0,49	2,68	0,28	0,37	11,97	
23					0,66	0,82	4,31	0,04	0,06	3,19				0,14	0,18	1,03	0,03	0,05	5,85	
24																				



Tooth ID															
2_C.opd	1,27	1,51	9,02	0,23	0,31	4,53			2_T2.opd	0,7	0,86	4,06	0,26	0,34	11,82
6_C.opd	3,48	4,26	21,73	0,29	0,37	8,08			6_T2.opd	0,62	0,78	3,82	0,3	0,42	12,08
7_C.opd	1,87	2,25	11,98	0,19	0,25	4,97			7_T2.opd	1,11	1,38	7,01	0,36	0,52	13,08
9_C.opd	1,18	1,47	7,68	0,18	0,23	4,02			9_T2.opd	0,7	0,87	4,71	0,3	0,48	22,72
10_C.opd	2,38	2,72	11,18	0,21	0,27	5,66			10_T2.opd	0,73	0,98	6,18	0,26	0,34	9,69
12_C.opd	1,66	1,96	8,61	0,2	0,26	4,51			12_T2.opd	0,38	0,44	1,99	0,29	0,42	9,03
15_C.opd	2,35	2,87	13,85	0,18	0,23	9,44			15_T2.opd	0,55	0,66	3,26	0,21	0,28	9,02
16_C.opd	1,96	2,38	14,18	0,18	0,23	8,5			16_T2.opd	0,52	0,61	2,76	0,21	0,27	4,67
18_C.opd	2,96	3,91	22,25	0,39	0,64	17,02			18_T2.opd	0,52	0,62	3,21	0,24	0,31	7,03
20_C.opd	0,49	0,64	3,88	0,21	0,27	4,43			20_T2.opd	0,75	0,89	4,09	0,29	0,38	13,61
24_C.opd	2,07	2,73	14,19	0,29	0,39	8			24_T2.opd	0,51	0,6	2,68	0,28	0,38	15,52
25_C.opd	1,89	2,44	12,75	0,33	0,44	11,83			25_T2.opd	1,26	1,65	10,61	0,39	0,54	16,28
28_C.opd	4,52	5,22	19,15	0,23	0,31	9,73			28_T2.opd	0,77	0,93	4,61	0,28	0,37	12,1
29_C.opd	0,89	1,11	6,5	0,2	0,27	4,59			29_T2.opd	0,65	0,77	3,63	0,25	0,32	9,53
33_C.opd	3,58	4,5	20,86	0,22	0,31	10,93			33_T2.opd	0,94	1,14	6,95	0,3	0,4	8,97
34_C.opd	1	1,2	5,5	0,11	0,16	4,06			34_T2.opd	0,7	0,85	4,57	0,23	0,3	9,83
37_C.opd	1,45	1,85	9,59	0,18	0,24	5,53			37_T2.opd	0,66	0,83	4,14	0,24	0,33	10,96
38_C.opd	2,81	3,75	20,75	0,21	0,28	8,37			38_T2.opd	0,39	0,47	2,42	0,31	0,44	12,15
41_C.opd	1,92	2,32	11,86	0,25	0,35	18,26			41_T2.opd	0,62	0,71	3,55	0,28	0,4	9,57
42_C.opd	1,31	1,96	17,02	0,27	0,36	9,52			42_T2.opd	0,71	0,83	3,54	0,25	0,33	8,47

```
Atomic percent (%)

Spectrum                 Na     P   Cl    Ca
-------------------------------------------
11 control             2.85 36.05 1.19 59.90
pilot control          3.35 35.04 0.74 60.87
13 control             1.83 34.89 1.11 62.17
11 handpiece           1.27 33.18 1.26 64.30
pilot handpiece        1.45 33.93 1.22 63.40
13 handpiece 430       2.10 34.61 0.98 62.31
9 control              0.71 32.41 1.05 65.82
10 control 430         0.95 33.48 1.06 64.52
18 control 430         1.07 32.38 1.37 65.18
18 stripping 430       2.25 35.39 1.22 61.14
10 stripping 430       0.67 28.12 0.50 70.72
9 stripping 430micron  0.85 32.44 1.27 65.43
-------------------------------------------
Mean value:            1.61 33.49 1.08 63.81
Sigma:                 0.87  2.10 0.25  2.91
Sigma mean:            0.25  0.61 0.07  0.84


Norm. mass percent (%)

Spectrum                 Na     P   Cl    Ca
-------------------------------------------
11 control             1.81 30.80 1.17 66.22
pilot control          2.12 29.92 0.72 67.24
13 control             1.15 29.58 1.08 68.19
11 handpiece           0.79 27.94 1.21 70.06
pilot handpiece        0.91 28.65 1.18 69.26
13 handpiece 430       1.32 29.35 0.95 68.38
9 control              0.44 27.17 1.01 71.38
10 control 430         0.59 28.16 1.02 70.23
18 control 430         0.66 27.19 1.32 70.83
18 stripping 430       1.42 30.10 1.19 67.29
10 stripping 430       0.41 23.30 0.47 75.82
9 stripping 430micron  0.53 27.22 1.22 71.03
-------------------------------------------
Mean value:            1.01 28.28 1.05 69.66
Sigma:                 0.56  2.00 0.24  2.56
Sigma mean:            0.16  0.58 0.07  0.74
```