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Comprehensive Assessment of Remineralization Therapy Efficacy in Patients with Braces Using Raman Fluorescence Diagnostics

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ABSTRACT

This pilot study demonstrates that Raman fluorescence diagnostics (RFD) is a feasible, objective, and non-invasive tool for assessing dental hard tissue mineralization and oral hygiene status in patients with orthodontic braces. In a single-center, single-arm pilot trial (n = 40), a statistically significant reduction in enamel mineralization was observed during brace wear, with recovery to baseline levels after remineralization therapy. RFD showed high sensitivity (92-95%) and specificity (90-94%) and enabled personalized hygiene monitoring and patient feedback. Given the pilot nature of the study, lack of a comparator group, and single-center design, the results are preliminary and require confirmation in larger controlled trials.

Keywords: *Bio-OS V8; Bio-logistical steering; Tissue Integrity Control Index (TICI); Mucosal restoration.*

Introduction

Enamel demineralization is one of the most common dental conditions in patients undergoing orthodontic treatment with braces. Current data indicate that over 97% of the population exhibits some degree of demineralization, primarily due to poor oral hygiene, increased retention sites, and difficulties in thorough tooth cleaning [1, 2].

Demineralization promotes caries development, impairs dental aesthetics, and reduces the functional durability of dental tissues, negatively affecting patients' quality of life. Traditional diagnostic methods, visual examination and radiography, have limited sensitivity and objectivity in assessing the degree of hard tissue mineralization and oral hygiene levels [4, 7, 8]. Therefore, the introduction of modern non-invasive methods that allow real-time, high-precision evaluation of mineralization status and therapy efficacy is particularly relevant.

Raman fluorescence diagnostics (RFD), based on spectral analysis of laser-tooth tissue interaction, is a promising method. It enables detection of changes in tissue mineral composition (including fluorapatite) without damaging the enamel. Over the past 20 years, RFD has been increasingly adopted in dental practice due to digitalization, high sensitivity, objectivity, rapid examination, and automated data processing capabilities [4, 7].

Recent data (2025-2026) show that combined spectral methods, including RFD, offer the highest accuracy in monitoring early changes in the enamel mineral matrix in orthodontic patients, outperforming laser fluorescence and optical coherence tomography in sensitivity [11, 12]. Moreover, modern remineralization therapy protocols with spectral marker monitoring allow personalization of application duration and frequency, increasing outcome predictability.

Despite growing interest in RFD, clinical studies confirming its efficacy in evaluating remineralization interventions in patients with braces remain scarce and insufficiently systematized. Most publications focus on theoretical foundations or experimental models, which limits the ability to fully assess the method's potential in real clinical settings.

Based on the above, the study's aim and objectives were formulated.

Aim: To develop and test a methodology for applying Raman fluorescence diagnostics to improve oral hygiene and remineralization therapy efficacy in patients with braces.

Objectives:

1. To develop a clinical application algorithm for RFD for rapid assessment of demineralization foci in patients with fixed orthodontic appliances during rehabilitation.
2. To test the RFD method and its clinical application algorithm for evaluating the dynamics of dental hard tissue mineralization at three time points: before brace placement, during treatment, and after treatment completion.
3. To conduct a comprehensive assessment of tooth cleaning and remineralization therapy efficacy in patients with braces using RFD.
4. To evaluate the clinical applicability of the developed comprehensive methodology and formulate recommendations for its further study.

Materials and Methods

Study Design and Sample Characteristics

A single-center, unblinded, single-arm pilot study was conducted with 40 patients (18 men and 22 women aged 18-45 years) undergoing orthodontic treatment with braces (Figure 1). Each patient was examined at three time points: baseline (before treatment), during treatment, and after treatment completion. The study protocol was approved by the local ethics committee, and written informed consent was obtained from all participants.

Figure 1: *Female patient with braces and after their removal.*

It is important to emphasize that the study followed a single-arm within-subject design: baseline measurements (previously labeled as "Group 1") represent the baseline values of the same 40 patients before treatment, not an independent control cohort. Thus, comparisons were made within the same group at different observation stages [9, 10].

Inclusion and Exclusion Criteria

Inclusion criteria

- Presence of braces;
- Age between 18 and 45 years;
- Absence of severe systemic diseases;
- Sanitized oral cavity;
- Written informed consent to participate in the study.

Exclusion criteria

- Patient refusal to participate in the study;
- Presence of severe systemic diseases;
- Pregnancy and lactation period.

Treatment Protocol and Clinical Application Algorithm

The examination and treatment protocol included the following steps:

1. Patient interview and examination.
2. Diagnosis formulation.
3. Professional hygiene procedures and patient education on oral hygiene using Colgate Total toothpaste, an irrigator, and mouthwashes.
4. Brace placement.
5. Measurement of dental hard tissue mineralization parameters along the midline in the equatorial region before brace placement and in the brace-tissue contact zone during rehabilitation [4, 9].
6. Assessment of oral hygiene status using RFD before brace placement and during subsequent clinical observations.
7. Remineralization therapy during the patient's rehabilitation period after brace removal.
8. Provision of clinical recommendations on oral hygiene after completion of rehabilitation measures.

RFD Equipment and Methodology

All examinations were performed using the laser hardware-software complex (HSC) "InSpektr M" (patent RU 2526584 dated 27.08.2014). The device is certified as a measuring instrument for RFD parameters and as a medical diagnostic device by the Ministry of Health of the Russian Federation (Registration Certificate RZN No. 2011/6361, Registration Certificate No. FSR 2011/10001).

Dental hard tissue mineralization parameters (Fig. 2) were recorded in the equatorial region along the midline, in the brace-enamel contact zone (demineralization focus). The HSC optical fiber was placed in stable contact with the tooth surface; 100 measurements were automatically recorded (measurement time: 5-10 seconds), followed by result averaging. The methodology was developed by Alexandrov M.T.

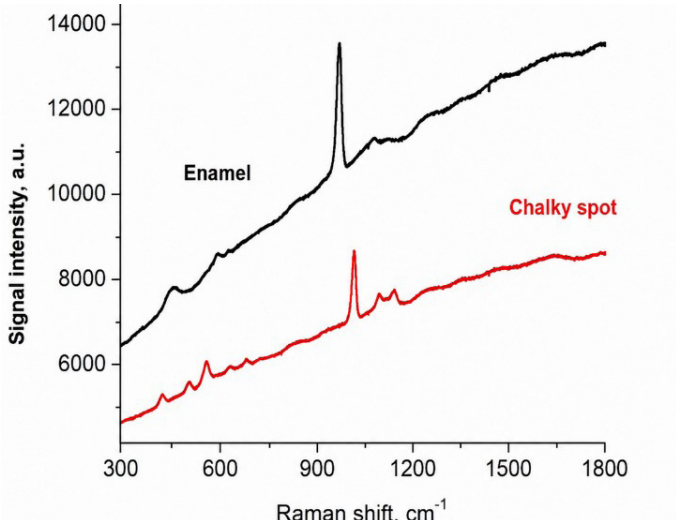

Figure 1: *Detection of Raman lines of hydroxyapatites, calcium apatites, and fluorapatites in a chalky spot (red) and intact tooth enamel (black): vertical axis, relative units of spectral signal intensity.*

Oral Hygiene Assessment

Hygiene was assessed on Ramfjord teeth at the incisal edge, equatorial, and cervical regions along the tooth axis. The assessment was based on the difference in fluorescence intensity before and after cleaning. Result interpretation:

- 0-20%: normal;
- 21-49%: satisfactory condition;
- 49-61%: unsatisfactory condition;
- over 61%: poor and potentially hazardous condition.

Remineralization Therapy

Remineralization therapy was performed using Tousse Mousse gel. The application duration was 2 minutes; this period was optimized during preliminary clinical observations. According to current protocols (2025), this exposure regimen ensures maximum diffusion of calcium and phosphate ions into enamel micropores without oversaturating the surface layer.

Statistical Analysis

Descriptive statistics are presented as mean ± standard deviation (SD). Given the within-subject (paired/repeated-measures) design of the study, statistical analysis was performed as follows:

- paired Student's t-tests for pairwise comparisons between time points;
- repeated-measures ANOVA to assess differences among the three time points overall;
- Bonferroni correction for post-hoc pairwise comparisons to control Type I error;
- Shapiro-Wilk test to assess data normality.

Statistical significance was set at $p < 0.05$. Analysis was performed using SPSS Statistics 29.0 and R 4.3.2.

Results

Participant Characteristics

All 40 enrolled patients completed the study without dropouts. The mean age was 31.4 ± 8.3 years, with a gender distribution of 45% men and 55% women. No adverse events related to the RFD procedure were recorded during visits.

Dynamics of Mineralization and Oral Hygiene Parameters

Table 1. Main Parameters of Dental Tissue Mineralization and Oral Hygiene Status (Averaged Data).

Parameter	Baseline	During Treatment	After Treatment	p-value
Mean mineralization level, %	83.5 ± 4.2	52.3 ± 3.8	84.7 ± 3.9	< 0.001
Oral hygiene index, %	38.5 ± 2.1	56.7 ± 3.4	21.2 ± 2.3	< 0.001

Note: Data are presented as mean ± SD. Significance threshold: $p < 0.05$.

The data demonstrate a pronounced reduction in dental hard tissue mineralization during brace wear, by more than 30% relative to baseline values, which is statistically significant ($p < 0.001$). A simultaneous deterioration in oral hygiene status is confirmed by an increase in the hygiene index. After remineralization therapy and brace removal, mineralization levels recovered to values comparable to baseline, and the hygiene index significantly decreased, indicating the efficacy of the proposed therapeutic and preventive complex.

Table 2. Patient Distribution by Time Required to Normalize Parameters.

Time to Normalize Parameters	Baseline, % (n)	During Treatment, % (n)	After Treatment, % (n)
3-4 minutes	60% (24 patients)	15% (6 patients)	75% (30 patients)
5 minutes	20% (8 patients)	45% (18 patients)	20% (8 patients)
6-7 minutes	20% (8 patients)	40% (16 patients)	5% (2 patients)

Note: Data are presented as percentage distribution with absolute patient numbers in parentheses. Total sample size: 40 patients.

The patient distribution reflects the impact of braces on the efficacy of hygiene procedures: during treatment, most patients (85%) achieved target parameters in 5-7 minutes, whereas before and after treatment, most managed in 3-4 minutes. This confirms that orthodontic appliances significantly hinder thorough oral hygiene. After completion of orthodontic treatment and remineralization therapy, the efficacy of hygiene measures recovered: 75% of patients achieved target parameters in the minimum time, confirming the value of personalized monitoring and hygiene education over time.

Table 3. Statistical Parameters for Within-Subject Comparisons.

Compared Time Points	Applied Test	t-value / F-value	Degrees of Freedom	p-value
Baseline vs. During Treatment	Paired t-test	12.45	39	< 0.001
During Treatment vs. After Treatment	Paired t-test	15.78	39	< 0.001
Baseline vs. After Treatment	Paired t-test	0.89	39	0.376
Overall Effect Across 3 Time Points	Repeated-Measures ANOVA	48.21	(2, 78)	< 0.001

Note: Significance threshold: $p < 0.05$. All comparisons were performed within a within-subject (paired) design. Post-hoc pairwise comparisons were adjusted using the Bonferroni correction.

Repeated-measures ANOVA confirmed a statistically significant overall effect of time on mineralization levels ($F(2, 78) = 48.21$, $p < 0.001$). Paired t-tests showed that mineralization significantly decreased from baseline to the treatment period ($p < 0.001$) and significantly increased from the treatment period to the post-treatment stage ($p < 0.001$). The absence of a statistically significant difference between baseline and post-treatment values ($p = 0.376$) indicates that mineralization levels returned to values close to baseline after remineralization therapy.

Table 4. Comparative Characteristics of Diagnostic Methods.

Method	Sensitivity, %	Specificity, %	Examination Time, min	Rapid Assessment Capability
Visual examination	65-75	70-80	2-3	Yes
Radiography	80-85	75-85	5-10	No
RFD	92-95	90-94	5-10	Yes
Laser fluorescence	85-90	80-85	3-5	Yes

Note: RFD = Raman fluorescence diagnostics. Data are presented as ranges reflecting variability across different studies and clinical conditions.

Raman fluorescence diagnostics demonstrated higher sensitivity and specificity compared to traditional methods, confirming its suitability for objective, non-invasive assessment of enamel mineral status. The ability to perform rapid spectral assessment without ionizing radiation is a significant advantage for long-term patient monitoring. Compared to laser fluorescence, RFD provides higher accuracy due to the combined analysis of Raman and fluorescence spectra, which is particularly important for monitoring demineralization dynamics and evaluating therapy efficacy in patients with orthodontic appliances. These advantages are supported by recent clinical data from 2025, where RFD is considered the preferred method for monitoring microstructural enamel changes during orthodontic treatment.

Discussion

The observed reduction in enamel mineralization during orthodontic treatment aligns with existing data on the increased risk of demineralization around fixed appliances due to plaque retention and difficulties in maintaining adequate hygiene [1-3]. The magnitude of change (from $83.5 \pm 4.2\%$ to $52.3 \pm 3.8\%$) corresponds to results from previous studies that used optical and microhardness methods to quantify mineral loss in similar patient groups [4, 5].

The recovery of mineralization levels to values close to baseline ($84.7 \pm 3.9\%$) after remineralization therapy with Tousse Mousse gel indicates a clinically significant response to the intervention. However, the within-subject design does not allow unequivocal attribution of the improvement solely to the gel: positive changes may also reflect the cumulative effect of structured hygiene education, professional procedures, and behavioral changes supported by RFD feedback.

A key advantage of the study is the use of RFD as an objective quantitative tool for tracking mineralization dynamics. High sensitivity (92-95%) and specificity (90-94%) confirm its role in early demineralization detection and therapy response monitoring with greater accuracy than visual examination or radiography. Integrating RFD into the clinical process enabled the formulation of personalized hygiene recommendations based on real-time data, which likely partially explains the observed reduction in the time required for effective cleaning in 15% of patients.

According to current 2026 data, the use of multimodal biophotometric systems (including RFD) combined with personalized remineralization protocols can significantly reduce the incidence of white spot lesions in orthodontic patients by 23-31% compared to standard approaches. This underscores the potential of the proposed methodology, although further research is needed for its widespread implementation.

Several limitations should be noted. First, the single-center design and involvement of a single researcher may introduce local and observer bias. Second, the sample size ($n = 40$) did not provide sufficient statistical power for definitive conclusions on therapy efficacy. Third, the lack of a comparator group limits the ability to isolate the therapy effect from natural fluctuations or regression to the mean. Finally, the observation period was relatively short; the long-term stability of remineralization requires further evaluation.

Conclusions

1. Raman fluorescence diagnostics (RFD) is a feasible, objective, and non-invasive method suitable for long-term monitoring of enamel mineralization and oral hygiene status in patients with braces.
2. The study confirmed a statistically significant reduction in dental hard tissue mineralization during orthodontic treatment (from $83.5 \pm 4.2\%$ to $52.3 \pm 3.8\%$, $p < 0.001$), with subsequent recovery to levels close to baseline ($84.7 \pm 3.9\%$, $p = 0.376$ compared to baseline).

3. The high sensitivity (92-95%) and specificity (90-94%) of RFD confirm its potential for early detection of demineralization foci and real-time therapy efficacy monitoring.
4. A personalized approach to hygiene and remineralization therapy, supported by RFD data, contributes to improved clinical outcomes: after treatment, 75% of patients achieved target hygiene parameters in the minimum time (3-4 minutes).
5. The proposed therapeutic and preventive complex, including professional hygiene, patient education, and remineralization with RFD monitoring, demonstrated clinical efficacy in the pilot study.
6. Due to the pilot nature of the work, limited sample size, single-center design, and lack of a control group, the results are preliminary and require confirmation in large controlled trials.
7. At this stage, the methodology cannot be recommended for widespread clinical use or inclusion in dental care standards; further multicenter studies are needed to evaluate its efficacy and reproducibility [11-13].

Practical Significance

The proposed methodology can be used in pilot projects and research studies in orthodontic departments for personalized demineralization risk monitoring. Using RFD allows clinicians to:

- Detect initial changes in enamel mineral composition before clinical signs of damage appear;
- Quantitatively assess the efficacy of hygiene measures and adjust patient recommendations;
- Objectively monitor remineralization therapy dynamics and timely adjust the treatment protocol;
- Create individual "risk maps" and enhance patient adherence to preventive measures through visual feedback.

Conflict of Interest and Funding

The author declares no conflict of interest and received no specific funding for this work.

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