

## RESEARCH

# Effect of diode laser application on root surface carbon, calcium and phosphorus levels: A SEM-EDX study

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### ABSTRACT

**Effect of diode laser application on root surface carbon, calcium and phosphorus levels: A SEM-EDX study**

**Background:** Preservation of the organic ingredients on the root surfaces during the periodontal therapy has been emphasized in the literature. Scanning electron microscopy energy-dispersive X-ray (SEM-EDX) analysis is an accepted approach to evaluate organic and inorganic components on investigated samples in terms of carbon, calcium and phosphorus levels. The aim of this study was to evaluate the effects of diode laser application with periodontal pocket decontamination energy setting to the organic content of intact root surfaces via SEM-EDX analysis.

**Materials and Methods:** Each proximal root surfaces of the root surfaces of 10 teeth which were extracted due to orthodontic reasons were divided as test (n=20) and control (n=20) sites. Diode laser (810 nm) applications were done to the test sites of root surfaces. All specimens were analyzed using SEM-EDX with respect to carbon, calcium and phosphorus levels.

**Results:** Diode laser applied root surfaces exhibit significantly lower carbon levels comparing with control sites. Additionally, increased calcium and phosphorus levels were detected in the test sites.

**Conclusion:** Within the limitations of this study, it can be concluded that, diode laser applications may alter organic content of intact root surface. Further studies are required to elucidate potential effects of diode laser application to specific protein components on the root surfaces.

### KEYWORDS

**Diode laser, element analysis, root surface, SEM-EDX**

### ÖZ

**Diyot lazer uygulamasının kök yüzeyi karbon, kalsiyum ve fosfor seviyelerine etkisi: Bir SEM-EDX çalışması**

**Amaç:** Periodontal tedavi sırasında kök yüzeylerindeki etkilenmemiş organik unsurların korunması gerekliliği literatürde vurgulanmaktadır. Taramalı elektron mikroskopisi ve enerji yayımlı X-ışını (SEM-EDX) analizi ile karbon, kalsiyum ve fosfor element seviyelerinin değerlendirilmesi, kök yüzeyindeki organik ve inorganik yapıların tayininde uygulanan bir yaklaşımdır. Bu çalışmanın amacı, periodontal cep dekontaminasyonu enerji düzeyinde gerçekleştirilen diyot lazer uygulamasının kök yüzeyindeki organik içeriğe etkisinin SEM-EDX yöntemi ile incelenmesidir.

**Gereç ve Yöntemler:** Ortodontik nedenlerden ötürü çekilmiş olan toplam 10 adet dişin her bir aproksimal yüzeyi test (n=20) ve kontrol (n=20) alanları olmak üzere iki bölgeye ayrıldı. Test bölgelerine diyot lazer (810 nm) uygulamaları gerçekleştirildi. Bütün örnekler karbon, kalsiyum ve fosfor element seviyeleri açısından SEM-EDX yöntemi ile analiz edildi.

**Bulgular:** Diyot lazer uygulanan kök yüzeylerinde, kontrol alanlarına göre anlamlı düzeyde düşük karbon seviyeleri gözlemlendi. Kalsiyum ve fosfor seviyeleri test bölgelerinde kontrol alanlarında göre daha yüksek seviyede gözlemlendi.

**Sonuç:** Bu çalışmanın sınırları kapsamında, diyot lazer uygulamalarının kök yüzeyindeki organik yapıya etki edebileceği ortaya konmuştur. Diyot lazerinin kök yüzeyindeki spesifik protein yapılarına olan etkilerini inceleyen sonraki çalışmalara ihtiyaç vardır.

### ANAHTAR KELİMELER

**Diyot lazer, element analizi, kök yüzeyi, SEM-EDX**

Laser is defined as the amplified form of monochromatic and coherent photon emission, namely intensive light energy.<sup>1</sup> Since this technology presents various advantages in surgical processes such as relatively less intra-operative bleeding and favorable post-operative outcomes, it became an emerging treatment modality worldwide.<sup>2</sup> The expected ablation effect on the laser-applied tissues occurs via absorption of the energy by the related tissue components which are sensitive to wavelength of applied laser light. Accordingly, various laser types with different wavelengths are

utilized based on the target tissue characteristics.<sup>2</sup> Among those, diode lasers are used for soft tissue applications due to its energy is mostly absorbed by pigmented tissue ingredients. Additionally, diode lasers are also used in periodontal practice for bacterial decontamination of periodontal pocket.<sup>3</sup>

Periodontitis is characterized by the inflammatory destruction of supportive tissues of the teeth which is initiated by bacterial dental plaque. Since presence of any contaminated root surface hinders complete tissue healing, effective removal of the pathogenic deposits

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from tooth surface is the major objective of the periodontal therapy.<sup>4</sup> Laser supplementation to mechanical debridement is an accepted treatment modality of periodontitis. Accordingly, diode laser applications to the periodontal pocket are used to achieve efficient root surface decontamination and improve non-surgical and/or surgical periodontal treatment outcomes.<sup>5</sup>

Although complete removal of bacterial deposits from the tooth surface is crucial, it has been known that preservation of intact cementum is also important during periodontal debridement.<sup>6</sup> Cementum and its organic components are thought to contribute to the repair of periodontal tissues during the healing period. Extracellular matrix contents of the cementum including the collagen fibers and other protein-based structures contribute cell adhesion, differentiation and proliferation.<sup>7</sup> It has been suggested that preservation of collagenous and/or non-collagenous organic ingredients during the periodontal therapy may improve wound healing.<sup>8,9</sup> Therefore, avoidance of unnecessary instrumentation which may lead damage the integrity of residual collagen fibers and organic matrix of the intact cementum should be taken into consideration by the clinicians.

There is limited information about the influence of diode lasers on root surfaces. In addition, potential effects of diode laser irritation on organic content of intact root surface have not been scrutinized. SEM-EDX (scanning electron microscopy and energy dispersive X-ray spectroscopy) analysis is an accepted approach for the assessment of tooth surface organic and inorganic content via evaluation of carbon, calcium and phosphorus levels.<sup>10</sup> Accordingly relative increase in carbon levels comparing with calcium and phosphorus indicates the increase of organic content.<sup>11</sup> The aim of this study was to analyze the effect of diode laser applications on intact root surface carbon, calcium and phosphorus levels.

## **MATERIALS AND METHODS**

This study was approved by Ethics Committee of Kocaeli University (GOKAEK 2014/225). The study was conducted on both mesial and distal aspects of 10 single rooted teeth which were extracted for orthodontic reasons. Inclusion criteria of each tooth were: (1) having probing depth up to 3 mm at all aspects (2) having no attachment loss at all aspects (3) having no gingival recession at all aspects (4) having no sign of any caries lesions (5) having no dental restoration (6) no periodontal therapy within last 12 months (7) having no sign of tooth fracture disturbing the tooth integrity. All extracted teeth in sodium azide solution (0.2%) until the experiment date.

## **Laser irradiation**

This study was conducted using a gallium-aluminum-arsenide (GaAlAs) (810 nm wavelength) diode-laser system (Gigaa Laser, China) with 400- $\mu$ m diameter fiber optic tip. Each intact root surfaces at both mesial and distal aspects of the teeth were randomly assigned as test (n=20) and control (n=20) sites in middle part of the root surfaces. Two indicator dots were made using a small diameter bur at coronally and apically to determine a visionary border for test and control sites. Thus, adjacent sites were selected as test and control sites to standardize application areas. Energy settings of applied laser irradiation the protocol was compatible with the report of Euzebio Alves et al (2013).<sup>12</sup> Accordingly, the fiber optic was placed parallel to the long axis of the tooth and it was moved with apical-cervical scanning movements per 20 seconds in the test sites, using a power of 1.5 W, 20 s and power of density of 1,193.7 W/cm<sup>2</sup>. All laser irradiations and SEM-EDX analysis were made at the same experiment date.

## **SEM-EDX analysis**

After drying, the teeth were coated with gold using sputter-coater. SEM-EDX analysis was done by using Tescan Vega II XMU SEM system in Sakarya University, Thermal Spray Research Laboratory. SEM-EDX analysis was done at x100 magnifications in both groups. EDX analysis was conducted at randomly assigned five points of each experimental site. Accordingly obtained each carbon, calcium, phosphorus and oxygen scores as weight% were measured. The sum of obtained results of each investigated element in each group was calculated and underwent statistical analysis.

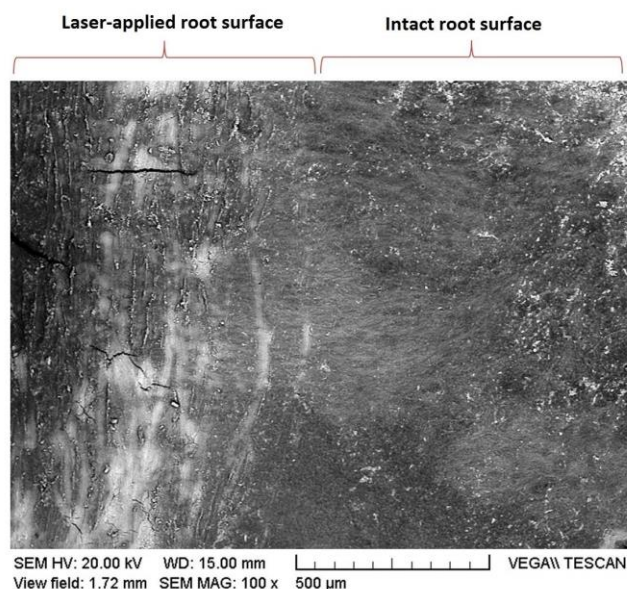
## **Statistical analysis**

Statistical analysis was performed with SPSS for Windows 15.0 (SPSS Inc., Chicago, IL, USA). Kolmogorov-Smirnov test was used for testing the normality of data. Differences were compared with the Mann-Whitney U test between the groups at a significance level of  $p < 0.05$ . Correlations were analyzed with Spearman's Rank Correlation Coefficient Test.

## **RESULTS**

According to SEM observations, laser applied root surfaces exhibit altered surface characteristics comparing with the control sites. Accordingly, considerably rough surfaces, irregular notches and some cracks were seen in the laser applied root surfaces (Figure 1). Carbon levels were significantly low in test sites comparing with control sites. Contrarily, calcium and phosphorus levels were

significantly high in test sites. Oxygen levels did not differ among the groups. Carbon/calcium and carbon/phosphor ratios were also significantly low in control sites (Table 1). Strong negative correlations were detected between carbon and calcium, and also carbon and phosphor levels in laser applied sites (Table 2). However, there was no correlation between any investigated elements in intact root surfaces (Table 3).



**Figure 1.**

SEM view of both laser-applied and intact root surfaces at x100 magnification. The border between laser-applied site and control site is clearly seen

**Table 1.**

**Comparison of the levels of investigated elements on both intact and laser-applied root surfaces**

	Intact root*	Laser-applied root*	p-value
Carbon	63.9 ± 5.52	40.13 ± 3.47	0.0001
Calcium	10.17 ± 3.96	25.24 ± 3.96	0.0001
Phosphor	8.9 ± 2.78	16.43 ± 2.79	0.0001
Oxygen	17.13 ± 1.8	18.51 ± 2.63	0.143
Carbon/Calcium	8.4 ± 6.55	1.64 ± 0.41	0.0001
Carbon/Phosphor	7.87 ± 2.75	2.52 ± 0.54	0.0001
Calcium/Phosphor	1.16 ± 0.47	1.58 ± 0.43	0.043

**Table 2.**

**Correlations among the levels of investigated elements on laser-applied root surfaces**

	Carbon	Calcium	Phosphor	Oxygen
Carbon	1.00			
Calcium	-0.806* (0.005)**	1.00		
Phosphor	-0.721 (0.019)	0.455 (0.187)	1.00	
Oxygen	-0.188 (0.603)	-0.079 (0.829)	-0.224 (0.533)	1.00

\*Correlation coefficients

\*\*p-values

**Table 3.**

**Correlations among the levels of investigated elements on intact root surfaces**

	Carbon	Calcium	Phosphor	Oxygen
Carbon	1.00			
Calcium	-0.624* (0.054)**	1.00		
Phosphor	-0.455 (0.187)	-0.018 (0.96)	1.00	
Oxygen	0.345 (0.328)	-0.236 (0.511)	-0.588 (0.074)	1.00

\*Correlation coefficients

\*\*p-values

## DISCUSSION

Tooth is a composite material which comprises both organic and inorganic ingredients.<sup>13</sup> While calcium-hydroxyapatite predominantly constitutes the inorganic structure, collagen is a prominent organic component of the teeth.<sup>14</sup> In particular, intact root surface is rich in collagen which originates from extrinsic fibers of cementum.<sup>15</sup> In addition, root surface comprises various organic components. Collagen bundle extensions from the root surface and other organic gradients constitute attachment apparatus. Some other non-collageous proteins including osteocalcin and bone phosphoprotein which are implicated in bone regeneration exist on the root surface.<sup>16</sup> One of the major aims of periodontal therapy is the regeneration of missing periodontal attachment. In addition to complete removal of bacterial toxins and other deposits, preserving the beneficial organic components on the root surface is also an important task of the periodontal treatment.<sup>17</sup> Protecting the integrity of exposed collagen bundles on the root surface may enhance initial cell colonization and thus periodontal wound healing.<sup>18</sup> Polson and Caton (1982) reported that deterioration of collagen fibers extensions on the root surface resulted in the absence of any connective tissue attachment.<sup>19</sup> The results of this study revealed that diode laser application led significantly reduction of the root surface carbon levels and increase of calcium and phosphor levels emphasizing on the reduction in the organic content rates of root surface.

The potential effects of laser applications on the root surfaces have been subject of various studies. Morlock et al (1992) and Schwarz et al (2001) reported that direct laser energy transmission to the root surfaces may lead some damages such as cracks and grooves due to instant rehydration caused by heat.<sup>20,21</sup> Baburao et al (2014) reported that root surfaces may be negatively affected as energy of diode laser irradiation.<sup>22</sup> On contrary, Theodoro et al (2003) investigated the thermal effects of diode laser applications with two different energy states on scaled and planed root surfaces by SEM *in vitro*.<sup>23</sup> In addition, they measured pulpal temperature simultaneously. According to their results, they have not noticed any high pulpal temperature degrees. Additionally, they have concluded that there were no significant alterations on applied root surfaces. In this study, some irregularities were seen in laser applied root surfaces comparing with adjacent control sites.

Assessment of the organic content of an investigated sample via carbon, calcium and phosphorus levels is an accepted methodological approach. SEM-EDX analysis is a referred methodology for detection of element content of investigated sample surfaces.<sup>10,24,25</sup> Henmi et al (2014) investigated bone matrix calcification during the embryonic and post-embryonic period via SEM-EDX carbon, calcium and phosphorus levels.<sup>11</sup> They have stated that the decrease of carbon levels may reflect the decreasing concentrations organic ingredients. Accordingly, decrease of C/Ca and C/P ratios also refers the decrease of organic content. They have also confirmed the consistency of SEM-EDX results in terms of the assessment of organic and inorganic content with X-ray diffraction analysis and fourier transform infrared spectrometry. Similarly, Arnold and Gaengler (2006) investigated the distribution of carbon, calcium and phosphorus levels in pre-dentin, dentin and enamel which exhibit different mineralization patterns.<sup>26</sup> According to their results, carbon levels gradually decreased in pre-dentin, dentin and enamel, respectively. Similarly, they have observed that as the tissues became more mineralized calcium and phosphorus levels gradually increased. All above-mentioned studies refer the reliability of the SEM-EDX analysis for the assessment of organic and inorganic content of both bone and tooth. In our study, carbon levels were negatively correlated with calcium and phosphorus levels in laser applied sites, unlike intact root surfaces (Table 2, Table3). Accordingly, The results of our study indicated that diode laser application may deteriorate organic content of root surface. Accordingly, increased calcium and

phosphorus levels on root surfaces may be explained with the prominence of inorganic root surface characteristics following the laser irritation.

In this study, potential influences of diode laser applications on organic and inorganic components were evaluated via intact root surfaces. Considering the results, carbon levels were reduced since the thermal effects of laser irradiation led the deterioration organic components on root surface. Consequently, underlying calcium and phosphorus, which constitutes the inorganic structure, became more prominent. Reduced carbon/calcium and carbon/phosphorus levels in laser applied sites may support this argument. The importance of the preservation of organic ingredients has been emphasized previously.<sup>7</sup>

Diode laser applications on root surface may reduce the rates of organic content of root surface. Further studies are required to evaluate potential effects of such technologies specifically on organic content of root surfaces which are affected by periodontitis.

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