Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2017 January 28; 10044: . doi:10.1117/12.2256734.

Influence of Multi-Wavelength Laser Irradiation of Enamel and Dentin Surfaces on Surface Morphology and Permeability

Nai-Yuan N. Chang^a, Jamison Jew^a, Jacob C. Simon^a, Kenneth H. Chan^a, Robert C. Lee^b, William A. Fried^a, Jinny Cho^a, Cynthia L. Darling^a, and Daniel Fried^a

^aUniversity of California, San Francisco, San Francisco, CA 94143-0758, U.S.A

^bUniversity of Washington, Seattle, WA 98195, U.S.A

Abstract

UV and IR lasers can be used to specifically target protein, water, and the mineral phase of dental hard tissues to produce varying changes in surface morphology. In this study, we irradiated enamel and dentin surfaces with various combinations of lasers operating at 0.355, 2.94, and 9.4 μm , exposed those surfaces to topical fluoride, and subsequently evaluated the influence of these changes on surface morphology and permeability. Digital microscopy and surface dehydration rate measurements were used to monitor changes in the samples overtime. The surface morphology and permeability (dehydration rate) varied markedly with the different laser treatments on enamel. On dentin, fluoride was most effective in reducing the permeability.

Keywords

lasers; enamel; dentin; dehydration; permeability

1. INTRODUCTION

The nature of dental decay or dental caries in the U.S. has changed markedly due to the introduction of fluoride to the drinking water, the use of fluoride dentifrices and rinses, and improved dental hygiene. Despite these advances, dental caries continues to be the leading cause of tooth loss in the U.S.¹⁻³ The development of new conservative methods for inhibiting tooth decay are needed. Several studies over the past forty years have demonstrated that lasers can be used to thermally modify the mineral phase, change the surface morphology, and increase the incorporation of fluoride to increase the acid-resistance of dental enamel. Infrared laser radiation from Er:YAG and CO₂ lasers is highly absorbed by water and the minerals of the tooth structure. High-energy laser irradiation has been shown to cause thermal decomposition and crystalline transformation of carbonated hydroxyapatite to a purer-phase hydroxyapatite, which has demonstrated increased acid resistance⁴⁻¹⁰. Although alternative mechanisms for caries inhibition by laser irradiation has been proposed, infrared spectroscopy and x-ray crystallography have been used to clearly establish the correlation of carbonate loss from the mineral phase due to thermal decomposition with the threshold for increased acid-resistance. Furthermore, fluoride application both before and after Er:YAG and CO₂ laser irradiation has been shown to further improve the acid resistance of enamel over laser irradiation alone^{8,11-14}. Although

the mechanism of synergy between fluoride application and laser irradiation has not been firmly established, it is most likely due to the increased retention of fluoride to the laser treated surfaces. UV light at 0.355 μm from a frequency-tripled Nd:YAG laser has been shown to greatly increase the efficacy of fluoride¹⁵. We hypothesize that the UV laser etches the protein and lipid around the enamel crystals to expose the mineral. This initially increased the enamel permeability and decreased its acid-resistance, however after adding fluoride, the UV laser + topical fluoride treatment was significantly more effective in inhibiting acid dissolution than fluoride alone.

Similar laser treatments that are effective for enamel have not been effective on dentin. IR spectroscopy and microradiography show that CO₂ laser irradiation transforms the dentin into a more highly mineralized (harder) enamel-like hydroxyapatite, similar to the laser-irradiated enamel^{16,17}. However, dentin contains 50% collagen and the loss of that collagen matrix induces a large contraction of volume. The surface layer of enamel-like transformed dentin is permeated by large cracks through which the acid can penetrate so that the laser treatments are not effective¹⁸. We treated dentin surfaces in multiple studies using laser pulse durations varying from a microsecond to seconds to modify progressively thicker layers of dentin, but none of the treatments were effective. This was unfortunate, since more effective methods are needed for the treatment of root caries. There are several papers in the literature that have claimed that laser treatments are effective for inhibiting decay in dentin. However, these studies are not valid since the hardness of the outer layer of dentin is increased after loss of the collagen and only surface hardness measurements were used to assess acid resistance and the subsurface mineral loss was not measured as was done in our studies.

The three lasers investigated in this study have distinctly different effects on the surface morphology of enamel. The CO₂ laser operating at 9.3–9.6 μm , coincident with the strongest absorption by the mineral phase, produces a glass-like layer of melted enamel with an order of magnitude increase in the size of the enamel crystals, and this layer is highly effective in inhibiting acid dissolution. In contrast, the Er:YAG laser is most strongly absorbed by water and the subsurface expansion of water leads to the ablation of enamel without melting the enamel, and the ablated surface does not have the same characteristic melted layer observed for the CO₂ laser. Even though we have shown that the surface temperature is much higher for the CO₂ laser than for erbium lasers at the onset of ablation, Er:YAG lasers have been shown to increase the acid resistance of enamel but they are not as effective as the CO₂ laser, and the effect is mostly due to peripheral heat accumulation under undesirable irradiation conditions, such as without water. The UV laser at 0.355 μm appears to selectively etch the protein and lipid around the enamel prisms creating yet another unique surface morphology.

The purpose of this study was to explore the influence of laser irradiation and topical fluoride application on the surface morphology and permeability to shed light on the mechanism of interaction. In addition, to irradiating samples with the individual laser systems, we also explored irradiating the samples previously irradiated by the Er:YAG and UV lasers a second time by the CO₂ laser after fluoride application. We hypothesize that prior irradiation by the Er:YAG and UV lasers both preferentially removes protein and lipids including the collagen from dentin, and that this can both enhance the adhesion of topical

fluoride and reduce volume contraction (crack formation) during the subsequent thermal transformation by the CO₂ laser for more effective laser treatments.

In addition, we now have new tools for assessing the laser induced changes to the enamel and dentin surfaces. Changes in the surface roughness and permeability are important for both acid resistance and fluoride retention. In this study, we employed digital microscopy and optical dehydration measurements. High-resolution digital microscopy is valuable since it allows us to view changes on rough surfaces at 1000x magnification. This approach is advantageous over scanning electron microscopy, which requires a high vacuum environment that can induce morphological changes in dental enamel, such as the formation of microcracks, making it difficult to differentiate the effects caused by the laser from those caused by the SEM environment.

The permeability of the laser treated surfaces is also important, since a higher permeability will lead to deeper subsurface demineralization. We have investigated optical methods for monitoring changes in the permeability since such measurements can be used to assess the activity of carious lesions. When lesions become arrested by mineral deposition in the outer layers of the lesion, the diffusion of fluids into the lesion is inhibited. Previous studies have demonstrated that the optical changes due to the loss of water from porous lesions can be exploited to assess lesion severity with QLF and thermal and NIR imaging^{19–21}. We have investigated optical methods for assessing water diffusion rates from lesions since the porosity of the outer layers of active lesions is significantly greater than for arrested lesions. This can be indirectly measured via thermal and NIR reflectance methods²². Hence, the rate of water diffusion out of the lesion reflects the degree of lesion activity. NIR reflectance imaging showed better performance than thermal imaging for monitoring the dehydration of enamel lesions, but did not perform well on dentin surfaces due to the higher light scattering of sound dentin. Water in the pores at the surface of the lesion absorbs the incident NIR light and reduces surface scattering, causing reduced lesion contrast. Loss of that water during dehydration causes marked increase in reflectivity and lesion contrast. During lesion dehydration, the vaporization of water from the lesion pores causes a decrease in surface temperature in the lesion area, which can be measured with a thermal imaging camera. Thermal imaging at wavelengths from 8000 to 13000 nm had the highest diagnostic performance on artificial dentinal lesions and natural root caries lesions²³. Unlike NIR dehydration measurements where a NIR light source is also needed to illuminate the tooth, thermal imaging does not require an additional light source. Here, we employ both thermal and NIR reflectance methods to measure the surface dehydration rates of the laser treated surfaces before and after exposure to demineralization.

2. MATERIALS AND METHODS

Four sets of samples were used: 2 sets of enamel blocks and 2 sets of dentin blocks grouped by the wavelengths of laser irradiation. Figure 1 shows the workflow of the study design employed.

2.1 Sample Block Preparation

Enamel and dentin blocks (n=24 each), approximately 10–12 mm in length with a width of 2 mm and a thickness of 2 mm were prepared from extracted bovine incisors acquired from a slaughterhouse. For dentin samples, the bovine enamel was removed from the outer surface towards the dentinoenamel junction (DEJ) exposing the dentin. Both enamel and dentin blocks were ground to a 9 μm finish. Each sample was partitioned into seven fiducial windows by etching small incisions 1.5 mm apart across each of the blocks using a laser. Incisions were etched using a radio-frequency (RF)-excited industrial CO₂ laser, a Coherent Diamond J-5V Series (Coherent Inc., Santa Clara, CA), operating at 9.4 μm with a pulse duration of 25 μs and a pulse repetition rate of 200 Hz (Fig. 2).

2.2 Sample Laser and Fluoride Treatments

Three different lasers were used to irradiate the sample windows: the 3mikronTM Er:Y₃Al₅O₁₂ garnet (Er:YAG) laser (Pantec Engineering AG, Liechtenstein) operating at 2.94 μm with fluence of 7.5 J/cm² (for enamel) and 3.75 J/cm² (for dentin) at 100 Hz and a scanning velocity of 5 mm/s; the Tempest Nd:YAG laser (New Wave Research, Sunnyvale, CA) operating at 0.355 μm (UV wavelength) with a fluence of 1.43 J/cm² (for both enamel and dentin) at 30 Hz and a scanning velocity of 1.25 mm/s; and the same CO₂ laser with fluences of 0.88 J/cm² (for enamel) and 0.52 J/cm² (for dentin) at 100 Hz and a scanning velocity of 2 mm/s. The enamel and dentin blocks were further separated into 2 subgroups (n = 12 each), the Er:YAG-treated group and UV-treated group. Certain fiducial windows are also treated with acidulated phosphate fluoride (APF) (Gelato APF Fluoride Gel, Keystone Industries, Gibbstown, NJ) for one minute in combination to serial laser treatments. The seven fiducial windows for each sample were subjected to different treatments as follows: 1) fluoride only, 2) UV or Er:YAG laser + fluoride, 3) UV or Er:YAG laser + fluoride + CO₂, 4) CO₂ laser + fluoride, 5) control (no treatment), 6) CO₂ laser only, and 7) UV or Er:YAG laser + CO₂ laser (Fig. 2).

2.3 Demineralization

A thin layer of acid-resistant varnish in the form of nail polish, Revlon 270 (New York, NY), was applied to all sides except the top surfaces of the enamel and dentin blocks before exposure to the demineralization solution. Samples were exposed were immersed in 40 mL aliquots of the demineralization solution for 24 hours 3 times with time points of 24, 48, 72 hours. The demineralization solution, which was maintained at 37°C and pH 5.0, was composed of 2.0 mmol/L calcium, 2.0 mmol/L phosphate, and 75 mmol/L acetate. This dissolution model is a “Surface Softened” dissolution model designed to produce subsurface dissolution while maintaining an intact surface, and it simulates highly active early lesions.

2.4 Visible Light Reflectance

Visible light reflectance images were taken by Dino-lite digital microscope Edge AM4815ZTL with extended depth of field (BigC, Torrance, CA).

2.5 Near-IR and Thermal Dehydration Measurements

Each sample was placed in a mount connected to a high-speed XY-scanning motion controller system (Newport, Irvine, CA) ESP301 controller and 850G-HS stages, coupled with an air nozzle and a light source. Each sample was immersed in the water bath for 30 seconds while being vigorously shaken to enhance water diffusion. After the sample was removed from the water bath, an image was captured as an initial reference image and the air spray was activated. The air pressure was set to 15 psi and the computer controlled air nozzle was positioned 4 cm away from the sample. Each measurement consisted of capturing a sequence of images at 4 frames per second for 30 seconds. For each measurement, the air nozzle and the light source were centered on the region of interest (ROI) which encompasses the entire enamel/dentin sample. The dehydration setup was completely automated using LabVIEW software (National Instruments, Austin, TX).

A Model SU320KTSX InGaAs focal plane array (Sensor-Unlimited, Princeton, NJ) with a spectral sensitivity range from 900 nm to 1750 nm, a resolution of 320×256 pixels and an InfiniMite lens (Infinity, Boulder, CO) was used to acquire all the images during the dehydration process. Light from a 150 W fiber-optic illuminator FOI-1 (E Licht Company, Denver, CO) was directed at the sample at an incident angle of approximately 60° to reduce specular reflection and the source to sample distance was fixed at 5 cm. A FEL LP series long-pass filter at 1400 nm (Thorlabs, Newton, NJ) was used for a wavelength range from 1400–1700 nm. Near-IR reflectance images were processed and automatically analyzed using a dedicated program constructed with LabVIEW software. ROI encompassing the whole sample was used and measurement was recorded for each time point. The intensity difference between the final and initial images, $I(t=30)$, was calculated using $I_{30} - I_0$, where I_{30} is the mean intensity at $t = 30$ seconds and I_0 is the mean intensity prior to turning on the air nozzle.

An infrared (IR) thermography camera, Model A65 from FLIR Systems (Wilsonville, OR) sensitive from $7.5 - 13 \mu\text{m}$ with a resolution of 640×512 pixels, a thermal sensitivity of 50 mK and a lens with a 13 mm focal length was used to record temperature changes during the dehydration process. The ambient room temperature, flowing air temperature and water bath temperature were approximately 21°C (294.15 K) and were consistent throughout the experiment. The object emissivity was set to 0.92, and the atmospheric temperature was set to 294.15 K²⁴. While humidity values were not recorded, every sample was measured under the same condition, where relative humidity was set at a default value of 50%. Previous studies have shown the area enclosed by the time-temperature curve, Q , can be used as a quantitative measure of porosity and can be used to discriminate between sound and demineralized enamel *in vitro*^{19,20,23}. Thermal images were processed and analyzed using a dedicated program written in LabVIEW. The thermography camera outputs a series of temperature measurements over time. Calibration was carried out via matching the measurements from the initial reference image to the ambient temperature. Q was calculated and averaged among the samples within the subgroup.

3. RESULTS

3.1 Surface Morphology

Digital light microscope images of the laser irradiated of enamel and dentin surfaces are shown in Fig. 2. These are depth convolution images with all the surfaces in focus. The surface morphology varies markedly with the different laser systems. The enamel and dentin surfaces irradiated by Er:YAG laser appeared rough. The exposed mineral tubules could be seen under digital microscopy for samples irradiated by UV laser. However, melting was not apparent for either irradiation. For the enamel windows irradiated by the CO₂ laser, there are lamellar structures produced by recrystallization of the melted enamel. The size of the lamellar structures varies with larger structures for the samples that were also irradiated by the UV and Er:YAG lasers. Similar structures were observed for dentin; however, the tubules are more clearly visible after UV irradiation and the lamellar melt structures after CO₂ laser irradiation are smaller for dentin and not as dramatically altered by UV and Er:YAG irradiation.

3.2 Dehydration Measurements

NIR reflectance dehydration measurements for the enamel samples are shown in Fig. 3. Note that Er:YAG-treated enamel windows demonstrated the greatest amount of porosity regardless of demineralization, where windows treated with fluoride only demonstrated the lowest amount of porosity. NIR reflectance dehydration measurements for dentin samples are not shown here, as no significant changes were detectable; the high collagen content of dentin may have interfered with the NIR-reflectance imaging. There was an overall increase in I after 3-days of demineralization for all the windows.

From Fig. 3, note the apparent differences in the shape of the enamel NIR dehydration time-intensity curve for Er:YAG-treated windows compared to the rest of the windows. Er:YAG-treated windows exhibited a more sigmoidal-like shape most visibly after 3 days of demineralization. The UV-treated windows also demonstrated time-intensity curves that are sigmoidal shaped, however less prominent compared to the Er:YAG-treated windows. Moreover, the CO₂-treated windows, even in combination with UV (but not with Er:YAG), exhibited more logarithmic-shaped curves. From these curves, we calculated I , the intensity differences at $t = 30$ seconds after the onset of the air nozzle. Intensity differences increased after 3 days of demineralization for most of the sample windows.

The thermal dehydration measurement results for the dentin samples are shown in Fig. 4. Note that the Er:YAG and CO₂ laser treated windows demonstrated the most prominent changes in temperature, where the control windows demonstrated the least amount of changes in temperature throughout the dehydration process.

From Fig. 4, the shape of the dentin thermal dehydration time-temperature curves is similar for all the windows with an initial temperature dip followed by progressive increase in temperature. Prior to acid challenge, fluoride-only dentin windows and control windows manifested more complex curves. However, after three days of demineralization, all curves exhibited similar shapes, and all the samples with added fluoride have a lower temperature rise than the control. From these curves, we calculated Q , or the area under the time-

temperature curve from initial turning-on of air nozzle to time equals 30 seconds. Overall, temperature differences during dehydration increased after 3 days of demineralization.

4. DISCUSSION

In all, different laser treatments dramatically influenced the surface morphology and permeability of both enamel and dentin. Er:YAG and UV lasers, showed marked differences in surface roughness without melting the enamel or dentin. Er:YAG irradiation led to significantly rougher enamel and dentin surfaces and led to higher permeability. Topical fluoride induced changes in the permeability of dentin, but not enamel. CO₂ laser treatment principally gave the best results for the inhibition of demineralization.

We hypothesized that Er:YAG laser and UV laser irradiation of enamel and dentin surfaces will expose the mineral structure by removing water and proteins, respectively, to increase fluoride incorporation. Subsequent CO₂ laser irradiation melts the surface to incorporate the applied fluoride. We hypothesized that this process will increase acid resistance however no enhancement of acid resistance was observed for the multiple laser treatments. The results here also further suggest that UV laser irradiation followed by fluoride application might also decrease permeability upon acid challenge, especially on dentin surfaces; however, the results were not statistically significant. In our previous study of the inhibition of demineralization by the UV laser and fluoride, the reduction in the rate of surface dissolution by fluoride alone was only 10% while the reductions were more than 50% for the UV laser + fluoride¹⁵. It is interesting that we did not observe a similar enhancement of the efficacy of fluoride with the Er:YAG laser. This suggests that simply roughening the surface without melting the enamel is not sufficient. These results also demonstrate that the inhibition of demineralization is dominated by the thermally induced mineral changes by CO₂ laser irradiation.

Acknowledgments

The authors acknowledge the support of NIH grants R01-DE14698 and R01-19631.

References

1. Chauncey HH, Glass RL, Alman JE. Dental caries. Principal cause of tooth extraction in a sample of US male adults. *Caries Res.* 1989; 23:200–205. [PubMed: 2661000]
2. Lm, Kaste LM, et al. Coronal caries in the primary and permanent dentition of children and adolescents 1–17 years of age: United States, 1988–1991. *J Dent Res.* 1996; 75(Spec No):631–641. [PubMed: 8594087]
3. Winn DM, et al. Coronal and root caries in the dentition of adults in the United States, 1988–1991. *J Dent Res.* 1996; 75(Spec No):642–651. [PubMed: 8594088]
4. Meurman JH, Hemmerlé J, Voegel JC, Rauhamaa-Mäkinen R, Luomanen M. Transformation of hydroxyapatite to fluorapatite by irradiation with high-energy CO₂ laser. *Caries Res.* 1997; 31:397–400. [PubMed: 9286525]
5. Fowler BO, Kuroda S. Changes in heated and in laser-irradiated human tooth enamel and their probable effects on solubility. *Calcif Tissue Int.* 1986; 38:197–208. [PubMed: 3011230]
6. Dg N, Wl J, Jd F. Laser irradiation of human dental enamel and dentine. *N Z Dent J.* 1986; 82:74–77. [PubMed: 3461347]

7. Featherstone JDB, Barrett-Vespone NA, Fried D, Kantorowitz Z, Seka W. CO₂ Laser Inhibition of Artificial Caries-like Lesion Progression in Dental Enamel. *J Dent Res*. 1998; 77:1397–1403. [PubMed: 9649168]
8. Hsu DJ, Darling CL, Lachica MM, Fried D. Nondestructive assessment of the inhibition of enamel demineralization by CO₂ laser treatment using polarization sensitive optical coherence tomography. *J Biomed Opt*. 2008; 13:054027-054027–9. [PubMed: 19021407]
9. Fried D, Featherstone JDB, Visuri SR, Seka WD, Walsh J, Joseph T. Caries inhibition potential of Er:YAG and Er:YSGG laser radiation. *Proc SPIE Vol*. 1996; 2672:73–78.
10. Apel C, Schäfer C, Gutknecht N. Demineralization of Er:YAG and Er, Cr:YSGG Laser-Prepared Enamel Cavities in vitro. *Caries Res*. 2003; 37:34–37. [PubMed: 12566637]
11. Kwon YH, Lee JS, Choi YH, Lee JM, Song KB. Change of enamel after Er:YAG and CO₂ laser irradiation and fluoride treatment. *Photomed Laser Surg*. 2005; 23:389–394. [PubMed: 16144482]
12. Tagomori S, Morioka T. Combined Effects of Laser and Fluoride on Acid Resistance of Human Dental Enamel. *Caries Res*. 1989; 23:225–231. [PubMed: 2790854]
13. Fox JL, et al. Combined Effects of Laser Irradiation and Chemical Inhibitors on the Dissolution of Dental Enamel. *Caries Res*. 1992; 26:333–339. [PubMed: 1468096]
14. dos Santos MN, Featherstone JDB, Fried D. Effect of a new carbon dioxide laser and fluoride on sound and demineralized enamel. *Proc SPIE Vol*. 2001; 4249:169–174.
15. Wheeler CR, Fried D, Featherstone JDB, Watanabe LG, Le CQ. Irradiation of dental enamel with Q-switched $\lambda=3355$ -nm laser pulses: Surface morphology, fluoride adsorption, and adhesion to composite resin. *Lasers Surg Med*. 2003; 32:310–317. [PubMed: 12696100]
16. Gao XL, Pan JS, Hsu CY. Laser-Fluoride Effect on Root Demineralization. *J Dent Res*. 2006; 85:919–923. [PubMed: 16998132]
17. Steiner-Oliveira C, Nobre-dos-Santos M, Zero DT, Eckert G, Hara AT. Effect of a pulsed CO₂ laser and fluoride on the prevention of enamel and dentine erosion. *Arch Oral Biol*. 2010; 55:127–133. [PubMed: 20031117]
18. Le CQ, Fried D, Featherstone JDB. Lack of dentin acid resistance following 9.3 μ m CO₂ laser irradiation. *Proc SPIE Vol*. 2008; 6843:J1–5.
19. Kaneko, K., Matsuyama, K., Nakashima, S. Proceedings of the 4th annual Indiana conference. Indiana University School of Dentistry; Indianapolis, Indiana: 1999. Quantification of early carious enamel lesions by using an infrared camera in vitro; p. 83-100.
20. Zakian CM, Taylor AM, Ellwood RP, Pretty IA. Occlusal caries detection by using thermal imaging. *J Dent*. 2010; 38:788–795. [PubMed: 20599464]
21. Usenik P, Bürmen M, Fidler A, Pernuš F, Likar B. Near-infrared hyperspectral imaging of water evaporation dynamics for early detection of incipient caries. *J Dent*. 2014; 42:1242–1247. [PubMed: 25150104]
22. Lee RC, Darling CL, Fried D. Assessment of remineralization via measurement of dehydration rates with thermal and near-IR reflectance imaging. *J Dent*. 2015; 43:1032–1042. [PubMed: 25862275]
23. Lee RC, Darling CL, Fried D. Assessment of remineralized dentin lesions with thermal and near-infrared reflectance imaging. *Proc SPIE Vol*. 2016; 9692:1–5.
24. Lin M, Liu QD, Xu F, Bai BF, Lu TJ. In vitro investigation of heat transfer in human tooth. *Proc SPIE Vol*. 2009; 7522:1–7.

Fig. 1. Workflow of the experimental setup. APF = Acidulated Phosphate Fluoride, NIR = near-infrared.

Fig. 2. Images of a Er:YAG-treated and UV-treated samples of enamel (top) and dentin (bottom) under visible light reflectance after laser and fluoride treatments and 3 days of demineralization (scale-bar = 1 mm).

Fig. 3.

Average of NIR reflectance dehydration intensity curves for enamel samples (Er:YAG: $n = 12$, UV: $n = 12$). Left: no acid challenge; Right: after 3 days of demineralization. The intensity differences between the initial and midpoint NIR reflectance images, $I(t=30)$, for each window were calculated using $I_{30} - I_0$, where I_{30} is the mean intensity at $t = 30$ seconds and I_0 is the mean intensity prior to turning on the air nozzle.

Fig. 4.

Average of thermal dehydration time-temperature curves for dentin samples (Er:YAG: $n = 12$, UV: $n = 12$). The area enclosed by the time-temperature curve, Q , from initiation of air nozzle to $t = 30$ seconds were calculated.