

Changes in chemical composition and collagen structure of dentine tissue after erbium laser irradiation

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Received 1 June 2004; received in revised form 29 September 2004; accepted 30 September 2004

Abstract

Erbium laser radiation has a great affinity for the water molecule, which is present in quantity in biological hard tissues. The objective of this work is to identify chemical changes by infrared spectroscopy of irradiated dentine by an Er:YAG-2.94 μm laser. The irradiation was performed with fluences between 0.365 and 1.94 J/cm². For the infrared analysis a Fourier transform infrared spectrometer was used. After the irradiation were observed: loss of water, alteration of the structure and composition of the collagen, and increase of the OH⁻ radical. These alterations can be identified by a decrease in intensity of the water band between 2800–3800 cm⁻¹, OH⁻ band at 3575 cm⁻¹ and bands ascribed to organic matrix between 2800–3400 cm⁻¹ and 1100–1400 cm⁻¹.

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Keywords: FTIR; Spectroscopy; Water caries prevention; Infrared

1. Introduction

Lasers with emission in the infrared region are used in many odontological procedures and laser–tissue interaction research. Considering only applications, which involve hard dental tissue irradiation, laser radiation can be used in dental cavities preparation [1], dentinal hypersensitivity reduction [2], intracanal bacterial reduction [3], caries prevention [4,5] and tooth bleaching [6].

An erbium laser application to dentine tissues in a sub-ablative regime can be an intracanal irradiation in order to obtain a bacterial reduction. The morphology of the intracanal surface was studied in previous work [3] and showed a major exposition of the dentinal tubules and a smoother surface after the erbium irradiation. The bacterial reduction is possible because the temperature at the surface is around 300 °C and at the periodontal tissue below 7 °C [7]. Besides these morphological evaluations it is necessary to determine

the chemical and physical changes produced in the irradiated tissues or in the adjacent regions. Infrared spectroscopy shows a possibility to identify chemical changes in the water content, organic and mineral matrices after the laser irradiation.

The objective of this work is to identify alterations of the chemical composition of dentine after subablative and, in part ablative erbium laser irradiation. The results of this study will have great importance in the development of clinical erbium laser applications.

2. Materials and methods

The sample used in this work was bovine incisor; after extraction the teeth were stored in sodium chloride solution at 0.9 wt.% until sample preparation and the experimental procedures. The samples were prepared in slices and not pulverized in order to preserve the natural characteristics of the tooth in an oral cavity, and also to permit the laser irradiation. In the first step, the teeth were cut longitudinally into slices

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of 0.5 mm thickness using a diamond blade system; the slices were sanded with a diamond paste to thickness below 80 μm ; for this work a very thin slice ($\sim 50 \mu\text{m}$) was selected in order to decrease the absorption signal in the infrared region and thus to avoid the infrared signal saturation. The sample irradiation was performed with an erbium laser (Er:YAG) with emission at $2.94 \mu\text{m}$ (3401 cm^{-1}), pulse width of 400 μs , and repetition rate of 2 Hz. To irradiate the entire sample area (diameter $\sim 2 \text{ mm}$) 1 up to 20 pulses were applied onto the surface.

Fourier transform infrared spectrometer (Magna-IR System 850 series II, Nicolet, Madison, United States of America) was used. The spectral region analyzed was between 400 and 4000 cm^{-1} (2.5 and $25 \mu\text{m}$), and a sample holder with specific diameter holes was used in order to select the region in the irradiated sample. The spectra were registered in the transmission mode with 0.482 cm^{-1} resolution.

The spectral acquisition was alternated with the sample irradiation—first the spectrum of the non-irradiated sample was acquired, then sequentially the irradiation was applied to the sample, from the least fluency successively up to 1.94 J/cm^2 . The same sample was used for all the applied fluences: 0.365, 0.651, 0.937, 1.271, 1.581 and 1.94 J/cm^2 . To obtain the same thermal dissipation as in clinical application the irradiation was carried out with the sample on a dentine block. No thermal paste was applied between the sample and the block to avoid possible chemical contamination of the sample.

Before the measurement was made in the spectrometer, a dry air flux for 20 min was applied to remove the ambient water vapor and CO_2 gas.

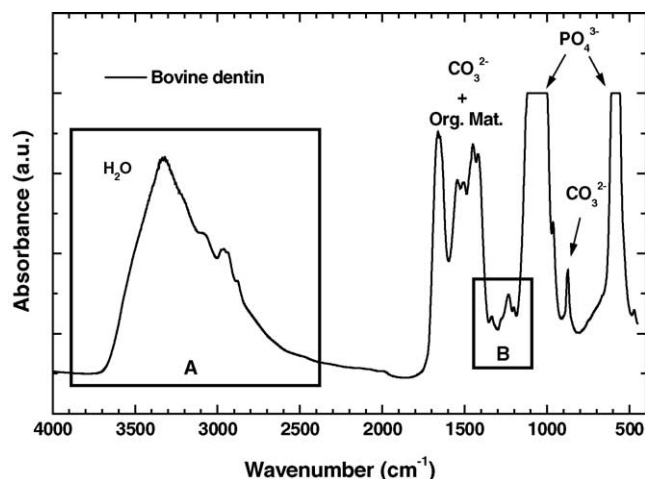


Fig. 1. Infrared absorption spectrum of bovine dentine near $400\text{--}4000 \text{ cm}^{-1}$ with the intense bands identified as: a broad water band near $2500\text{--}3700 \text{ cm}^{-1}$; carbonate radical near $1400\text{--}1700 \text{ cm}^{-1}$ and at 875 cm^{-1} ; organic matrix bands near $2800\text{--}3400 \text{ cm}^{-1}$ and $1100\text{--}1400 \text{ cm}^{-1}$; phosphate radical bands near $950\text{--}1150 \text{ cm}^{-1}$ and at 600 cm^{-1} ; the two regions marked by the boxes show changes after erbium laser irradiation and can be better visualized in Fig. 2 (region A) and in Fig. 3 (region B).

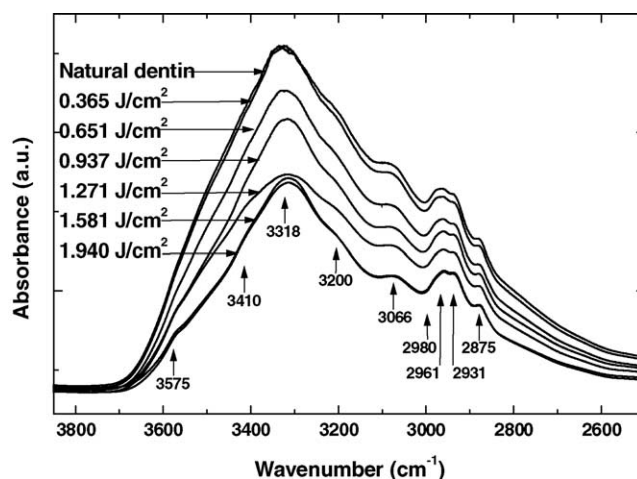


Fig. 2. Infrared absorption spectra near $2500\text{--}3800 \text{ cm}^{-1}$ of natural dentine and after erbium laser irradiation with fluences up to 1.94 J/cm^2 ; the arrows indicate the absorption band positions which are assigned to water vibration modes, OH^- stretching, amide A, amide B and C–H stretching (see Table 1).

3. Results

The absorption spectra (Fig. 1) of the tissues result from the sum of the absorption bands of the chemical compounds present in them. The dentine spectrum between 2.5 and $25 \mu\text{m}$ is composed of bands from water; from mineral matrix: phosphates, carbonates and hydroxyls; and from organic matrix: collagen and non-collagen proteins. In Fig. 1 the spectrum of bovine dentine near $400\text{--}4000 \text{ cm}^{-1}$ can be seen. In these spectra the more intense bands are indicated: a broad water band near $2500\text{--}3700 \text{ cm}^{-1}$; carbonate radical and organic material bands near $1400\text{--}1700 \text{ cm}^{-1}$ and at 875 cm^{-1} ; phosphate radical bands near $950\text{--}1150 \text{ cm}^{-1}$ and around 600 cm^{-1} ; for more details see reference [8].

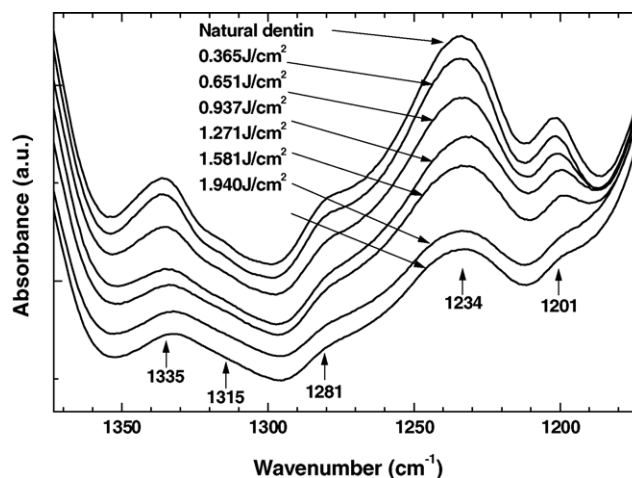


Fig. 3. Infrared absorption spectra near $1100\text{--}1400 \text{ cm}^{-1}$ for natural dentine and after erbium laser irradiation with fluences up to 1.94 J/cm^2 . The arrows indicate the absorption band positions which are assigned to collagen protein (see Table 2 and Table 3).

Table 1

Position of infrared absorption bands of bovine dentine near 2500–3800 cm^{-1} acquired from Fig. 2. Comparison with known water and collagen absorption bands and their respective assigned vibrational modes is also done

Dentine (cm^{-1}) (this work)	Literature (cm^{-1})		Assignments [9] [10] [11]
	Water [9]	Collagen [10] [11]	
3575	—	—	OH^- stretching (ν_1)
3410	3400/3490	—	(ν_3) Water mode
3318	3280	3330/3293	($\nu_1 + 2\nu_2$) Water mode or amide A
3200	—	—	—
3066	—	3075/3067	Amide B
2980	—	—	—
2961	—	2960	—
2931	—	2925	C—H stretching
2875	—	2875	—

After erbium laser irradiation significant changes in the absorption bands of the dentine tissue were observed in two regions indicated in Fig. 1: near 2600–3800 cm^{-1} (region A) and near 1170–1370 cm^{-1} (region B). The two mentioned regions can be better visualized in Fig. 2 and Fig. 3, respectively, for the natural sample and after irradiation with all the fluences. Besides the mentioned changes, the amide II band at 1545 cm^{-1} showed an area reduction also, but no conclusions could be drawn because a large noise signal was present in this spectral region.

In Fig. 2 (2600–3800 cm^{-1}) can be seen the spectra of natural and irradiated dentine, the absorption bands are indicated by arrows. These bands, with their assignments to chemical compounds, are compared with literature values at Table 1 [9–11]. The band positions indicated in Fig. 2 and listed in Table 1, are precisely determined from the first derivative spectrum (not shown). The amide A band is ascribed to the stretching vibrations of NH-groups involved in interchain hydrogen bonding and the amide B to the amide II (N–H bending and C–N stretching) overtone [11,12].

In Fig. 2 can observe the broad water band decreasing after laser irradiation with increasing fluence. The water loss (normalized values) is visualized in Fig. 4, where the area

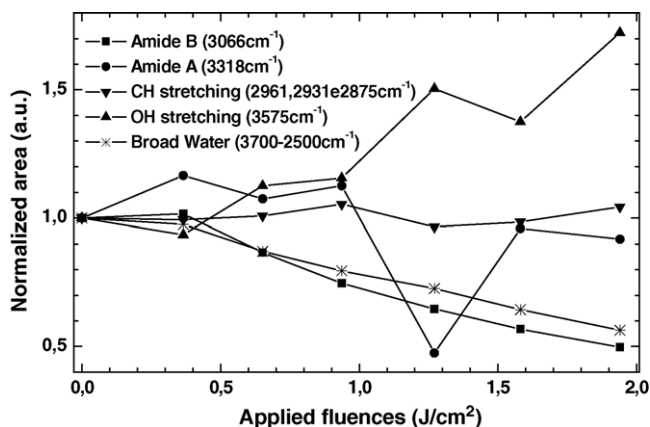


Fig. 4. Normalized area from dentine absorption bands of natural and erbium laser irradiated tissue with different fluences: near 2120–3760 cm^{-1} (broad water band); at 3575 cm^{-1} (OH^- stretching); at 3318 cm^{-1} (amide A or water) at 3066 cm^{-1} (amide B); sum of 2961, 2931 and 2875 (CH stretching).

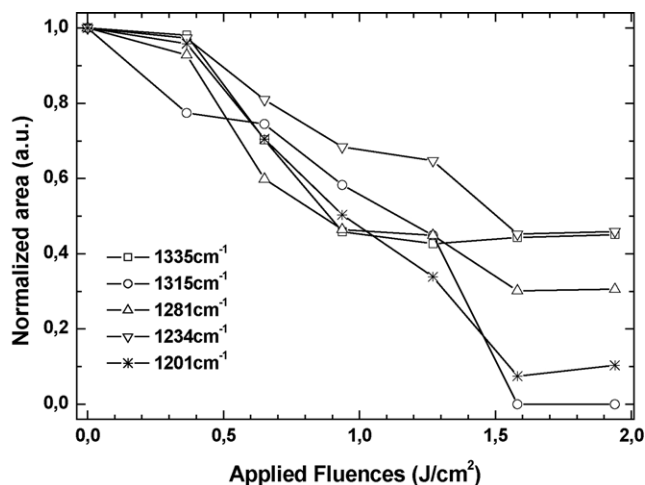


Fig. 5. Normalized area from dentine absorption bands of natural and erbium laser irradiated tissue with different fluences: at 1335 cm^{-1} (CH_2 deformation), 1315 cm^{-1} (CH_2 wagging or $\text{C}(\text{CH}_2)$ twisting), 1281 or 1234 cm^{-1} (amide III) and 1201 cm^{-1} (CC ring stretching); for precise assignment see Table 2 and 3.

under the broad water band is plotted against the applied fluences. The same figure are showed the band area (normalized values) versus laser fluences for the band at 3575 cm^{-1} (OH^- stretching), at 3318 cm^{-1} with possible association to amide A or water; at 3066 cm^{-1} assigned to amide B and the bands at 2961, 2931 and 2875 cm^{-1} assignment to C–H stretching.

In Fig. 3 it can be observed near 1110–1400 cm^{-1} in the natural tissue and the sample irradiated with different fluences. For this region the bands are assigned only to organic material, and a concordance between the band positions of different tissues occurs [13,14]. The normalized area of all the bands for this region (Fig. 5) decreased with increasing fluence.

4. Discussion

The region (2500–3800 cm^{-1}) shows overlapping of water, organic material and OH^- bands. Some observed bands

Table 2

Position of infrared absorption bands of bovine dentine near 1100–1400 cm^{-1} acquired from Fig. 3; a comparison with collagen absorption bands from rat skin and human bone can be made

Dentine (cm ⁻¹) (this work)	Literature (cm ⁻¹) [13]				
	Rat skin		Human bone		Assignments
Slice	Powder	Slice	Powder	Slice	
1335	1334	1337	1334	1342	Protein structure
1315	–	1306	–	–	CH ₂ wag
1281	1277	1281	1276	–	Protein structure
1234	1233	1232	1238	1233	CONH (amide III), CN stretching and NH deformation.
1201	1206	1201	–	1204	Protein structure

are not exactly assigned and the evaluation after laser irradiation is difficult. However the bands ascribed to OH^- and C–H stretching, as well as the water band are identified and its behavior after irradiation is very clear. Otherwise, the amide A and amide B bands show weak and broad bands near 3066 and 3318 cm^{-1} , respectively. Nevertheless, we identify the behavior of the amide A and B after Er:YAG laser irradiation with different fluences. Therefore, the following discussion about organic matrix in erbium irradiated dentine is not based only on the behavior of amide A and B but mainly on the C–H bounds, amide III and other bands ascribed to the collagen molecule CH_2 deformation, $\text{C}(\text{CH}_2)$ twisting, CC ring stretching.

The determined band positions can be compared in Table 2 with the collagen absorption band values from rat skin and human bone, and in the Table 3 with nematode cuticle [15], human stratum corneum, pig and goat tissue [14].

The assignments in Table 2 [13] identify the CH_2 wag, amide III and three bands that the authors do not assign to a chemical vibration mode, but only attribute to the collagen structure. Otherwise at the Table 3 [14] the authors identify similar absorption bands and give a more precise assignment. The three bands (1201, 1281 and 1335 cm^{-1}) that were attributed to the collagen structure [13] are better assigned respectively to CH_2 deformation, amide III and CC stretching [14].

In the literature, the amide III was first attributed to the band near 1233 cm^{-1} [13] and more recently to the band near 1281 cm^{-1} [14]. In this work, we will consider the assignment of the more recent publication, which correlates the amide III to the band near 1281 cm^{-1} [14]. The bands

between 1100 and 1400 cm^{-1} are sensitive to the molecular conformation and this spectral region is also called “finger-print” region, because changes in the bands are attributed to different conformations, which the same molecule can show. It is important to note that, despite literature assignment diverges for some bands, changes in its intensity and position are attributed to changes in the collagen molecule conformation.

In the literature the maximum temperature rise of the hard dental tissue during erbium laser irradiation, with fluences below 7 J/ cm^2 , is reported to be approximately 300 °C on the surface and values below that maximum in subsurface layers [16]; these values can change for different tissues and laser parameters. For this reason, the infrared signal registered in our experiment is a composition of signals from the first layer, submitted to temperatures near 300 °C, and from sub-surfaces layers, submitted to lower temperatures. Therefore, the final infrared signal is a mean of tissue submitted to higher temperatures (~ 300 °C) and tissue submitted to lower values. As a consequence the observed results indicate a partial collagen degradation. In our results, despite it is not possible to distinguish the changes at the surface from the sub-surfaces layers, we can infer that the changes, collagen degradation, loss of water and OH^- increase, are higher at the surface and lower at sub-surfaces regions.

In thermal analysis the first chemical compound released from the tissue upon heating is water. The literature shows that the adsorbed water is totally released from the tissue at 200 °C [17], the remaining water is bonded to the tissue structure and is released only when heated between 400 and 1300 °C [18][19]. The different thermal stability of the water

Table 3

Comparison of dentin absorption bands (1100 and 1400 cm^{-1}) with fresh nematode cuticle, human stratum corneum, pig and goat tissue; at the right column are listed the approximate description of vibration mode

Dentine (cm^{-1}) (this work)	Literature (cm^{-1})				Assignments [14]
	Nematode cuticle [15]	Hum. stratum corneum [14]	Pig tissue [14]	Goat tissue [14]	
1335	1337	1336	1336	1338	CH_2 deformation
1315	1315	1296	1299	1295	$\text{C}(\text{CH}_2)$ twisting
1281	1281	1274	1276	1275	CONH (amide III): CN stretching and NH deformation.
1234	1237	–	–	–	–
1201	1206	–	1208	–	CC ring stretching

is attributed to different bond energies between the molecule and its site in the tissue [20].

In the temperature range (100–400 °C) the organic material of the dentine is released and a maximum loss around 320 °C is identified [21]. Another compound that is released from the tissue is the carbonate radical; in the dentine tissue the loss starts at 100 °C, is more intensive at 700 °C, and around 1000 °C it is totally released from the tissue [22].

The thermal analysis results from the literature show three chemical compounds set which are sensitive to heat treatment below 300 °C. Beyond direct erbium laser radiation absorption by the water, the consequent temperature elevation in adjacent tissue regions produces a similar chemical compound loss as observed in thermal analysis. In this work, only changes in the organic material and water were observed. The carbonate radical concentration after the irradiation was evaluated from the band area at 875 cm^{-1} , but no significant changes were observed for the analyzed fluences.

The observed effects after laser irradiation and heating the tissue to temperatures below 300 °C are probably responsible for the dentin discoloration (browning) [23]. This discoloration is not exactly determined but some models are proposed. Otherwise, in heated enamel, the discoloration (opaque) is attributed to the water loss [24].

Type I collagen molecules in soft tissues denature irreversibly at 43–60 °C [25] whereas the calcified collagen molecules in bone break down approximately at 150 °C [26]. Differences between the properties of dentinal or collagen and soft tissue collagen are attributed to differences in terms of an additional level of stabilization in the structure of dentine collagen [27,28].

The collagen fibrils in soft tissue are highly orientated in directions parallel to one another, so as to form bundles at increasing orders of size. In bone and dentine, however, fibrils are arranged more haphazardly so as to form more tightly knit structures. The presence of enzymatic and non-enzymatic cross-links alters the structural stability of collagen [29], the higher thermal stability of collagen in hard tissues is probably a consequence of this. No direct comparisons of dentine and bone collagens appear to have been made, but such evidence suggests that dentine collagen is more highly cross-linked; therefore, the denaturation temperature of dentine would be little higher than the bone denaturation temperature. Another feature that results in increased thermal stability of the collagen molecule in hard tissues is its interaction with mineral crystals [30], in other words, the degree of mineralization influences the collagen temperature denaturation of the tissue. Therefore, in our samples irradiated with erbium laser, the reached temperature, values between 300 °C and ambient temperature, denatures partially the collagen structure of the sample, as showed by the behavior of the organic matrix infrared bands.

The thermal effect observed after the irradiation of any visible or infrared high intensity laser originates from the laser radiation absorption by some chemical compounds in the tissue and the consequent conversion to thermal energy; in

this work the erbium laser, which is highly absorbed by water, was used. Other lasers such as CO₂ with wavelengths between 8–11 μm , Ho:YLF or Ho:YAG lasers with emission around 2 μm , Nd:YAG or Nd:YLF lasers with emission around 1 μm , other erbium lasers, Er, Cr:YASGG at 2.78 μm or visible lasers can produce similar effects to those observed in this study. All the lasers that produce some thermal effect can induce alterations in the physical and chemical composition such as observed in this work after the Er:YAG irradiation. The intensity and extension of the effects will depend on the laser parameters: fluence, frequency, wavelength; and the tissue optical and thermal characteristics.

The two highest fluences (fluences above the ablation threshold) produced tissue removal. Above the ablation threshold the irradiation reduces the thickness of the sample and consequently contributes to sharpening the water and organic material bands.

Correlation of this work with results in vivo or in vitro experiments needs some consideration, because differences between such experiments and the present study occur. Upon irradiating the dentine tissue of a tooth inserted in the alveolar cavity, the maximum temperature rise in the superficial and sub-superficial layers is lower than the temperature during in vitro irradiation. This lower temperature results from the large thermal dissipation that occurs in the tissue when the tooth is whole and inserted in the alveolar cavity. The effects observed in this work are mainly a function of the temperature produced by the laser; if a major thermal dissipation occurs in vivo, the effects will happen in the same form but with lower intensity and more restrict to the irradiated local.

The erbium laser as well as all the solid-state lasers applicable in odontological procedures operate normally with the beam composed majority with a fundamental mode. The intensity transversal profile of this laser beam is composed with a high intensity values at the center region and lower intensity values at the surrounding regions [31]. As a consequence the resulting effects produced on the tissue will following this beam intensity profile. This dependence between intensity and resulted effects will produce a inhomogeneous on the irradiated tissue surface. Beyond this intensity profile inherent to the laser beam, a reduction of the temperature values on the tissue occurs also as a function of the distance from the irradiated local. With these inhomogeneous effect on the laser intensity and generated temperature on the analyzed sample, the observed effects in this work (water loss, organic matrix degradation and OH⁻ increase) will be more intense in the central irradiated area (produced with one laser pulse) and on the superficial tissue layer; and less intense in the surrounded irradiated area and deeper tissue layers.

The erbium laser (2.94 μm) produces changes in the composition and conformation of the organic matrix (collagen), OH⁻ radical and the water present in the dentine after irradiation with fluences below 1.9 J/cm². These changes are identified by the decrease of the water band near 2800–3800 cm^{-1} , amide B at 3066 cm^{-1} , CH₂ deformation at 1335 cm^{-1} , CH₂ wagging or C(CH₂) twisting at 1315 cm^{-1} , amide III at 1281

or 1234 cm^{-1} , CC ring at 1201 cm^{-1} and the increase of the OH^- stretching band at 3575 cm^{-1} .

Acknowledgments

We thank the Centro de Lasers e Aplicações - CLA of the Instituto de Pesquisas Energéticas e Nucleares IPEN and the Institut für Lasertechnologien in der Medizin und Messtechnik-ILM of the Ulm Universität for technical support and facilities. FAPESP, CNPq and RHAE supported this research.

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