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Collagen absorption bands in heated and rehydrated dentine

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Abstract

The objective of this work is identifying changes in the collagen bands in heated and rehydrated dentine. We use bovine dentine slices that were heated in oven between 100 and 300 °C. The sample hydration was conducted in sodium chloride solution at 0.9 wt.%; the spectra were acquired by a Fourier transform infrared spectrometer in the spectral range of 4000–400 cm^{-1}. Our results show a temperature range ($T \leq 175$ °C) where the dentinal collagen can be partially denatured and reverted to initial conformation; a second region (175 °C < $T \leq 225$ °C) where this process occurs partially and a third region ($T > 225$ °C) where the collagen is denatured and no reversion is observed after rehydration. This work identifies an important characteristic that dentinal collagen can assume when the tissue is heated and rehydrated; these results indicate the denaturation temperature of dentinal collagen to be near 175–200 °C.

Keywords: Dentine; Collagen; FTIR; Spectroscopy; Denaturation; Hydration

1. Introduction

The dentine vibrational spectrum (4000–400 cm^{-1}) is mainly composed of water, hydroxyapatite and organic matrix bands [1]. The hydroxyapatite bands are originated from hydroxyl, carbonate and phosphate groups and the major organic matrix bands arise from the groups presented in the collagen molecule.

The bands in spectral region between 1400 and 1100 cm^{-1} are sensitive to the collagen molecular conformation and is also called "fingerprint" region, because changes in bands of this region are attributed to different conformations of a same molecule [2]. The collagen molecule consists of three polypeptide chains; each one is twisted in a self-axis with a pitch of about 9.3 Å and three amino acids units per turn [3]. Each twisted chain is twisted again with a longer pitch, at about 28.6 Å and 10 amino acids per turn. Finally, the arranged three polypeptide chains, with a twisted phase of 120° between each chain, build the collagen "super-helix". The molecule is a rod-like structure with length of about 330 nm and diameter of about 1.35 nm. Packing a large number of molecules builds up a fibril with undefined length and diameter of about 100–200 nm.

The collagen band assignment for the region between 1400 and 1100 cm^{-1} was previously discussed [4,5] and the most acceptable assignments for dentine are: CH_2 deformation at 1335 cm^{-1}; $C(CH_2)$ twisting at 1315 cm^{-1}; CN stretching and NH deformation at 1281 cm^{-1}; no assignment for 1234 cm^{-1} and CC ring stretching for 1201 cm^{-1}. In this spectral region, the intensity of dentinal collagen bands has been reduced after irradiation with Er:YAG laser (radiation exposure less than 2 J cm^{-2}), while at the same radiation exposure the CH covalent bonds stay unchanged [4], indicating changes in the collagen conformation after laser irradiation but the preservation of the CH covalent bonds.

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This difference occurs because the Er:YAG laser irradiation with 2 J cm^{-2} produces a temperature rise to below 300 °C [6], therefore the temperature reached is underneath the organic material degradation maximum, which occurs in dentine at about 310 °C [7].

The Er:YAG irradiated samples at previous work [4] were stored in sodium chloride solution, and it was observed that the infrared absorption bands, between 1400 and 1100 cm^{-1}, were restored after rehydration (not published). Consequently, we expect that changes in collagen structure, which occurs with heating, can be reverted to natural conformation after rehydration. Therefore, the objective of this work is to identify the bands reduction after heating ($T < 300$ °C) and the reversion after rehydration, thus determining at which temperature range this reversion can occur.

2. Materials and methods

Bovine incisors were selected for this work; the teeth were kept immediately after extraction in sodium chloride (NaCl) solution at 0.9 wt.% of concentration. The teeth were free of caries, pigments or other dental anomalies. The roots were cut in slices of about 0.5 mm using a diamond blade system and then sanded down to thickness about 100 μm. Nine slices were prepared and each one will be heated to a specific temperature value.

The spectral acquisition was conducted in a Fourier transform infrared spectrometer (MB-Series, Bomem Hartmann & Braun, Quebec, Canada). The spectral range of 4000–400 cm^{-1} (2.5–25 μm) was analysed and registered in the transmission mode with resolution of 2 cm^{-1}. As our interest was only the evaluation of dentine organic matrix, we registered the spectral region between 1400 and 1100 cm^{-1}. The area under the bands was determined after subtracting a straight line (background elimination). The graphics of normalized area were composing dividing the area of heated sample by the area of natural sample.

The samples were heated in an oven by heat treatment temperatures (HTTs) between 100 and 300 °C at steps of 25 °C. A spectrum of each sample was recorded before heating. After heating (30 min) the samples were cooled down at ambient temperature for ∼15 min and then analysed in the spectrometer. After the described measurements, the samples were rehydrated in NaCl solution and the spectrum of each sample was recorded again after 1–5 days of hydration.

3. Results

In Fig. 1, is possible to observe the region between 1370 and 1150 cm^{-1} of the spectrum of natural dentine with the bands that were monitored after heating and hydrating: 1201, 1234, 1281 and 1335 cm^{-1}. The band in 1315 cm^{-1} was not monitored because of its weak intensity. After heating the samples at 100 °C, a decrease in the intensities of the monitored absorption bands was observed. Fig. 2 shows that the observed decrease in intensity is strongly temperature dependent, reaching almost zero after 300 °C.

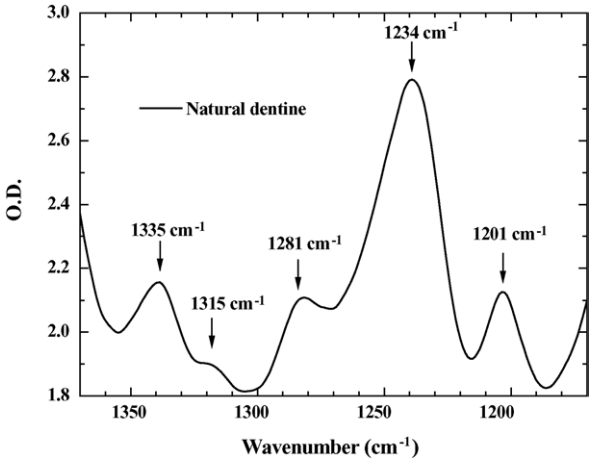

Fig. 1. Infrared absorption spectrum of natural dentine between 1370 and 1150 cm^{-1}, arrows indicate the absorption bands assigned to the organic matrix. The band at 1315 cm^{-1} was not monitored because it is very weak and difficult to identify after heating.

Shown in Figs. 3–8 are the normalized areas monitored after different HTTs and hydrating during 5 days. After hydration, the dentine bands can be classified as total reverted in samples heated with temperatures equal or below 175 °C; partially reverted in samples heated at temperatures between 200 and 225 °C; and non-reverted in samples heated with temperature equal or above 250 °C.

In the samples heated at 100 °C (Fig. 3), 125 °C (not shown), 150 °C (Fig. 4) and 175 °C (not shown) the intensities of the bands are reduced, by different proportion, and totally reverted by hydration to values near those observed before thermal treatment. The results for samples heated at 125 and 175 °C are not shown since they present the same behaviour as those heated at 100 and 150 °C.

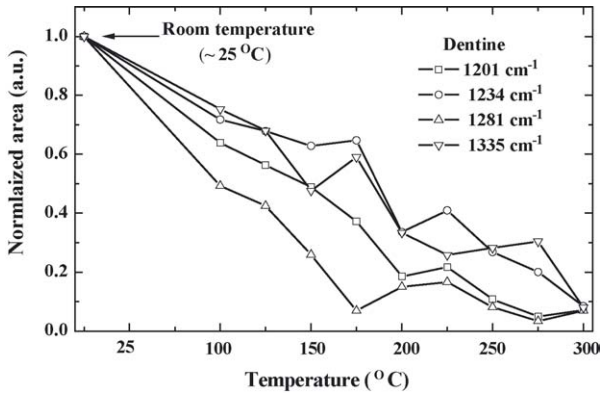

Fig. 2. Normalized area of the monitored bands as a function of the temperature of the thermal treatment (100–300 °C). It is possible to observe a linear behaviour on the area reduction of the monitored bands, reaching to values near zero after heating the tissue at 300 °C.

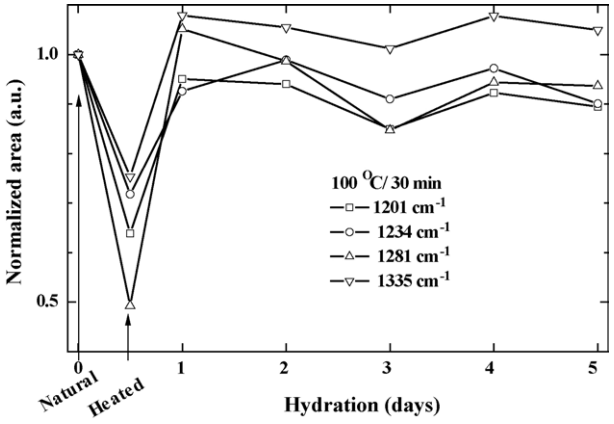

Fig. 3. Normalized area of dentine bands before heating, after heating at 100 °C (30 min) and after hydration in NaCl solution during 5 days. The area is reduced up to 50% of the initial observed area and after hydration all observed bands increase and return to similar values to those observed for unheated dentine.

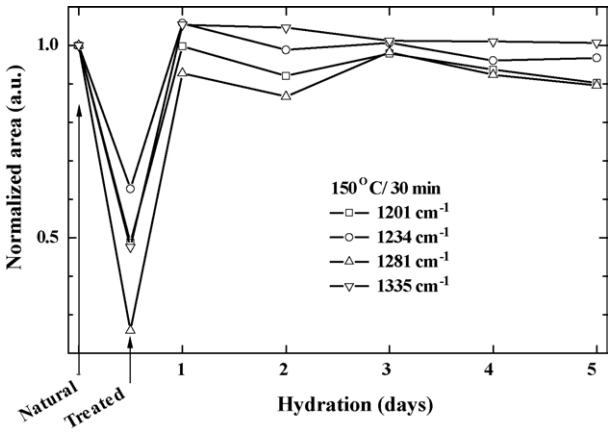

Fig. 4. Normalized area of dentine bands before heating, after heating at 150 °C (30 min) and after hydration in NaCl solution during 5 days. As described for the sample heated at 100 °C, at 150 °C the bands area are also reduced and after hydration returns to initial values. The same behaviour is observed for the sample heated at 125 and 175 °C (not shown).

A partial reversion to values before thermal treatment is observed in samples heated at 200 °C (Fig. 5) and 225 °C (Fig. 6). For these two HTTs, the intensity of the bands are reduced and even after 5 days of hydration they do not return to the initial values observed in untreated samples. At higher HTTs, only an accentuated reduction in the intensity of the monitored bands is observed, which stay constant up to the fifth day of hydration. Figs. 7 and 8 show the dentine samples heated at 250 and 275 °C, respectively; the sample heated at 300 °C shows a similar behaviour and is not presented.

4. Discussion

The intensities of the collagen bands decrease about 25–50% when the tissue is heated to 100 °C. The band inten-

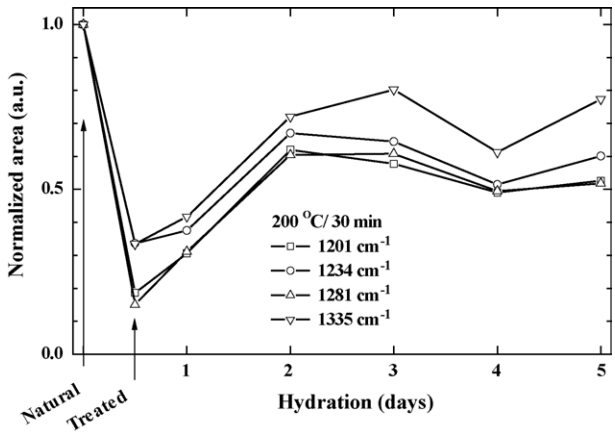

Fig. 5. Normalized area of dentine bands before heating, after heating at 200 °C (30 min) and after hydration in NaCl solution during 5 days. At this temperature the band areas are drastically reduced (about 20–30%) and after hydration the bands are partially reverted. It is possible to observe that after the second day the area is almost constant at about 60% of the initial area.

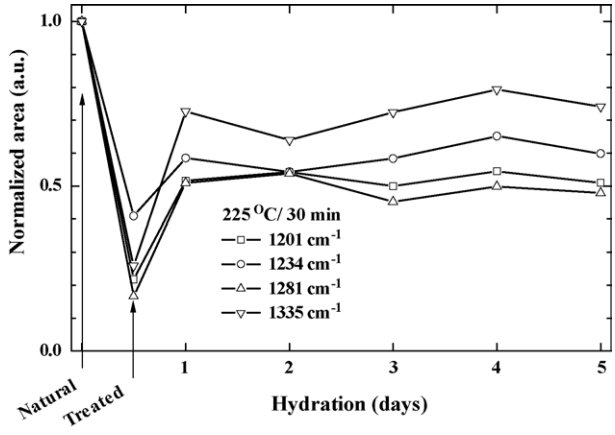

Fig. 6. Normalized area of dentine bands before heating, after heating at 225 °C (30 min) and after hydration in NaCl solution during 5 days. As described for the sample heated at 200 °C, here at 225 °C the behaviour is similar; the reversion is partial and to about 70–50% of the initial area values.

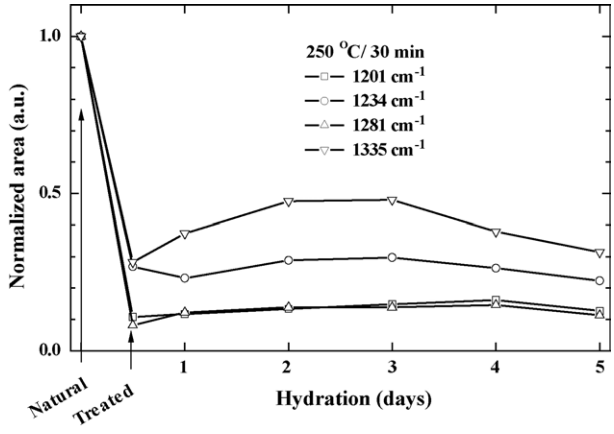

Fig. 7. Normalized area of dentine bands before and after heating at 250 °C (30 min) and after hydration in NaCl solution during 5 days. At 250 °C the bands are reduced at about 10–30% of the initial area and after hydration only a little reversion is observed.

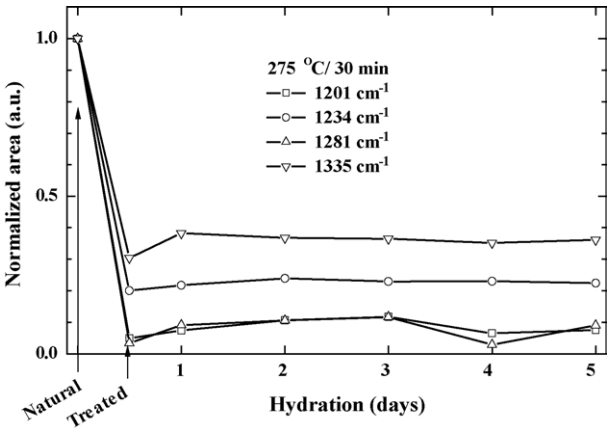

Fig. 8. Normalized area of dentine bands before, after heating at 275 °C (30 min) and after hydration in NaCl solution during 5 days. As described for the sample heated at 250 °C, the sample heated at 275 °C is also drastically reduced and it is not reverted. The sample heated at 300 °C shows the same behaviour.

sity at 1335 cm^{-1} (CH_2 deformation) reduces 25% and the band intensity at 1281 cm^{-1} (CN stretching and NH deformation) reduces 50%, while the other bands intensities show percental reductions between 25 and 50% when the tissue is heated to 100 °C. Increasing the HTT we observe a progressive reduction of the intensities of all collagen bands, reaching nearly zero when the tissue is heated at 300 °C (Fig. 2). In dentine, the organic matrix degradation occurs first at 310 °C [7], but the results of this work show that even below this temperature the monitored collagen bands are fallen to zero. Our results are in agreement with the literature because the value of 310 °C [7] is the temperature where the elimination of organic material from the tissue occurs.

The return after hydration of monitored bands can be classified in three temperature regions: (a) $T \leq 175$ °C: with total reversion of the bands; (b) 175 °C < $T \leq 225$ °C: with partial reversion; and (c) $T > 225$ °C: with non-reversion of the collagen bands.

The tissue heated to a temperature below 175 °C has its bands reduced after heating and reverted to the area observed in natural tissues soon after the first hydration day and the area keeps this value up to the fifth day. In the second temperature region (175 °C < $T \leq 225$ °C), the area is reduced more drastically after heating and with the first hydration day the reversion is partial and for the following days the area increases a little but does not reach to the initial area observed in natural tissues.

The polypeptide chain proximity in the molecule allows the formation of inter-chain bonds; the bonds can be weak hydrogen bonds or strong covalent bonds [3]. Though the hydrogen bonds are very weak, its presence in polypeptide chain occurs at each three amino acids, i.e., they are collectively sufficiently strong to hold the three chains together. Additional covalent cross-links that are also present in collagen (hydroxyproline, amide ester cross-links) will increase the structure stabilisation [8,9]. Additionally, cross-links between different molecules within a fibril will also stabilise the fibril structure.

Looking at the collagen molecule structure and the great quantity of possible hydrogen bonds that this molecule can produce at intra- and inter-molecular configuration, the water loss by heating will reduce the structure stabilisation and can then cause conformational changes. Therefore, the breakdowns of the hydrogen bonds are probably the main processes responsible for changing the collagen conformation. With less water content, the hydrogen bonds that determine the collagen alpha-helix structure stabilisation is lost. These bonds can be restored after rehydration and bring back the collagen structure as shown in the results for samples heated at temperatures below 175 °C. For HTTs between 175 and 225 °C the restitution of hydrogen bonds is probably partial and the collagen structure reversion is also partial.

Type I collagen molecules in soft tissues denature irreversibly at 43–60 °C [10], whereas the calcified collagen molecules in bone break down at approximately 150 °C [11]. Differences between the properties of dentinal or bone collagen and soft tissue collagen are attributed to differences in terms of an additional level of stabilisation in the structure [9,12]. Therefore, it is possible to observe collagen absorption bands in samples of heated dentine up to 175 °C. As the collagen molecules are stable at the temperature range below 175 °C, we can identify 175–200 °C as the denaturation temperature of the dentinal collagen. This value is a little higher than the denaturation temperature of the bone collagen, which is about 150 °C [11]. Considering that the dentinal collagen has higher cross-linkage than bone collagen [12], the higher observed denaturation temperature for dentine is in agreement, i.e., the higher cross-links in dentine will increase the collagen stabilisation and as a consequence the denaturation temperature will also increase.

In the present work was determined a temperature range ($T \leq 175$ °C) where the dentinal collagen can be partial denatured and reverted to initial conformation; and a second region (175 °C < $T \leq 225$ °C) where this process occurs partially. This work identifies an important characteristic that dentinal collagen can assume when the tissue is heated; the observed characteristics of the collagen molecule can be used in favour to clinical therapy.

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