Archives of Oral Biology (2004) 49, 233–238

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Archives of Oral Biology

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SHORT COMMUNICATION

Dental discolouration after thermal treatment

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Accepted 14 August 2003

KEYWORDS
Enamel; Dentin;
Discolouration;
Thermal treatment;
Laser irradiation

Summary Enamel and dentin discolouration have extrinsic and intrinsic origins. Possible causes include pigmented food or drink, caries, clinical chemical treatments, trauma and, high temperature. In the oral cavity, dental hard tissues can be heated when irradiated with high-intensity lasers. This paper, reports initial results on the discolouration of enamel and dentin induced by thermal treatment. The samples used in this work were bovine incisor teeth. Enamel and dentin discolouration were verified using microscopy and transmission spectroscopy. Thermal treatment was carried out at temperatures of 140 and 200 °C. The natural transparent aspect of the enamel became opaque after thermal treatment, it whitened following treatment at 140 °C, and turned completely opaque after treatment at 200 °C. With the same thermal treatment, dentin became light brown after treatment, at 140 °C, and the brown pattern was more evident after treatment at 200 °C. Although there is no conclusive evidence, non-enzymatic browning, collagen denaturation or oxidation of some chemical component of the dentin, may be intensified or produced by the thermal treatment. In enamel, water loss and the consequent increase in light scattering explain the observed opacity.

Introduction

Several authors have studied the discolouration of enamel and dentin, and have classified it as extrinsic and intrinsic.[1] Possible causes include pigmented food or drink such as coffee, tea or wine,[2] chlorhexidine treatment,[3,4] caries,[5] presence of metals such as iron,[6,7] haemoglobin[8,9] and temperature.[10,11] The literature does not discuss the origin of tissue discoloured by thermal treatment, but reports discolouration of light yellow to bluish-white passing through brown following thermal treatment between 150 and 700 °C.[10,11] In the oral cavity, the dental hard tissues can be heated when irradiated by high-intensity lasers.[12,13] When applying laser irradiation using safe parameters, the change in temperature in the pulp and periodontal tissues is below the damage threshold.[14–16] However, at the irradiated site, the temperature rises to higher values (300 °C).[17] For example, during enamel irradiation with an Er:YAG laser (7 J/cm^2), the surface temperature is around 300 °C, 4 ms after the laser stops, the temperature at the surface is still above 150 °C. Similar values are expected in irradiated dentin. These values only correspond to the surface tissue; in subsuperficial layers, the maximum temperature is always lower than the temperature at the surface; i.e. for the example above, the maximum temperature in the subsuperficial layers will be between the ambient temperature and 300 °C. This paper reports initial results on enamel and dentin discolouration induced by thermal treatment in bovine incisor teeth.

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0003-9969/$ – see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.archoralbio.2003.08.005

Materials and methods

The samples used in this work were bovine incisor teeth (animals approximately 2 years old), extracted and stored immediately in sodium chloride solution at 0.9 wt.% to keep their natural properties. For microscopy, tissue samples were selected from transverse cuts in the pulp region, and three slices were selected for each evaluated temperature (140 and 200 °C). For optical spectroscopy, dentin roots were cut longitudinally with a thickness of 500 μm using a diamond blade system. The slices were sanded with carbide sandpaper to a thickness of around 90 μm, and then polished with 0.3 μm diamond paste. For each temperature, three samples were tested and one of these spectra was selected. The absorbance spectra presented in this study were not corrected for slice thickness because all tested samples had similar values (90 μm). To minimise possible thermal effects during sawing and sanding of the samples, these procedures were conducted using water as irrigation.

A light microscope was used to verify the discolouration of enamel and dentin (Carl Zeiss, Germany). For additional analysis, an ultraviolet and visible transmission spectrometer (Cary-17, USA) was used in dentin. Thermal treatment was carried out at temperatures below 200 °C and, treatment times up to 6 h were used. In the oven, a glass plate was used to support the samples, and the temperature was monitored using a mercury bulb thermometer. For spectroscopic evaluation, samples treated at 140 and 200 °C were selected and kept in the oven for 1, 2 and 6 h. Microscopic analysis was performed on samples that had received the same thermal treatment as above, but for a 2 h period. After thermal treatment, samples were handled in air without rehydration. The time between thermal treatment and analysis by microscope or spectrometer did not exceed 15 min. The temperatures of 140 and 200 °C were chosen on the basis of the temperature rise produced by Er:YAG laser irradiation. Thermal treatments of longer than 6 h resulted in a very slow increase in absorbance, so this was not evaluated. For thermal treatments of less than 1 h, absorbance increased quickly and was not evaluated because the time necessary to cool and measure the sample (∼15 min) was too long compared with the aimed treatment time (values below 1 h), and could have led to errors in the results.

Results

Figure 1 shows enamel and dentin tissues before thermal treatment, and the transparent aspect of the enamel and the light yellow aspect of the dentin can be observed. Figures 2 and 3 show the enamel and dentin tissues after 2 h of treatment at 140 and 200 °C, respectively. The tissues expanded and contracted according to the heating or cooling applied. As the tooth slice is composed of two tissues with different thermal expansion coefficients, cracks were observed in the enamel-dentin junction after

Figure 1 Enamel and dentin before thermal treatment, showing the transparent aspect of enamel and the light yellow aspect of dentin.

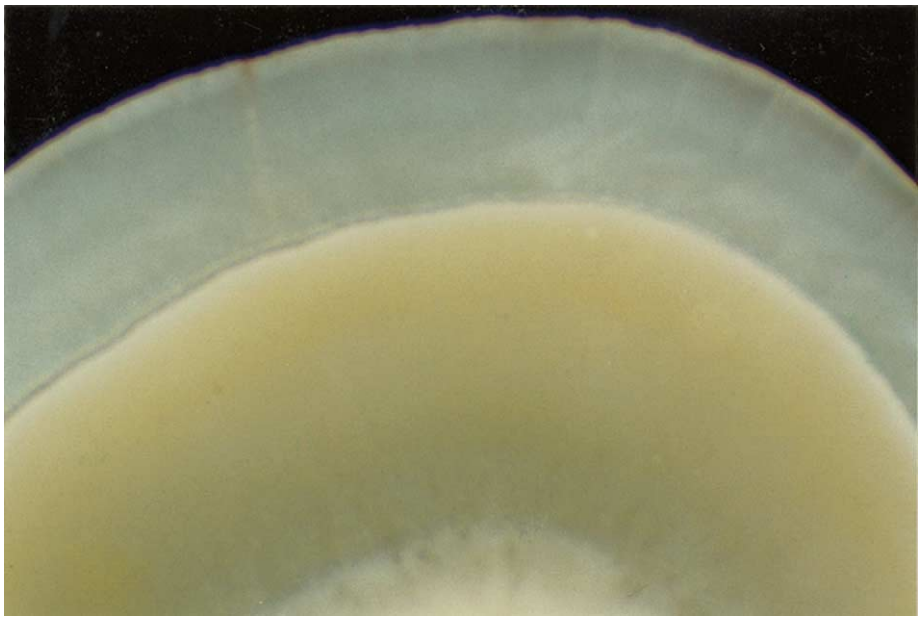

Figure 2 Enamel and dentin after 2 h of thermal treatment at 140 °C, showing slight enamel whitening and browning of dentin.

thermal treatment. The temperatures, used in this study (140 and 200 °C) did not cause shrinkage of the samples.

The black support behind the natural enamel sample is visible in Figure 1 because of the translucency of the enamel. Following thermal treatment, the enamel became opaque; treatment at 140 °C (Figure 2) caused the enamel to whiten and it turned completely opaque following treatment at 200 °C (Figure 3). Dentin showed diverse behaviour, and turned brown following thermal treatment. This discolouration was very slight following 2 h of treatment at 140 °C (Figure 2). However, following 2 h of treatment at 200 °C (Figure 3), the effect was more evident, resulting in dark brown dentin. The absorbance variation of dentin after 1, 2 and 6 h of treatment at 140 °C can be visualised on the spectra shown in Figure 4. Figure 5 shows the results

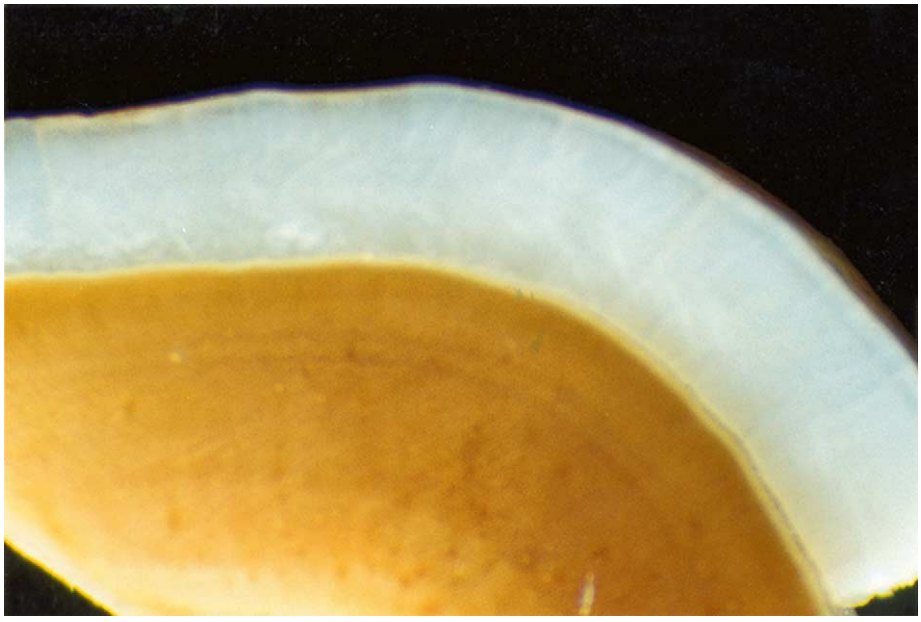

Figure 3 Enamel and dentin after 2 h of thermal treatment at 200 °C, showing very strong enamel whitening and browning of dentin.

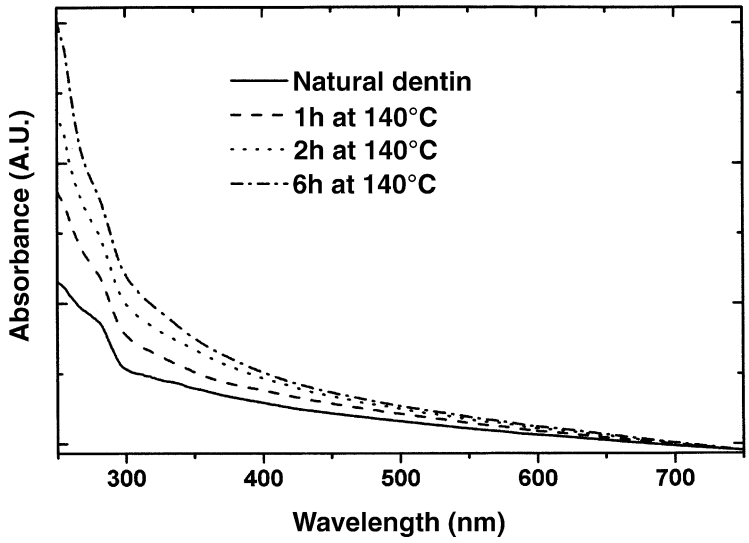

Figure 4 Absorbance spectra between 250 and 750 nm of natural dentin, treated for 1, 2 and 6 h at 140 °C.

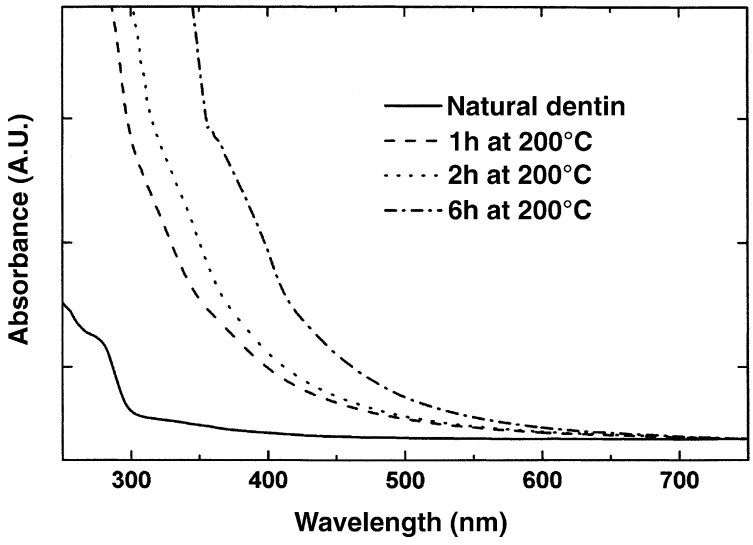

Figure 5 Absorbance spectra between 250 and 750 nm of natural dentin, treated for 1, 2 and 6 h at 200 °C.

following treatment at 200 °C. The absorbance spectra were normalised to be equal in intensity at 750 nm.

The discolouration process of dentin started slowly at 140 °C and became more intensive at 200 °C, producing the brown tissue. This dentin discolouration originated from higher absorption or scattering of the blue and violet wavelengths, as observed in Figures 4 and 5. Absorbance, in the blue and ultraviolet region, increased in the first hour of treatment, but this increase was not so pronounced in subsequent hours.

Different chemical composition and morphologic features of tissues can lead to different colour aspects, even within the same sample. Examples are pulp vascularisation, dentinal tubule size, crystal orientation, secondary dentin, caries process and other features that occur during tooth development.

Discussion

The colour changes observed following the thermal treatment in these bovine samples are also likely to occur in human tissues. Samples from different individuals or animals are composed of different relative concentrations of chemical radicals (OH^-, PO_4^{3-}, CO_3^{2-}), water, organic material and trace elements.[18] If discolouration is assigned to one of these elements or radicals, its intensity will depend on this concentration and, as a consequence, the discolouration intensity will differ between human

or bovine tissues. However, it will always occur to some degree. Thermal treatment by an oven or laser irradiation showed some differences and similarities that must be discussed. Differences include the length of time that the temperature acts on the tissue and the spatial distribution into the tissue; laser irradiation only exposes tissue to a determined temperature for milliseconds, whereas thermal treatment in an oven, as in this study, exposes tissue to the temperature for a longer period.

Another difference between the two treatments was the spatial thermal distribution into the tissue. For any fixed time following laser irradiation, a spatial thermal gradient occurred in the tissue with a maximum temperature at the point of irradiation. However, samples treated in an oven have a constant temperature throughout the sample.

Similarities between thermal treatment using laser irradiation or an oven included the temperature that the tissues were exposed to i.e.; the chosen temperature (140 and 200 °C) corresponded with a specific tissue layer localized beneath the Er:YAG laser irradiated surface. The effects observed after thermal treatment at 140 and 200 °C were predicted in subsuperficial regions of irradiated tissues where the maximum temperature was also 140 and 200 °C. If it is considered that a chemical reaction needs a determined activation energy to occur, this reaction is independent of the length of exposure and depends only of the temperature, i.e. the same product can be formed with both oven thermal treatment and laser pulse treatment. As laser irradiation produces a spatial thermal gradient, the reaction products have the same spatial gradient. The effects will be more intense at the site of irradiation than in the subsurface layers, and the product will decrease gradually, eventually reaching a layer without any observable effects.

Enamel whitening occurs due to water elimination. Before treatment, the enamel was transparent because of the presence of water in its liquid state. After thermal treatment, partial elimination of water resulted in interstitial regions filled with air; this could increase light scattering, resulting in the white pattern observed in Figure 3. A similar opaque behaviour has been observed in enamel dried with air;[19] following rehydration, the enamel became transparent again.

In dentin, water elimination and consequently the whitening process may also occur, but the browning effect was more evident. Thermal treatment below 200 °C produced alterations in the tissues that were restricted to the organic matrix and water.[20] Water in the tissue may be adsorbed or strongly bound to the structure, and the structural water is released when the tissue is heated to 400 °C.[21] In the spectral region where absorbance increases with temperature, absorption bands from the electronic structure of many chemical elements composing the tissue were present. Alterations in the electronic structure, and consequently in the absorption spectra, could produce discolouration such as the browning effect observed here.[22]

A more probable origin of the browning effect observed in dentin is some chemical element present in the organic matrix of the tissue. The browning effect was not observed in enamel following the same thermal treatment.

Considering an organic origin, the brown dentin could be caused by non-enzymatic browning (Maillard reaction), oxidation or changes in the collagen structure or composition that can produce an absorption band in the ultraviolet-blue spectral range. In the literature, the Maillard reaction is only predicted in demineralized dentin by the caries process.[5] The Maillard reaction takes place when components such as reducing sugars, carbohydrates, amino acids or proteins react together. One of the consequences of this reaction is the tissue discolouration observed in the caries process. Its intensity depends mainly on the concentration and nature of the reactants, pH, time and temperature.[23,24] Despite the possibility of a reaction between carbohydrates and collagen in sound dentin and its enhancement with thermal treatment, the Maillard reaction can not be associated with the browning of dentin in this study because this reaction only occurs in caries dentin.[5]

For dentin, the proposed model for collagen molecule and hydroxyapatite crystal interlocking is described as the hydroxyapatite occupying the intraspaces of the collagen fibrils and the interfibril spaces;[25,26] the collagen-hydroxyapatite system is linked with both covalent and non-covalent bonds. Thermal denaturation of collagen corresponds with the melting of its periodic organisation. The temperature rise provides more freedom to the molecules that exhibit random configurations, destroying the original periodic arrangement and unfolding the protein. In our system, after thermal denaturation up to 200 °C, non-covalent interactions between different atoms break and the helical structure of the collagen is lost.[27] The separation of collagen-hydroxyapatite or collagen unfolding can introduce changes in the electronic structure, and absorption bands in the ultraviolet and visible spectra can occur. However, the browning pattern observed after thermal treatment could have several other origins, and evaluations with other techniques are necessary in order to obtain a more conclusive analysis.

Acknowledgements

This research was supported by FAPESP, CNPq and RHAE. We thank the Centro de Lasers e Aplicaç ões (CLA) of the Instituto de Pesquisas Energéticas e Nucleares (IPEN) for the technical support and facilities.

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