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Abstract : Heating effects on dentin from mammoth tusks were examined by means of X-ray diffraction (XRD) and Fourier transform infrared spectrometric (FTIR), techniques. Dentin samples were sectioned into rectangular plates of about 10×5×1mm in size, using a low speed diamond saw. Heating treatment was carried out using a differential thermal analyzer. It was shown that the organic materials of the dentin were about 32 wt%, and combusted at a temperature range of 200℃ to 600℃, showing the coupled exotherm resulting in the release of the inorganic apatite phase from the closely related dentin collagen, and that the exotherm at 700℃ related to the crystalline phase transformation from the intermediate phase to crystalline β-TCP. This study suggested that the magnesium components in mammoth tusk dentin manage or control the crystalline phase transformation.

Introduction

Dentin has been widely used as a native biological material in a variety of research fields, such as caries mechanism[1] and adhesive experiments[2]. Recently laser applications in dental clinic have grown radically. The study of mechanical and/or thermal ablation mechanisms of lasers to dentin is needed, and we need to know the heating effects or thermal changes of dentin in more detail[3]. However, only a limited number of studies have been carried out on the thermal properties of dentin[4-13]. Besides the structure of dentin, human dentin has a varied chemical composition[14]. Therefore, standard dentin material for experimental use is required immediately.

Dentin from mammoth tusk may be a good candidate for experimental use because of its volume and uniformity. It is widely used for cell culture, though little is known about elephant tusk dentin structure and chemical composition[15-17]. This study aimed to clarify the effects of heating on mammoth dentin, to provide basic knowledge of the thermal behavior of dentin.

Materials and Methods

The mammoth tusk used in this study (about 10 cm in diameter) was obtained through an importer. Based on the pattern of the sectioned plane of tusk, the material was identified as that of fossil *Mammuthus* sp. The surface was colored brown, but the inside was white and had a lustre similar to ivory. Plates, about 10×5×1mm, were sectioned from the outer part of the dentin using a Buhler low speed diamond saw.

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Table 1 Results of TG, XRD, FTIR and colors after the heating treatments *

		TG	XRD			FTIR	
Sample	Color	Wt loss % in TG	HA	Intm	TCP	Organic	Inorganic
r.t.	Natural	-	Low XL	-	-	+ +	Ap
100 ℃	Natural		Low XL	-	-	+ +	Ap
200 ℃	Natural	16	Low XL	-	-	Start decay	Ap
300 ℃	Natural		Low XL	-	-	Decaying	Ap
400 ℃	Natural	21	Low XL	-	-	Weaken & broaden	Ap
500 ℃	Sooty gray			Low XL	-		Intm
600 ℃	Brownish	11	-	Low XL	-		Intm
700 ℃	Brownish		-	-	Low XL		TCP
800 ℃	White	1	-	-	XL		TCP
900 ℃	White		-	-	XL		TCP
1,000 ℃	White	< 0.2	-	-	XL		TCP
1,100 ℃	White		-	-	XL		TCP
1,200 ℃	White	< 0.1	-	-	XL		TCP

* : Low XL : low crystalline, XL : well crystalline, Ap : apatite, Intm : intermediate, TCP : β-tricalcium phosphate.

Heating treatment was carried out using a RIGAKU laser-furnace thermogravimetric-differential thermal analyzer, TG-DTA. The samples were heated to the set-up temperatures, 100℃ interval between 100℃ and 1,100℃, with an ascending rate of 5℃/min in a static atmosphere. The heating was quickly stopped when the sample reached the set-up temperature and rapidly cooled to room temperature using an air blow system.

X-ray diffraction (XRD) data was obtained by a RIGAKU-PSPC, position-sensitive-proportional-counter micro-XRD system under the following conditions : X-ray generator : rotary type Cu target, accelerating voltage : 50kV, current : 300 mA, X-ray beam collimeter : 30 μm, sample position : 10 degree to the incident beam, sample movement : rotation at 30 degree/sec around the sample holder ϕ-axis and rocking at 10 degree/sec around the sample holder χ-axis, counting time : 15 min. Diffraction patterns from three points in the heated and unheated tusk dentin plates were obtained and averaged. The diffraction patterns were analyzed by JADE software.

FTIR was carried out using a HORIBA FT-530 micro-FTIR under the following conditions : detector : 16× MCT with LN_2 cooling, measuring area : 10μm × 10—50μm depending on the sample condition, resolution : 4cm^{-1}, scanning : 20 cycles, gain : auto, function : H-G. The obtained raw reflection spectrum was transformed into pseudo-transferred absorption pattern by the K-K function.

Results

Table 1 lists the color change of the heated tusk dentin. Below 400℃, the tusk dentin showed no color change and a natural white color. Above 500℃ the color changed to sooty gray. Above 700℃ the color faded to brownish, and then above 900℃ the color became white again.

The TG-DTA curve showed coupled strong exotherms from 300℃ to 500℃ and a weak exotherm at about 700℃, except for a broad endotherm with a weight loss of 16% below 200℃ attributed to

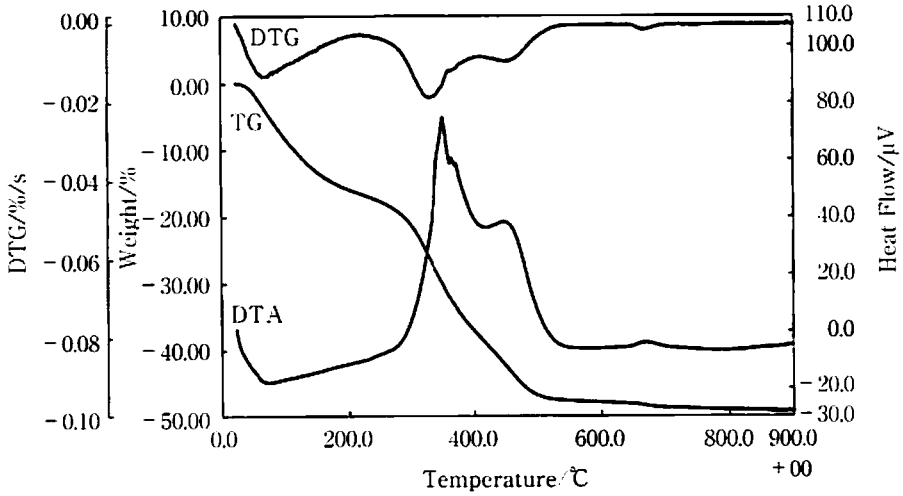

Fig. 1 TG-DTA curves of the mammoth dentin

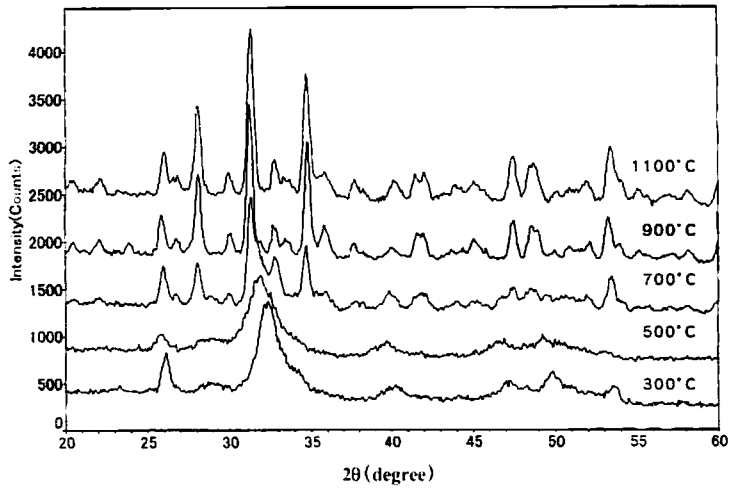

Fig. 2 XRD patterns of the mammoth dentin, after 300℃, 500℃, 700℃, 900℃ and 1,100℃ heating, from bottom to top, respectively.
Each pattern was shifted in order to make it legible.

dehydration (Fig. 1). The weight loss was about 21 wt% from 200℃ to 400℃, 11 wt% from 400℃ to 600℃, 1 wt% from 600℃ to 800℃, and less than 0.2 wt% from 800℃ to 1200℃ (Table 1).

The XRD patterns at 300℃, 500℃, 700℃, 900℃ and 1,100℃ are shown in Fig. 2. The other XRD patterns were a transition pattern between both ends. At 300℃ or below, the XRD pattern was a low crystalline apatite, typical of biological apatites. At 400℃ and above, the broad peak maximum shifted to the lower angles located among peaks of apatite and tricalcium phosphate. Above 700℃, a crystalline β-tricalcium phosphate phase, β-TCP, occurred in stead of apatite. Above 800℃, β-TCP was the only crystalline phase and the calculated unit cell dimensions were a = 10.359 Å and c = 37.21 Å, at the middle point of values for calcium phosphate and calcium-magnesium phosphate (Table 2).

Table 2 Unit cell dimensions of the heat-producted TCP

				PDF[18] card no.
Whitlockite (syn)	$Ca_3(PO_4)_2$	10.4290	37.3800	9-169
Mammoth dentin	After Heated at 1,200 ℃	10.359 (0.004)*	37.21 (0.01)	
Ca-Mg-Phosphate	$Ca_{2.81}Mg_{0.19}(PO_4)_2$	10.3370	37.0680	70-682

*: Figures in the parenthesis are the estimated standard deviations calculated by JADE software.

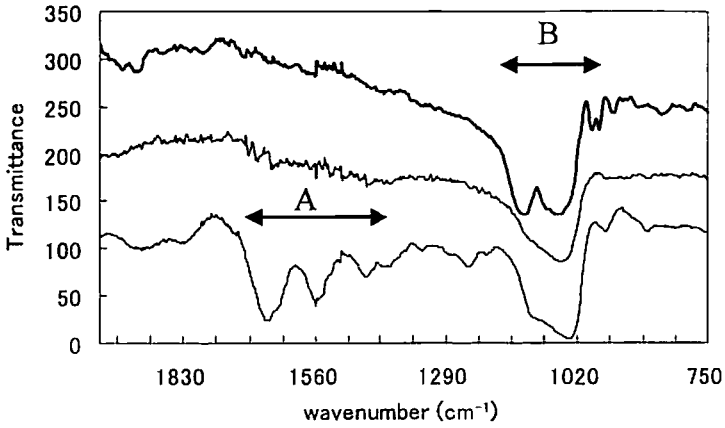

Fig. 3 FTIR spectra of the mammoth dentin, before the heating and after heating at 500℃ and 1,200℃, from bottom to top, respectively. A : Absorption due to amid, B : Absorption due to phosphate ions. The absorption at 964 cm^{-1} in the unheated sample was attributed to v1 of hydroxyapatite (ref.). Each pattern was shifted in order to make it legible.

Figure 3 shows the results of the FTIR study. These showed a correlation to a the organic and inorganic changes during heating, i.e., the organic compounds started to decay after 300℃, and completely combusted after 500℃, which was the temperature of the intermediate phase in the exchange to apatite (Table 1).

Discussion

The TG-DTA curve showed a large exotherm from 300℃ to 500℃ and a weak exotherm at about 700℃ corresponding to weight losses. The former exotherm was assumed to be due to combustion of dentin organic matrix[6-12], but the direct evidence had not been shown before. In this study TG-DTA analysis was coupled with FTIR and it was clearly shown that the absorption bands assigned to the organic composition diminished in the temperature range of 200℃ to 600℃ in accordance with the TG-DTA data. An organic content of about 32% in the mammoth dentin was comparable to the earlier report[7] and apparently larger than the value of about 20% for humans[10]. The reason for the high content of organic materials in mammoth tusk was not shown here, but it might be related to the size of tooth, because of the fact that the larger the tooth the higher the organic content[9,12].

Based on the XRD and FTIR data, the weak exotherm at about 700℃ indicated the crystalline phase transformation to crystalline β-TCP. This is harmony with the result of Lim and Liboff[5], who analyzed human dentin using a thermogravimeter, and concluded that between 700℃ and 800℃ apatite transformed into β-TCP.

In this study the apparent peak-shift found in the XRD pattern, and the broadened absorption in FTIR after 600℃ and 700℃ were attributed to the intermediate phase of the transformation from low crystalline apatite to the crystalline β-TCP. It has not been reported that the apatite crystal structure makes a solid-solution with tricalcium phosphate structure, while the apatite structure has the potential to include a wide variety of ionic substitutions[19,20]. The properties of the intermediate phase remained unclear and should be studied because the phase may play an important role in inorganic phase transformation.

This study highlighted a relation between organic combustion and the transformations of the crystalline phase. Collagen in dentin is tightly bonded to apatite as shown by Sakae, *et al*.[10]. They demonstrated the change in thermal behavior of collagen in mineralized and chemically de-mineralized dentin. In this study, FTIR results showed a crystalline phase transformation occurred with the diminishment of organic components, mainly collagen, by heating. This phenomenon is explained by the close relationship between the organic and inorganic components in dentin.

β-TCP in this study showed unit cell dimensions at the middle point of the values for calcium phosphate and calcium-magnesium phosphate. The unit cell dimensions usually reflect the chemical composition and ionic substitutions in a linear relationship[21], and therefore the roughly assumed chemical composition for the heat-producted β-TCP was $Ca_{2.81}Mg_{0.19}(PO_4)_2$ according to the estimation calculation known as Vegard's law[19] i.e. the unit cell dimensions correlate to the ratio of compositions which share the same atomic positions. For example, the a-axis dimension of the $(Ca, Mg)_3(PO_4)$ was written as $a = a(Ca_3(PO_4)_2) \cdot f(Ca_3(PO_4)_2) + a(Mg_3(PO_4)_3) \cdot f(Mg_3(PO_4)_3)$, where f is the fraction of the component.

The high concentration of Mg in the dentin might explain the rapid apatite diminishment by heating, unlike in human and bovine dentin, where the apatite phase remains after heating to 1,000℃ and higher[22,23]. In the comparative study of some animal dentin[9,10], there was a relationship between the magnesium content and the amount of the heat-product whitlockite. These studies showed that Probosidean dentin had a relatively large amount of magnesium, and that the formation of whitlockite in these dentin was at a lower temperature and had a larger volume after heating. These studies indicated that magnesium in dentin was a key trigger material that accelerates the thermal decomposition and transformation of dentin apatite into whitlockite.

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References

1) Robinson, C., Connell, S., Kirkham, J., Brooks, S.J., Shore R.C. and Smith, A.M. : The effect of fluoride on the developing tooth. Caries Res. 38 : 268—276, 2004.
2) Kameyama, A., Ihara, S., Amagai, T., Miake, Y., Kawada, E., Oda, Y., Yanagisawa, T. and Hirai, Y. : Adhesion of 4-META/MMA-TBB resin to heated dentin : effects of pre-treatments with $FeCl_3$ and/or HEMA. Dent. Mater. J. 22 : 1—9, 2003.
3) Fried, D., Zuerlein, M.J., Le, C.Q. and Featherstone, J.D. : Thermal and chemical modification of dentin by 9—11-μm CO_2 laser pulses of 5-100-μs duration. Lasers Surg. Med. 31 : 275—282, 2002.

4) Brauer, G.M., Termini, D.J. and Burns, C.L. : Characterization of components of dental materials and components of tooth structure by differential thermal analysis. J. Dent. Res. 49 : 100—110, 1970.
5) Lim, J.J. and Liboff, A.R. : Thermogravimetric analysis of dentin. J. Dent. Res. 51 : 509—514, 1972.
6) Sakae, T., Mishima, H. and Kozawa, Y. : Changes in bovine dentin mineral with sodium hypochlorite treatment. J. Dent. Res. 67 : 1229—1234, 1988.
7) Sakae, T., Akaishi, S., Mishima, H. and Kozawa, Y. : Thermogravimetric and differential thermal analysis of elephant tusk dentin. Nihon Univ. J. Oral Sci. 18 : 655—659, 1992. (in Japanese)
8) Larmas, M.A., Hayrynen, H. and Lajunen, L.H. : Thermogravimetric studies on sound and carious human enamel and dentin as well as hydroxyapatite. Scand. J. Dent. Res. 101 : 185—191, 1993.
9) Sakae, T., Mishima, H., Takahashi-Kunuki, Y., Suzuki, K. and Kozawa, Y. : Dentin apatites from teeth of different animals : crystallographic properties. Bull. de l' Institut Oceanographique, Monaco, n° Special 14 : 215—219, 1994.
10) Sakae, T., Mishima, H., Kozawa, Y. and LeGeros, R.Z. : Thermal stability of mineralized and demineralized dentin : a differential scanning calorimetric study. Connect. Tissue Res. 33 : 193—196, 1995.
11) Sakae, T., Yanaka, S., Watanabe, H. and Kozawa, Y. : Formation of porous and dense calcium phosphate structure by cintering of dentin. Nihon Univ. J. Oral Sci. 22 : 45—51, 1996. (in Japanese)
12) Sakae, T. : Toothed whale dentin collagen studied with thermal analysis technique. In : Biomineralization and hard tissue of marine organisms. (eds. Wada, K. and Kobayashi, S), pp. 143—149, Tokai Univ. Press, Tokyo, 1996. (in Japanese)
13) Bachmann, L., Thome Sena, E., Fernando Stolf, S. and Maria Zezell, D. : Dental discolouration after thermal treatment. Arch. Oral Biol. 49 : 233—238, 2004.
14) Rowles, S.L. : Chemistry of the mineral phase of dentin. In : Structural and chemical organization of teeth. (ed. Miles, A.E.W.), pp. 201—246, Academic Press, New York, 1967.
15) Schaedler, J.M., Krook, L., Wootton, J.A., Hover, B., Brodsky, B., Naresh, M.D., Gillette, D.D., Madsen, D.B., Horne, R.H. and Minor, R.R. : Studies of collagen in bone and dentin matrix of a Columbian mammoth (late Pleistocene) of central Utah. Matrix 12 : 297—307, 1992.
16) Raubenheimer, E.J., Brown, J.M., Rama, D.B., Dreyer, M.J., Smith, P.D. and Dauth, J. : Geographical variations in the composition of ivory of the African elephant (*Loxodonta africana*). Arch. Oral Biol. 43 : 641—647, 1998.
17) Raubenheimer, E.J., Bosman, M.C., Vorster, R. and Noffke, C.E. : Histogenesis of the chequered pattern of ivory of the African elephant (*Loxodonta africana*). Arch. Oral Biol. 43 : 969—977, 1998.
18) Powder Diffraction File (PDF) : International Centre for Diffraction Data, Pennsylvania, USA, card no. 9—169 and no. 70—682, 2000.
19) McConnel, D. : Apatite, pp. 22—32, Springer-Verlag, New York, 1973.
20) Elliott, J.C. : Structure and Chemistry of the Apatites and Other Calcium Orthophosphates. pp. 111—190, Elsevier, Amsterdam, 1994.
21) LeGeros, R.Z. : Calcium Phosphates in Oral Biology and Medicine. pp. 82—107, Karger, Basel, 1991.
22) Dickens, B., Schroeder, L.W. and Brown, W.E. : Crystallographic studies of the role of Mg as a stabilizing impurity in β-$Ca_3(PO_4)_2$. I. The crystal structure of pure β-$Ca_3(PO_4)_2$. J. Solid State Chem. 10 : 232—248, 1974.
23) Sakae, T., Mishima, H., Kozawa, Y., Yamamoto, H., Nagano, E. and Hirai, G. : Thermal study of the formation of magnesium whitlockite from hydroxyapatite and magnesium phosphate. Nihon Univ. J. Oral Sci. 14 : 463—468, 1988. (in Japanese)