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Effects of heating on the mechanical and chemical properties of human dentin

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ABSTRACT

Objectives. We had previously discovered that the flexural and tensile strengths of human dentin were 2–2.4 times greater after being heated to 140 °C, and deduced that the generation of higher-density structures and therefore dehydration probably promoted the increased strength. Our test hypotheses were that intertubular dentin, which constitutes a major part of organic components, was selectively affected by heating, and such changes could happen without critical damages to the basic structure of dentin type I collagen.

Methods. Micro-mechanical changes of human dentin by heating at 140 °C were investigated by nano-indentation. Chemical changes in dentin collagen after heating were also investigated by X-ray diffraction study, a microscopic Fourier transform infrared (micro-FTIR) and a laser Raman spectroscopic analyses, and a cross-linking analysis by high-performance liquid chromatography.

Results. The results of nano-indentation showed that the micro-hardness of intertubular dentin increased after heating at 140 °C to 1.8 times more than unheated dentin; on the other hand, peritubular dentin was unchanged. Results of X-ray diffraction showed that the lateral packing of collagen molecules shrank from 13.6 ± 0.3 to 10.6 ± 0.1 Å after heating, but the shrinkage reversed to the original after rehydration for seven days. After heating, no substantial chemical changes in the collagen molecules were detected in tests by micro-FTIR or Raman analyses, or by cross-linking analysis.

Significance. These results suggest that intertubular dentin, which contains most of the type I collagen, was selectively affected by heating at 140 °C without critical damage to its collagen.

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1. Introduction

In a previous significant clinical study [1], fracture was found to be the most frequent reason for tooth loss in persons involved in a long-term plaque control program. In this study, 62% of extractions were undertaken because of tooth fracture, compared with only 7% and 5% because of caries or periodontal disease, respectively. This research suggested that tooth fracture is a critical issue. Another etiological study on 227 vertical root fractures showed that 88% fracture happened in pulpless teeth [2]. Therefore, effective methods to strengthen pulpless teeth could significantly help in preventing tooth fracture and thus be of potentially great assistance to clinicians in their front-line battle to promote oral general health.

In our previous research [3], we had discovered that the flexural and microtensile strengths of human dentin were 2–2.4 times greater after heating to either 110 °C or 140 °C. X-ray diffraction analyses indicated that dehydration probably generated higher-density structures and caused the shrinkage of the lateral packing of the collagen triple-helices from 14 Å to 11 Å. This was the probable cause of the greater strength of dentin after heating. As the next step in this series of studies, we investigated the effects of heating on the mechanical properties of human dentin by focusing on what happened when dentin was dehydrated and rehydrated [4]. Several studies had reported that changes in the biomechanical properties of dentin after dehydration could be reversed by rehydration [5–7]. We did discover that rehydration reverses the strength of heated dentin. Given how dentin reacted when dehydrated and then re-hydrated, we speculated whether strengthening effect of dentin by heating may be reversible without critical damage to its organic components, particularly type I collagen.

In human bone, which has a similar composition to dentin, the type I collagen network plays an important role in its toughness, and the toughness and strength of bone decreases with increasing denaturation of the collagen [8,9]. Several studies have investigated how calcified and decalcified collagen in bone or dentin reacts to heating. Heat transforms the crystalline collagen triple helical structure into an amorphous random coil form [10]. Some studies reported that the degree of mineralization significantly affected the denaturation temperature of type I collagen [11,12]. Other studies showed that the calcified type I collagen molecules in bone broke down approximately at 150–175 °C [8,13,14], while the decalcified type I collagen molecules denature irreversibly at 35.9–60 °C [15–19]. The denaturation temperature seemed to vary depending on testing conditions and origin of materials.

Armstrong et al. [20] found the denatured temperature of human dentin collagen in mineralized versus demineralized teeth in relation to the extent of dehydration. They reported that the denaturation temperature of collagen in mineralized dentin was between 160 and 186 °C; but that of demineralized dentin exhibited a denaturation temperature of 65.6 °C that increased with dehydration to 176 °C. They suggested that presence of apatite crystallites in mineralized collagen or interpeptide bonding in demineralized collagen increased the denaturation temperature. Given that 176 °C is the denaturation temperature for dehydrated demineralized dentin, heating to 140 °C as shown in our previous study [3], can significantly strengthen human dentin without major damage to its collagen.

In this study, we focused on the performance of type I collagen in dentin, after the dentin had been heated at 140 °C to achieve the best strength. First, we tried to identify the exact changes in mechanical properties of heated dentin by conducting nano-indentation with combination to an atomic force microscope (AFM). This makes it possible to understand changes in the heated dentin at the microstructural level. Then, the thermal stability of dehydrated dentin collagen was investigated by various methods: an X-ray diffraction analysis, a microscopic Fourier transform infrared (micro-FTIR) spectroscopic analysis, a microscopic laser Raman spectroscopic analysis, and a cross-linking analysis by high-performance liquid chromatography (HPLC). Our test hypotheses were that intertubular dentin, which constitutes a major part of organic components, was selectively affected by heating, and such changes could happen without critical damages to the basic structure of dentin type I collagen.

2. Materials and methods

2.1. Preparation of dentin specimens

Human third molars free of caries were stored in Hanks' balanced salt solution (HBSS) at 4 °C and used within three months of extraction. Teeth were obtained from patients aged from 22 to 67. Protocols were approved by the ethics committee of Osaka University. Disk and beam-shaped dentin specimens were prepared (Fig. 1). Disk-shaped specimens with a thickness of approximately 1.5 mm were obtained from coronal central portions of the molars by sectioning perpendicular to the tooth axis. Beam-shaped specimens, measuring approximately 1.7 mm × 0.9 mm × 8.0 mm were obtained from the coronal central portions of the molars. Dentinal tubules in the beam-shaped specimens were organized to run perpendicular to the longitudinal surface along the specimen length.

2.2. Heat treatment and rehydration

The specimens prepared were treated according to the following protocols: control (wet) – soaked in HBSS; heat – heated from room temperature to 140 °C at a rate of 10 °C/min, since we had confirmed the mechanical properties of human dentin were strengthened greatest to that temperature [3]; rehydrated – heated, then soaked in HBSS at ambient temperature for seven days. Each experimental group contained 10 specimens.

2.3. Nano-indentation with an AFM

Each disk-shaped specimen was polished by using waterproof silicone carbide abrasive papers (#600, 1000, 1500, Buehler, Lake Bluff, IL) under running water with a grinding-polishing machine (Ecomet 3000, Buehler). Then, the specimen was cut in half. One was heated and the other was kept without heating as a control. Nano-indentation was conducted by using a pyramidal diamond tip with a diameter of 200 nm secured to a nano-indenter (Nano Indenter SA2, MTS Japan, Tokyo) at ambient temperature. The force for the indentation was