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The interrelationship of microstructure and hardness of human coronal dentin using reference point indentation technique and micro-Raman spectroscopy

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ABSTRACT

Objectives. The aim of this paper is to determine the interrelationship between the microstructure – in terms of chemical composition and crystallinity – to the microhardness of coronal dentin.

Methods. Dentin microhardness was tested by a novel reference point indenter and compared to the traditional Knoop hardness method. Micro-Raman spectroscopy was used to determine the chemical composition and crystallinity of dentin.

Results. From the occlusal groove to the border of the coronal pulp chamber, dentin hardness decreased from superficial dentin (SD) to deep dentin (DD). Mineral/organic matrix ratios (phosphate/C—H and phosphate/amide I) also decreased from SD to DD; however, this change was significant ($P < 0.05$) in the phosphate/amide I ratio only. The phosphate/carbonate ratio decreased significantly by varying position from SD to DD. The degree of the crystallinity, as measured by the full width at half maximum (FWHM) of the peak at 960 cm^{-1}, decreased significantly going from superficial to deep dentin.

Significance. For the first time, the interrelationship between the microstructure and the mechanical properties of coronal dentin was determined by using the novel reference point indentation technique and micro-Raman spectroscopy. We hypothesize that the decrease in hardness from superficial to deep dentin can potentially be explained by decreased mineral content and increased carbonate content, which is also associated with decreased crystallinity. Collectively, there is a positive association between dentin hardness and mineral content and a negative association between dentin hardness and carbonate content.

Abbreviations: RPI, reference point indenter; PBS, phosphate buffered saline; CEJ, cementoenamel junction; SD, superficial dentin; DD, deep dentin; DEJ, dentin-enamel junction; KHN, Knoop hardness numbers; BHN, BioDent hardness numbers; ANOVA, analysis of variance; SEM, scanning electron microscopy; SE, secondary electron detector; FWHM, full-width at half maximum; ISE, indentation size effect.

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1. Introduction

Tooth dentin is a hierarchical biocomposite composed of different structural elements, such as, dentinal tubules, highly-mineralized peritubular, intertubular dentin that is composed of type I collagen, and dentinal fluid [1]. The distribution of structural elements from superficial dentin, i.e., by the dentin-enamel junction (DEJ), to deep dentin, i.e., near the border of the coronal pulp chamber, results in an anisotropic biocomposite in which properties vary by location. Studies that have evaluated the structure and material properties of dentin suggest that the mechanical properties of dentin are dependent on its internal structure, composition, and external environment [2–4].

It is vital to understand the mechanical properties of the dentin in order to determine its structure–property relationships. Several informative reviews in the context of mechanical properties of human tooth are available describing different techniques and results [5–7]. Among these properties of human teeth, hardness is routinely reported in the literature. This is likely due to available testing methods. Hardness tests are fast, simple, user-friendly and can be correlated to other mechanical properties, e.g. Young's modulus [8], thin-film bond strength [9] and shear bond strength [10]. Hardness can also be determined by micro- and nano-indentation techniques [8,10,11]. However, reported hardness values vary, which may be due to the innate variability of human teeth and/or testing methods. To this end, studies have been addressing the precision and accuracy of the reported hardness values by either upgrading methods or evaluating new techniques [12,13]. Recently, the reference point indentation (RPI) technique has gained widespread interest as a microindentation tester in characterizing mechanical properties of bone [14]. Similar to the traditional microindentation hardness tester, RPI can be used to measure microhardness. However, the capability and accuracy of this technique in evaluating microhardness of coronal dentin is unclear. To this end, to validate the micro-scale hardness obtained by the RPI technique, the Knoop hardness tester was included as a reference.

The purpose of this study was to determine the interrelationship between the microstructure – in terms of chemical composition and crystallinity – to the microhardness of coronal dentin. Dentin microhardness was tested by using a novel reference point indenter technique compared to the traditional Knoop hardness method. Micro-Raman spectroscopy was used to characterize the chemical composition and crystallinity of dentin.

2. Materials and methods

2.1. Preparation of dentin specimens

Twenty-six, extracted third molars from individuals 17–21 years old were collected according to a protocol approved by an institutional board (IRB13-924NHS). Because these extracted teeth were not associated with any patient identifiers, their use in the project was categorized as not human subject research by the IRB. Excess soft tissue was removed and the teeth were stored at 4 °C in phosphate buffered saline (PBS, pH 7.4) with 0.002% sodium azide.

A slow-speed, water-cooled diamond saw (Buehler Ltd, Lake Bluff, IL, USA) was utilized to remove the roots, parallel to the occlusal table, approximately 5 mm below the cemento-enamel junction (CEJ). For hardness testing, 20 of the tooth crowns were sectioned bucco-lingually to create, two, paired 2-mm-thick sections from each tooth. Those forty sections were embedded in cold-curing epoxy resin (EpoxiCure 2, Buehler, Lake Bluff, IL, USA) and polished with a motorized wheel (Metaserv, Buehler, Lake Bluff, IL, USA) using decreasing grit-sized waterproof silicon carbide, polishing cloth papers, and finished with diamond paste. Specimens were washed ultrasonically after each step to remove any surface debris. For micro-Raman spectroscopy, the remaining six tooth crowns were sectioned bucco-lingually to generate one 2-mm-thick section from each tooth. Before analysis, the sections were sequentially polished under water using 600- and 1200-grit SiC paper.

2.2. Hardness test

Using the embedded sections, the hardness of coronal dentin was measured along a reproducible line starting at the occlusal groove to the border of the coronal pulp chamber; two locations were designated: superficial dentin (SD), 200-μm from the DEJ; and deep dentin (DD), 200-μm above the coronal pulp chamber boundary. A BioDent RPI technique and traditional Knoop hardness method were used respectively with one of the paired sections from twenty teeth.

A Knoop hardness test was performed using a Wilson Hardness Tukon 1202 (Buehler, Lake Bluff, IL, USA). Knoop hardness numbers (KHN) were calculated as the load divided by the length of the longest diagonal:

$$\mathrm{KHN} = 14.229\frac{P}{d^2}$$

where P is the applied load and d is the length of the longest diagonal measured from a secondary electron microscopy image. Knoop indentations were performed at two load levels (0.1- or 0.5-N) with a 10-s dwell time.

BioDent hardness numbers (BHN) were obtained using a BioDent RPI (Active Life Scientific, Santa Barbara, CA, USA) with Bone Probe 1 using two load levels (2- or 3-N) with a 10-s dwell time. This probe assembly features a sharp, beveled edge reference probe that anchors the probe assembly to the dentin surface. BHN were calculated by dividing the applied force by the area of the conical indentation pattern formed by the test probe and is given as:

$$\mathrm{BHN} = \frac{\mathrm{P}}{\pi \mathrm{r}\sqrt{\mathrm{r}^2 + \mathrm{h}^2}}$$

where P is the applied load, r is a radius of the indentation calculated from the SEM image, and h is the indentation distance as reported by the instrument.

2.3. Scanning electron microscopy

In order to generate hardness values, scanning electron microscopy (SEM) images (FEI-XL30, FEI Company, Hillsboro,

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