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Physico-mechanical properties determination using microscale homotopic measurements: Application to sound and caries-affected primary tooth dentin

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Abstract

Microscale elastic moduli, composition and density have rarely been determined at the same location for biological materials. In this paper, we have performed homotopic measurements to determine the physico-mechanical properties of a second primary molar specimen exhibiting sound and caries-affected regions. A microscale acoustic impedance map of a section through this sample was acquired using scanning acoustic microscopy (SAM). Scanning electron microscopy was then used to obtain mineral mass fraction of the same section using backscattered images. Careful calibration of each method was performed to reduce system effects and obtain accurate data. Resorption, demineralization and hypermineralization mechanisms were considered in order to derive relationships between measured mineral mass fraction and material mass density. As a result, microscale mass density was determined at the same lateral resolution and location as the SAM data. The mass density and the acoustic impedance were combined to find the microscale elastic modulus and study the relationship between microscale composition and mechanical properties.

Keywords: Dentin; Micromechanics; Elastic modulus; Density; Acoustic microscopy

1. Introduction

Properties of calcified tissues, such as bone and dentin, have been a subject of intense research for the past several decades [1]. With the advances in experimental methods, measurement of mechanical and compositional properties has been made possible at increasingly high spatial resolutions. In an effort to understand the relationship between these properties, recently, researchers have used complementary high-resolution experimental methods. However, when complementary methods are applied on highly heterogeneous materials, such as calcified tissues, it is especially important that the measurements are performed at the same location. In this paper, we introduce the term "homotopic" (Greek *homos* = identical and *topos* = place) to describe the measurement of properties performed at the same location of the same sample.

Relatively few attempts have been made to perform homotopic measurements of the microscale elastic moduli, composition and density of calcified tissues. Most of these investigations have used destructive indentation techniques for the micromechanical property determination. In the past decade, some investigators have combined ultra-microindentation or nanoindentation with backscattered

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scanning electron microscopy (BSEM) to study the relationship between mechanical properties and mineral content for a variety of calcified tissues [2–5]. Nanoindentation has also been used in combination with atomic force microscopy, Fourier-transform infrared microspectroscopy, small angle X-ray scattering and BSEM to relate hardness and elastic modulus to a variety of structural and compositional properties of dentin [6,7]. More recently, nanoindentation has been used with time-of-flight secondary ion mass spectroscopy to image the same regions of in vitro carious lesions in human dental enamel [8].

Non-destructive methods, as an alternative to nanoindentation, have rarely been used for homotopic measurements. The advantage of non-destructive methods is that the sample is preserved in its near natural state for analysis with other complementary techniques. Among the earliest efforts to use non-destructive methods is the work by Katz and Meunier [9], in which the same regions of human and canine osteons were imaged using BSEM and scanning acoustic microscopy (SAM). The contrast in the SAM images was explained upon the basis of mineral density variations observed in the BSEM images. Turner et al. [10] measured elastic moduli using SAM and compared them to nanoindentation measurements obtained at similar locations on adjacent sections. SAM has also been used in conjunction with synchrotron radiation microcomputed tomography [11,12] and in combination with Raman microspectroscopy and nanoindentation [13] to obtain site-matched mechanical and compositional properties.

We have used SAM and scanning electron microscopy (SEM) with backscatter electron detection to characterize a second primary molar specimen exhibiting sound and caries-affected regions. Caries-affected dentin is of clinical significance because composite restorations require the dentist to bond material to this substrate. SAM was used to obtain the microscale acoustic impedance at various locations on the sample, ranging from sound dentin to caries-affected dentin. A BSEM image of the same sample was then acquired and analyzed to determine the mineral mass fraction. Relationships between measured mineral mass fraction and material mass density were derived for sound, carious and caries-affected dentin. These relationships were then used to determine the microscale mass density at the same lateral resolution and location as the SAM data. The data from SAM and SEM were combined to obtain the microscale homotopic physico-mechanical properties. As a result, we can study the relationship between the microscale composition and elastic moduli of sound to caries-affected primary tooth dentin.

2. Background – dentin microstructure and properties

Dentin is the hydrated composite structure that constitutes the body of each tooth, providing both a protective covering for the pulp and serving as a support for the overlying enamel. Dentin is composed of approximately 45–50% inorganic material, 30–35% organic material and 20% fluid by volume. Dentin mineral is a carbonate-rich, calcium-deficient apatite [14]. The organic component is predominantly type I collagen, with a minor contribution from other proteins [15–17]. The apatite mineralites are of very small size and are deposited almost exclusively within the collagen fibril (see e.g. Arsenault [18]). The interactions between collagen and nanocrystalline mineralite give rise to the stiffness of the dentin structure. The consequent dentin elasticity is an important feature that determines the mechanical behavior of the tooth structure.

The structural characteristics of sound dentin are well known at the microscale (100 μm). Dentin is described as a system of dentinal tubules surrounded by a collar of highly mineralized peritubular dentin [19]. The tubules traverse the structure from the pulp cavity to the region just below the dentin–enamel junction (DEJ) or the dentin–cementum junction. The tubules, which are described as narrow tunnels a few microns or less in diameter, represent the tracks taken by the odontoblastic cells from the pulp chamber to the respective junctions. Tubule density, size and orientation vary from location to location. The density and size are lowest close to the DEJ and highest at the predentin surface at the junction to the pulp chamber. Thus, the porosity of dentin varies from zero to 0.25 from the DEJ to the pulp [20–22]. The rate of change in porosity with depth depends on the tooth type. In primary tooth dentin, the dentinal tubule density and size is, in general, larger than in permanent dentin [21].

The composition of the peritubular dentin is carbonated apatite with very small amounts of organic matrix whereas intertubular dentin, i.e. the dentin separating the tubules, is type I collagen matrix reinforced with apatite. Based upon electron microscopic studies, peritubular dentin in primary teeth has been found to be 2–5 times thicker than that of permanent teeth [23]. The composition of intertubular dentin is primarily mineralized collagen fibrils; the fibrils are described as a composite of a collagen framework and thin plate-shaped carbonated apatite crystals whose *c*-axes are aligned with the collagen fibril axis [24]. In sound dentin, the majority of the mineralized collagen fibrils are perpendicular to the tubules [25]. Water in dentin may be classified as either free or bound. Water is present within the dentinal tubules as pulpal fluid and within the interstitial spaces between collagen fibrils. Based upon experimental chemical microanalyses, bound water is likely present as hydroxyl groups bound to the mineral component [26–28].

Beginning in the 1960s, macroscale elastic moduli of dentin have been measured by a variety of methods as reviewed by Kinney et al. [29]. Using nanoindentation methods, Kinney et al. [30], have measured the elastic modulus of peritubular dentin and intertubular dentin. At somewhat larger, unspecified scales, Katz et al. [31] measured similar values of dentin elastic modulus using SAM. At even higher scales, Lees and Rollins [32] used longitudinal and shear wave velocity measurements, Kinney et al. [33] used resonant ultrasound spectroscopy to determine elastic moduli of millimeter scale samples and John