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Mesoscale porosity at the dentin-enamel junction could affect the biomechanical properties of teeth

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ABSTRACT

In this paper, the 3D-morphology of the porosity in dentin is investigated within the first 350 μm from the dentin-enamel junction (DEJ) by fluorescence confocal laser scanning microscopy (CLSM). We found that the porous microstructure exhibits a much more complex geometry than classically described, which may impact our fundamental understanding of the mechanical behavior of teeth and could have practical consequences for dental surgery.

Our 3D observations reveal numerous fine branches stemming from the tubules which may play a role in cellular communication or mechanosensing during the early stages of dentinogenesis. The effect of this highly branched microstructure on the local mechanical properties is investigated by means of numerical simulations. Under simplified assumptions on the surrounding tissue characteristics, we find that the presence of fine branches negatively affects the mechanical properties by creating local stress concentrations. However, this effect is reduced by the presence of peritubular dentin surrounding the tubules.

The porosity was also quantified using the CSLM data and compared to this derived from SEM imaging. A bimodal distribution of channel diameters was found near the DEJ with a mean value of 1.5–2 μm for the tubules and 0.3–0.5 μm for the fine branches which contribute to 30% of the total porosity (~1.2%). A gradient in the branching density was observed from the DEJ towards the pulp, independently of the anatomical location.

Our work constitutes an incentive towards more elaborate multiscale studies of dentin microstructure to better assess the effect of aging and for the design of biomaterials used in dentistry, e.g. to ensure more efficient bonding to dentin. Finally, our analysis of the tubular network structure provides valuable data to improve current numerical models.

1. Introduction

Dentin is a mineralized tissue located between the enamel and the pulp cavity in the tooth that plays an essential role in absorbing and distributing stresses generated by mastication [1]. Such functions are primarily determined by the tissue structure and composition. Dentin is essentially composed of type I collagen fibrils mineralized by hydroxyapatite nanocrystals and exhibits a natural porosity in the form of tubules produced during the dentinogenesis step of tooth formation. This process appears to be strongly directional, whereby odontoblasts, the tissue forming cells, move collectively from the dentino-enamel junction (DEJ) to the pulp [2]. As the extracellular matrix densifies, long slender odontoblasts processes form to maintain cellular contact along the path to the DEJ. The voids, necessary to accommodate the presence of odontoblastic processes, therefore appear as a dense organization of highly anisotropic tubules, radially oriented, with increasing density from the DEJ to the pulp [3]. This growth process also results in a strong tissue texture, with collagen fibrils organized in two preferential orthogonal directions: a minor fraction aligned along

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the tubules in a thin peritubular collar (<1 μm) and the remaining part lying in the orthogonal direction between tubules [4].

The impact of this microstructure on the mechanical properties has been thoroughly investigated from the macroscopic to the nanoscopic scales [5–11,1]. Those studies concluded to the existence of a zone of ~300 μm below the DEJ which is critical for the mechanical adaptation between the hard enamel and the softer pulp dentin. Important structural changes were reported in this zone in the form of a pronounced collagen disorganization and decrease in mineralization closer to the DEJ [4] and the appearance of microbranches stemming from the tubules towards the DEJ [12]. However, while the gradual changes in tissue structure have clearly been related to the adaptive functional behavior of this interphase, the potential impact of the complex branching morphology on the mechanical properties is unclear.

The microtubule organization has been extensively studied in 2D with increasing resolution levels using transmission brightfield light microscopy [12,13], scanning and transmission electron microscopy (SEM, TEM) [13–15] and atomic force microscopy (AFM) [16,17]. Although not always explicitly stated, a strong assumption supporting those 2D studies lies in the strong degree of co-alignment of the tubules. Thus, following well controlled sample preparation protocols, the tubules can be viewed along their main orientation axis (longitudinal section) or crosswise (transverse sections). Nevertheless, such analysis fails to provide quantitative information concerning out-of-plane details. So far, 3D quantifications of dentinal porosity were carried out using mercury intrusion porosimetry by Vennat et al. (2009) [18], but this procedure only provides average porosity values which cannot be directly related to the microstructure. Recent progress in the field of X-ray tomography making use of the phase contrast [19] and coherence [20] of very bright synchrotron sources has provided sufficient resolution to resolve the tubules in 3D. However, such measurements remain challenging and cannot be performed routinely. Similarly, focused ion beam instruments coupled with SEM (FIB-SEM) constitute an interesting alternative despite the destructive nature of this method [21]. Finally, the progress made in the last two decades in the field of optics, particularly in nonlinear imaging, has provided new insight on the tissue-microstructure relationship [22,23]. However, to this day, a precise 3D description and quantification of the tubule network geometry, connectivity and of the corresponding local porosity in the vicinity of the DEJ is still lacking.

In this paper, we investigate the complex tubular branching geometry in a region of ~350 μm from the DEJ using confocal laser scanning microscopy (CLSM). In the field of odontology, CLSM is essentially used to study infiltrated dentin and quantify the penetration depth into enamel [24–27]. Recently, Eltit et al. [28] also used confocal microscopy to image cracks resulting from mechanical testing in 3D. However, this technique has rarely been used to analyze the 3D porous microstructure of dentin at the micron level. This is somewhat surprising since, in comparison to the above mentioned X-ray and electron microscopy methods, CLSM allows large areas to be scanned in a relatively short time, is less destructive, easier to operate and can be accessed at a lower cost.

Our data provide an accurate visualization of the porosity network in 3D, with high spatial resolution over statistically representative sample volumes, which allows re-assessing the porosity in dentin with a higher precision than has been obtained so far. Using those measurements, we provide a geometrical description of the porous structure, introducing new quantitative microstructural parameters to describe the multiscale tubule branching. We show, using simple simulations, that such geometrical characteristics can greatly affect the mechanical properties of the dentinal substrate. This suggests that more realistic microstructures including the geometrical characteristics of the porosity should be used for accurate modeling of tooth biomechanical function.

2. Materials and methods

2.1. Sample preparation

Three non-carious human third molars were used for this study. The teeth were obtained following informed consent according to the protocols approved by the review board of the Dental Faculty of Paris-Descartes University at 4 °C from young donors of 15–20 years. After cutting with a diamond saw (Presi Mecatome T210), the samples were reduced to a thickness of 200 ± 10 μm by polishing on both sides using 4000 grade SiC abrasive papers (Presi, Minitech 233). Our embedding protocol ensures that only the outer surface of the teeth was in contact with the resin to facilitate the cutting (by avoiding direct contact between the tooth and sample holder and allowing more accurate orientation with respect to the saw) and polishing (by fixing the sample on the metallic sample holder by gluing only the surrounding resin with cyanoacrylate glue) steps without obstructing the porosity. The sample sections of S1, S2 and S3 were then stained in glycerol with 0.02 wt% Rhodamine B (RhB) for 48 h at room temperature and mounted between glass slides using the same medium. S1 was used to establish the proof of feasibility of the study and to perform the morphological observation and quantification (Section 3.1). S2 and S3 were used to confirm the findings revealed by the study of S1 and to investigate the porous network variation with location and depth (Section 3.2.2).

2.2. Light microscopy

The samples were first observed by transmission light microscopy (Olympus IX71) with or without cross-polarizers, in order to choose the locations to be imaged.

2.3. Confocal laser scanning microscopy (CLSM)

The polished sections were observed with a motorized inverted confocal laser scanning microscope (SP8, Leica). A 40× oil immersion lens (1.3NA) was used and glycerol was chosen as a good compromise between index matching to minimize the aberrations and artifacts with the objective and efficient mounting medium for the fluorescent die. The RhB was excited at 561 nm and the fluorescence emission was collected between 566 and 636 nm with a hybrid detector. Brightfield transmission images were simultaneously acquired on a second channel using a dedicated photomultiplier. Five locations per sample were imaged at the DEJ: two in the distal and mesial quadrants at the basis of the cervical third of the crown, two in the distal and mesial quadrants in the middle third of the crown (approximately at the axial position of the pulpal horns) and one in the middle of the occlusal third of the crown. For each location, a region of $387.5 \times 387.5\ \mu m^2$ in plane and 35 μm in depth was scanned in steps of 189.3 nm laterally and 350 nm axially. The theoretical resolution (in the absence of aberrations) was 230 nm (lateral) and 1.0 μm (axial).

2.4. Scanning electron microscopy (SEM)

To have a comparative reference value for the tubule size and porosity, S1 was observed by SEM using a Helios 660 (FEI) in back-scattered electron (BSE) collection mode to obtain a map of the chemical contrast. The sample (obtained following the protocol described in Section 2.1 and previously observed by CLSM) was