2850. Nondestructive Submicron 3D Interrogation of Dentine Using Nanotomography

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Introduction

Dentine hypersensitivity is a common, painful condition that is thought to affect up to 20% of the adult population, and it is generally believed to result primarily from the exposure of dentine either following loss of the protective enamel layer or via gum recession.

The most pronounced morphologic characteristic of human dentine is its tubular structure. Dentine tubules have diameters of 1–5 μm and connect the pulp to the enamel dentine junction, and in healthy persons these tubules are filled with fluid. According to hydrodynamic theory, changes in dentinal fluid flow play an active role in the transmission of pain stimuli across dentine to the underlying neurons. In order to design tailored antisensitivity actives, the ability to interrogate dentine nondestructively is paramount.

Computed tomography (CT) is a widely utilised technique that permits the three-dimensional (3D) visualisation of objects. In conjunction with noninvasive analytical techniques, the method provides a powerful way of interrogating the 3D structure of objects nondestructively. However, until recently all CT studies on dental hard tissue have been limited to an optimum resolution of 10–100 μm, which is insufficient for examining the intricate submicron structure of dentine.

Objective

The aim of the study was to assess the capability of a novel ultra-high-resolution X-ray CT to interrogate the submicron structure of dentine nondestructively.

Methods

Dentine sample preparation

Dentine slices, 300 μm^3 in size, were taken from the coronal section of unerupted human third molars. All teeth were supplied with informed patient consent and in accordance with current human tissue acquisition legislation. The samples were sterilised and polished. The organic smear layer and smear plugs were removed by etching in 10% w/w citric acid for 2 minutes.

Submicron high-focused X-ray CT

The SkyScan NanoCT 2011 (Aartselaar, Belgium) was employed. The system has a sealed microfocus X-ray tube offering submicron spatial resolution. All images were obtained with an X-ray source operating at 25.0 kV and 200 μA, with an exposure time of 2000 ms. Six hundred projection micrographs were obtained with a shift of 0.5 degrees between each image.

Results

X-ray CT of dentine at 295-nm resolution

- **Figure 1A** shows a single transaxial X-ray CT micrograph for a section of dentine obtained from just above the root horn. Numerous homogeneously dispersed elliptical features, distinguished by their contrast and hence low mineral density, were observed. These features were approximately 2–5 μm in diameter, at a density equivalent to 10^6 cm^{-2}.
- **Figures 1B** and **1C** show coronal and sagittal slices generated from the two-dimensional (2D) transaxial micrograph.

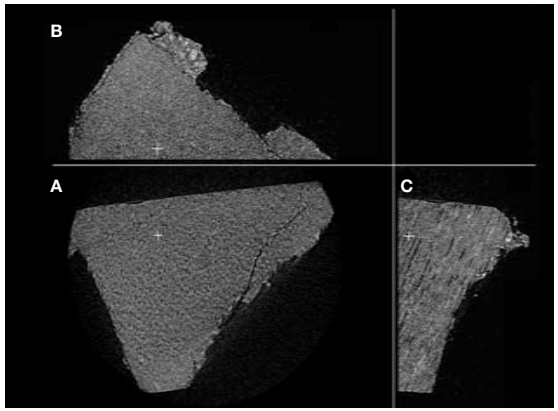

Figure 1. Coronal (B) and sagittal (C) 2D re-slices from the 3D transaxial data set (A).

The utility of CT permits sample sections to be recreated in any plane. These so-called 2D re-slices from the 3D data set allow comparisons between points in 3D space (as demonstrated by cross-hairs). The 2D re-slices demonstrate that the circular features observed in the transaxial image form highly orientated cylindrical features extending throughout the sample.

- **Figure 2** depicts a 3D-rendered image obtained from a defined section of the sample. The highly orientated nature of the tubules is observed.

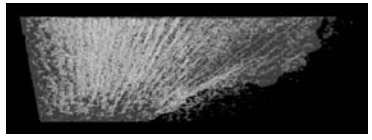

Figure 2. Rendered 3D image of dentine. For clarity the mineral matrix of the dentine has been made semi-transparent.

Impact of etching on dentine microstructure

Acid etching, which has been shown to be an effective method for removing the smear layer, has also been shown to remove the mineral content of intertubular dentine, resulting in a widening of the tubule cross-section.

- **Figure 3A** shows a coronal re-slice from the surface of the acid-etched dentine.
- **Figure 3B** shows a coronal re-slice at a depth of 20 μm into the sample.
- **Figure 3C** shows the corresponding sagittal image detailing the relative vertical positions of the two coronal images labelled **(i)** and **(ii)** and the transversely sectioned dentine tubules labelled **(iii)**.

The micrographs in **Figures 3A** and **3B** demonstrate that the diameter of the tubules at the surface is approximately 30% larger than that of tubules found deeper into the sample. These observations are in agreement that acid exposure results in widening of the tubule diameter.

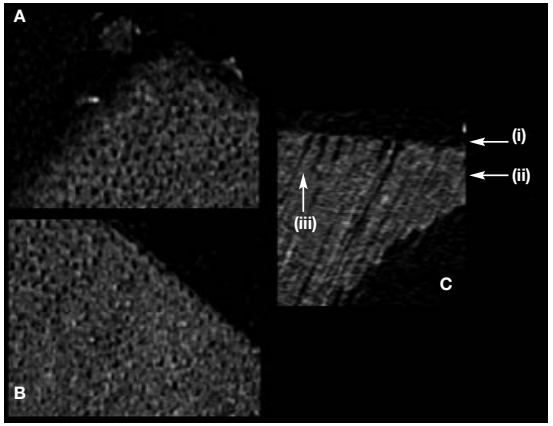

Figure 3. Transaxial micrographs obtained from the top most dentine surface (A) and 20 μm into the surface (B). Image C details a sagittal 2D re-slice.

Conclusion

Individual dentine tubules have been visualised nondestructively using ultra-high-resolution X-ray CT for the first time. The utility of this technique for interrogating individual dentine tubules has been demonstrated.