Characterization of the dentin microstructural components: a FIB-SEM analysis

U. MUENDI[1], T. REISS[1], E. DURSUN[2, 3], E. VENNAT[1, 2, a]

[1]MSSMat, CNRS, Centrale-Supélec, Université Paris-Saclay, Gif-sur-Yvette, France.
[2]URB2i, Université Paris Descartes, Montrouge, France.
[3]Hôpital Albert Chenevier, Créteil, France.

[a]elsa.vennat@centralesupelec.fr

Introduction

Dentin is the main tissue of the tooth. It is composed of peritubular dentin (PTD), intertubular dentin (ITD) and tubules (T). This microstructure has been mainly studied in 2D using optical microscopy [1], Scanning Electronic Microscopy (SEM) [2] and Atomic Force Microscopy (AFM) [3]. Among the few 3D studies of its architecture, tubules have been observed and quantified within the first 350 µm from the dentin-enamel junction (DEJ) using confocal laser scanning microscopy (CLSM) by Vennat et al. [4] It has been shown that the tubules have a complex geometry with many channels connecting them. Even in the localized area near DEJ, dentin shows properties gradients. In order to widen the knowledge of the dentinal microstructure, there is a need to investigate the three components of dentin (PTD, ITD and T) within the whole dentinal tissue.

As FIB-SEM allows imaging the three components of dentin in 3D, a FIB-SEM study has been led in order to investigate their organization in 3D. FIB-SEM has already been used on dentin [5], [6] but we argue that more parameters can be extracted from the data obtained, especially concerning the PTD and ITD and the objective of this paper is to prove it.

Materials and Methods

Samples. A human third molar was used in this study. The tooth was embedded in an epoxy resin and cut into four quarters. A quarter was selected and observed by light microscopy to locate the area to study in middle dentin.

FIB-SEM. Image acquisition was performed by using a FIB-SEM (FEI Helios 660). A protective carbon layer was deposited on the sample. The chosen area of 25 x 25 x 25 µm was isolated. Then, the BSE detector was used to image each layer of the sample. A gallium ionized beam was used to remove the sample layers one by one (Fig. 1), with a 20 nm distance in between two slices.

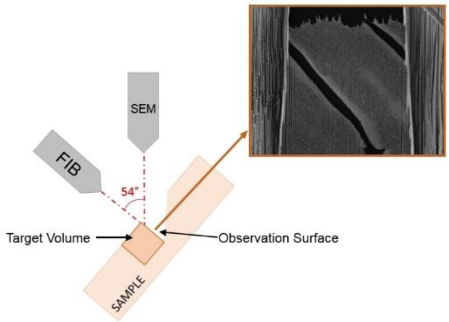

Figure 1 : Scheme of FIB-SEM "Slice and View" principle.

Image Analysis and 3D Visualization. The images obtained from FIB-SEM were treated and analyzed with ImageJ [7] (Fig. 2). 3D visualization was also performed with ImageJ.

Image Processing

Contrast and Noise. The images contrast was improved by using *Enhance Contrast* tool. Then, the "Curtaining Effect" or "Waterfall Effect" [8], was reduced by using the *Bandpass filter* tool.

Binarization. The stack was duplicated in three stacks, one for each dentin components (PTD, ITD and T). Then they were thresholded to separate each dentin component by thresholding. A median filter was applied. The stack containing all the information (on T, PTD and ITD) was obtained by using the *Merge channels* tool. Four stacks were finally obtained: T-Stack, PTD-Stack, ITD-Stack and Composite-Stack.

Measurements

Ratios of dentin components. To assess the porosity (that is to say the tubule ratio) of the chosen area, T-Stack was selected. The *MultiMeasure* tool was used with %area selected in *Set Measurement*. Ratios of PTD and ITD were obtained using the same method on the consistent stacks.

Density and Diameters. On the T-Stack, the *Reslice* tool was used to make the slices perpendicular to the tubules. Then the *Analyse Particles* tool was used. It allows the assessment of the tubule density and diameters.

3D Visualization. The dentin structure in 3D was imaged using the plugin *3D Viewer*.

Results

The porosity of the investigated area is 3 %. The ratios of PTD and ITD are 9.8 % and 87.2 %. The density is 28 300 tubules/mm² and the average tubule diameter is 1.5 µm. These values are in accordance with the values reported by Boushell *et al.* [9] and Marshall [3] for the middle dentin.

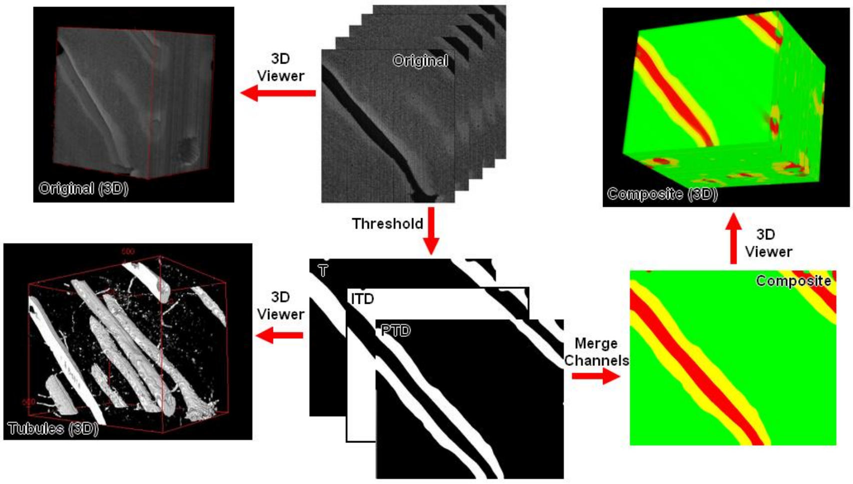

Figure 2: Scheme of the image treatment protocol.

On individual SEM images, it was observed that PTD was also surrounding the fine branches. As it was shown that lateral branches were weak areas in the tissue [4], PTD could constitute an interesting natural reinforcement. It was also noticed that the entanglement of collagen fibrils and hydroxyapatite crystals in ITD is observed. A study focused on an ITD area could improve the knowledge on the microstructure at the lower scale.

Conclusion

FIB-SEM with “Slice and View” method allows imaging in 3D a sample area with high resolution. Here, FIB-SEM has been proved to be able to image the three microstructural components of dentin (T, PTD and ITD). Some microstructural parameters of interest such as porosity, tubule diameter and ratio of PTD have been assessed. It was also found out that fines branches (constituting a secondary porous network) are surrounded by PTD: this has obviously an impact on the mechanical behavior of the tissue. A finite element study will be led to quantify this effect.

Here, the studied area is located in middle dentin but we argue it should be done in different areas in dentin to quantify the graded microstructure of dentin. Then, using different complementary means enabling 3D visualization such as FIB-SEM, CLSM and also nano-CT, a more comprehensive knowledge of the dentinal tissue could be reached.

References

[1] I. A. Mjör and I. Nordahl,: *The density and branching of dentinal tubules in human teeth*, Arch. Oral Biol., Vol. 41, nº 5 (1996) p. 401–412.

[2] W. Wang : *Caractérisation géométrique et mécanique multi-échelle de la dentine humaine*, Paris Saclay (2016).

[3] G. W. Marshall : *Dentin: microstructure and characterization*, Quintessence Int. Berl. Ger. 1985, Vol. 24, nº 9 (1993) p. 606-617.

[4] E. Vennat *et al* : *Mesoscale porosity at the dentin-enamel junction could affect the biomechanical properties of teeth*, Acta Biomater., Vol. 51 (2017) p. 418-432.

[5] J. S. Earl *et al.* : *Characterization of dentine structure in three dimensions using FIB-SEM*, J. Microsc., Vol. 240, nº 1 (2010) p. 1-5.

[6] S. Gaboreau *et al* : *Optimization of pore-network characterization of a compacted clay material by TEM and FIB/SEM imaging*, Microporous Mesoporous Mater., Vol. 224 (2016) p. 116-128.

[7] C. A. Schneider *et al* : *NIH Image to ImageJ: 25 years of image analysis*, Nat. Methods, Vol. 9, nº 7 (2012) p. 671.

[8] J. H. Fitschen *et al* : *Removal of curtaining effects by a variational model with directional forward differences*, Comput. Vis. Image Underst., Vol. 155 (2017) p. 24–32.

[9] L. W. Boushell *et al* : *Clinical significance of dental anatomy, histology, physiology, and occlusion* In : H. O. Heymann *et al* : *Sturdevant’s Art & Science of Operative Dentistry 6th edition* edited by Elsevier Health Sciences (2014).