The spatial arrangement of tubules in human dentin

J. H. KINNEY1*, J. OLIVEIRA2, D. L. HAUPT3, G. W. MARSHALL1, S. J. MARSHALL1
1Division of Bioengineering and Biomaterials, Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, San Francisco, CA 94143-0758
2Battelle Pacific Northwest Lab, Richland WA 99352
3Lawrence Livermore National Laboratory, Livermore, CA 94551
E-mail: jkinney@itla.ucsf.edu

We applied two-dimensional numerical methods to describe the spatial arrangement of tubules in human dentin. The methods considered were two-point correlation functions, entropy-like measures, and angular distributions between nearest neighbors. The correlation functions were based on Fourier transform methods. The latter two approaches were based on stochastic geometry, and involved developing the Delaunay tessellations of the tubule patterns and their dual Voronoi diagrams. We discovered that for analyzing the distribution of tubules the geometric methods of lattice tessellations were more sensitive to structural order of the tubules than were Fourier-based schemes. Analysis of the data indicated that dentinal tubules are highly ordered in normal dentin.

© 2001 Kluwer Academic Publishers

1. Introduction

Dentin is the hard, mineralized tissue in teeth that lies between the exterior enamel layer and the pulp. The most striking morphological feature of dentin is the tubule, which is a continuous cylindrical channel approximately 1–2 μm in diameter that runs between the dentin-enamel junction and the pulp. Tubules are surrounded by highly mineralized cuffs of peritubular dentin, and are imbedded in a matrix of mineralized collagen called intertubular dentin. Previous studies have described the distribution of tubules in human dentin in terms of the number density of tubules, tubule radius, and width of the peritubular cuff [1, 2]. This information has been used to interpret positional differences in the physical properties of dentin such as hardness and elastic modulus [3, 4].

In addition to the role that tubule density, orientation, and distribution might have in affecting mechanical behavior, tubule organization appears to play a significant role in demineralization and etching. In a previous study, we were able to explain the results of a controlled demineralization experiment in terms of the tubule density and orientation with respect to the etching direction [5]. We hypothesized that the etching rate and resulting surface morphology were controlled by the density and orientation of the tubules, which depend upon location within the dentin.

There are other reasons for a quantitative measure of tubule organization. Because the tubule lumens are the remnant homes of the odontoblast processes, knowledge of their organization might be helpful in understanding dentinogenesis or disease processes. A characteristic trait in dentinogenesis imperfecta, a heritable disorder of dentin, is few and irregular tubules in a disorganized collagen matrix. These histological observations have not been quantitative, largely because of the lack of a framework to describe tubule organization.

In cross section, the tubules look like an ensemble of circular holes. There are four generic ways that the tubules might be distributed on the cross section: (a) ordered on a periodic lattice; (b) disordered about periodic lattice sites; (c) clustered; or (d) randomly positioned within the intertubular matrix. Number density and size alone do not distinguish among these spatial distributions, so previous work is of limited value for understanding the spatial organization of the tubules.

In this study, we explored several two-dimensional numerical methods of describing point pattern distributions, and applied these methods to evaluate the tubule distributions near the dentin-enamel junction (primary outer dentin). The methods considered were two-point correlation functions, entropy-like measures, and angular distributions between nearest neighbors. The correlation functions were based on Fourier transform methods. The latter two approaches were geometric, and involved developing the Delaunay tessellations of the tubule patterns and their dual Voronoi diagrams. Though the principal focus was on the spatial arrangement of dentin tubules, the point pattern techniques described in this study are applicable to any materials problem where spatial arrangement of microstructure is important.

*Author to whom correspondence should be addressed: Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, 707 Parnassus Ave, MS 0758, San Francisco, CA 94143-0758.

2. Materials and methods
Three freshly extracted third molars from females aged 19–23 were used in this study. The teeth were sterilized with gamma irradiation prior to use [6]. Each tooth was mounted in polymethylmethacrylate and radiographed to determine the location of the dentin-enamel junction with respect to the occlusal surface. Then each tooth was sectioned parallel to the occlusal surface and just below the dentin-enamel junction using a modified water-cooled low speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL). The location of the specimens and the approximate orientation of the tubules are shown in Fig. 1. The cut disks were 0.75 mm thick. They were abraded flat on silicon carbide paper (grits 400, 600, and 800), and then polished using successive aluminum oxide slurries of 1.0-, 0.3-, and 0.05-µm particle size. Between polishing steps, each specimen was ultrasonicated to remove debris.

Each dentin disk was sectioned into four parts, and fiduciary marks were cut near the periphery close to the dentin-enamel junction. Each section was studied with a wet scanning electron microscope (modified ISI SX-40A SEM), which permitted imaging without prior desiccation or coating [7]. The instrument operated under a low pressure of approximately 10–100 mTorr, which suppressed charging but led to eventual desiccation of the sample. However, for mineralized dentin, no significant morphological changes have been detected during drying [8]. Selected areas of the four quadrants nearest the dentin-enamel junction were imaged and photographed at 20 kV and magnification of 2000 ×. The SEM images were digitized into 256-level gray scale images containing 512 × 384 picture elements (pixels). Magnification errors were minimized by checking microscope calibration against known standards, by viewing samples in an untitled configuration at the same working distance, and by determining the errors in magnification at different working distances.

2.1. Image processing
The SEM images were converted from gray scale to binary format with the pixels contained within the tubule lumens defined as occupied (pixel value of 1) and the dentinal hard tissue defined as unoccupied (pixel value of 0). These binary images were then segmented with a cluster-labeling algorithm so that each tubule was uniquely labeled (e.g. 1, 2, 3, . . ., N). The number density of tubules, defined as the number of tubules per unit area, was calculated after labeling.

The two-point correlation functions were calculated for each of the binary images. Next, the center of gravity of each tubule lumen was calculated and used to define a point lattice for the tubule distribution. These lattice points were used as vertices of a Delaunay tessellation from which a Voronoi diagram was generated. The tessellations were subsequently analyzed according to methods described below. This analysis procedure, as followed for a typical SEM image of dentinal tubules, is shown in Fig. 2.

2.2. Test patterns
Four generic, discreet point patterns were created to explore the range of tubule distributions that might be observed in dentin. These patterns were created on a rectangular image space containing 512 × 384 pixels (corresponding to the digital image of the SEM). The generic patterns were: (1) a square (periodic) lattice of \( m \times n \) points 50 pixels apart (\( D_o = 50 \)); (2) a disordered lattice obtained by randomly displacing the tubules in a circular region about the periodic lattice sites of the \( m \times n \) square grid; (3) a random lattice that approximated the complete spatial randomness of a Poisson point process; (4) groupings of points clustered about randomly positioned sites. Examples of these generic patterns are provided in Fig. 3.

Multiple images of the disordered, clustered, and random point patterns were created by varying the starting seed value of the random number generator. The disordered patterns were created by randomly distributing points in a circular region centered about the square lattice sites in terms of a maximum radius, \( D/D_o \). The maximum radius ranged from 0.1 to 1.0 in equal increments. For each value of \( D/D_o \), four point patterns were created starting with different seed values of the uniform random number generator. The root mean square tubule displacements differed slightly for each seed value, thereby allowing us to obtain a statistical measure of the accuracy of the methods used to describe the point pattern distributions.

Ten random point patterns were created with a uniform random number generator, the probability level for site occupancy set to approximate the range in tubule density seen with the SEM images (60–100 tubules per image). The same random number generator was also used to generate the lattices for the clustered patterns. For the clustered patterns, the probability level for site occupancy was set to provide anywhere from six to ten randomly distributed lattice sites per image. Ten points were then randomly distributed about each site in the same manner as used to construct the disordered point patterns, with the maximum displacement radius \( D = 0.5D_o \).

2.3. Data analysis
The first two spatial correlation functions, \( S_n \), are defined as