A morphometric analysis of the cross-sectional area of dentine occupied by dentinal tubules in human third molar teeth

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Summary

The number and the mean percentage tubular cross-sectional area of dentinal tubules per square millimetre were calculated in specimens of coronal dentine of 13 intact human third molar teeth from patients 18 to 28 years of age. The dentine was fractured at various known distances from the dentino-enamel junction. Near the dentino-enamel junction the number of tubules per square millimetre was 22,000 and the mean tubular cross-sectional area was 3.6%. Midway between the pulp wall and the dentino-enamel junction the number of tubules was 37,000 mm² and the mean tubular cross-sectional area was 6.2%. Close to the pulp the number of dentinal tubules was 48,000 mm² and the mean cross-sectional area of tubules was 10.2 percent. The number of tubules per square millimetre more than doubled and the area occupied by tubules increased threefold from the dentine close to the dentino-enamel junction, to that close to the pulp. These differences in tubular patterns at different depths in dentine are clinically significant in dentine permeability, the treatment of traumatized teeth, and pain transmission in dentine.

Keywords: dentinal tubules, dentine.

Introduction

Dentine forms the bulk of the tooth and is comprised of a network of collagen fibres and hydroxyapatite crystals through which pass dentinal tubules. Each tubule is formed by, and contains some portion of, a process from a cell from the odontoblast layer, which lies on the pulpal surface of dentine. The ratio between surface area of the dentino-enamel junction and that of the pulp is about 5:1 (Orban 1986). Accordingly, the tubules are further apart in the peripheral layer and more closely packed nearer the pulp. As the odontoblast retreats from the dentino-enamel junction during dentine formation, some activity produces a ring-shaped hypermineralized zone surrounding each tubule. Peritubular dentine is wider close to the dentino-enamel junction and narrower, or even absent, as the pulp is approached. Accordingly, the dentinal tubules are narrower at their outer ends and wider near the pulp.

While published data are available for dentine in general, not as much attention has been paid to tubule number relative to dentine depth. Ketterel (1961) observed that near the enamel, tubule numbers ranged from 9000 to 24,000 mm² and 64,000 to 70,000 tubules mm² were present 1 mm and less from the pulp. Intermediate areas showed about 35,000 tubules mm². Tronstad (1973) reported values of 7000 tubules mm² close to the dentino-enamel junction and 60,000 tubules mm² close to the pulp, while Brannstrom & Garberoglio (1972) reported values of 20,000 tubules mm² close to the dentino-enamel junction and 45,000 tubules mm² close to the pulp. A consistent finding in all of these studies has been that the number of dentinal tubules in a given cross-sectional area of dentine increases significantly as the pulp is approached.

The total cross-sectional area of dentine occupied by tubules is a function both of the numbers of tubules and of individual tubule diameter. Recent studies have shown this is an important parameter to consider when estimating the rate of diffusion of materials through dentine, as the rate of diffusion through dentine is directly proportional to the cross-sectional area of dentinal tubules (Pashley 1988). Tubular cross-sectional area may also be of importance when the hydrodynamic theory of pain in dentine is considered, and clinically when consideration is given to the treatment of fractured teeth. In deeper fractures not only is the fractured surface closer to the pulp but a greater number of larger dentinal tubules are involved in the fractured surface.
Two studies have estimated the cross-sectional area of tubules in human dentine. Hoppe & Stuben (1965), using decalcified human material, estimated that the total tubular volume is 21% of the total coronal volume of dentine, being 27.7% near the pulp and 19.1% near the dentino-enamel junction. Conversely, Garberoglio & Brannstrom (1976), in a mixed selection of undecalciﬁed human teeth, estimated that the tubular area was 10% of the total coronal area of dentine, being 28% near the pulp and 4% near the dentino-enamel junction.

In reviewing the literature on numbers of dentinal tubules and on tubular cross-sectional area at various levels in teeth, the authors considered that a further study investigating this aspect of dental anatomy was warranted. It was further considered that the use of scanning electron microscopy (SEM) coupled with morphometric analysis would give an accurate analysis of the true cross-sectional area of dentinal tubules at the various levels. In this study fractured undecalciﬁed sections of dentine were examined. The fracture technique was used to eliminate the possibility of tubule blockage due to grinding (Pashley 1985, 1988). Undecalciﬁed dentine was used as there is evidence that peritubular dentine is soluble to a large extent during decalcification (Isokawa et al., 1970a, b, Brannstrom & Garberoglio 1972).

Materials and methods

One-hundred and sixty specimens were obtained from 13 intact human permanent third molar teeth which had radiographic evidence of completed root formation. These were taken from patients aged between 18 and 28 years.

To obtain specimens for examination the molar teeth were fully split mesiodistally using a plumbers tube cutter (Fig. 1). An 0.5 mm diamond disc was then used to reduce each half of the tooth parallel to the fractured surface to a width of 2 mm. cutting from the outer (buccal or lingual surface) leaving the fractured surface intact (Fig. 2). This splitting and sectioning process was used in order that two longitudinal sections could be obtained from the middle of each tooth.

The two mesio-distal longitudinal slices of tooth structure were further sectioned in a bucco-lingual direction into smaller rectangular blocks using an 0.3 mm diamond disc. One such section is illustrated in Fig. 3. The direction of cutting was from the enamel surface towards the pulp. The angle of the disc was varied for each block so that the disc was held as parallel as possible to the tubules in the region of the tooth being sectioned. The direction of tubules in each part of the tooth was determined by a pilot study. Several sections were prepared from each tooth. Specimens were prepared from the occlusal surface of the tooth only and not from the marginal ridge area or from the root surface.

To produce specimens from these smaller blocks, a line (MN) (Fig. 4) was drawn along the dentino-enamel junction with a sharp diamond pencil. A second line (XY) was drawn at right angles to this, passing from the
Fig. 4. Diagrammatic representation of the fracturing and measuring technique used to obtain the specimens at various levels in the tooth. MN, line drawn along the dentino-enamel junction. XY, line drawn from the pulp at right angles to MN intersection at point A on the dentino-enamel junction. A, point of intersection of two lines at the dentino-enamel junction. B, plane of section. AB, distance from dentino-enamel junction.

pulp to meet the first line at the point A on the dentino-enamel junction. The distance (YA) was measured with a Combike digit outside micrometre. The average length of this distance was 3.4 mm. The enamel was then fractured from the tooth block using wire cutters. The dentine block was then fractured transversely close to the dentino-enamel junction at a new level B to provide a cross-sectional specimen for examination. The distance YB was measured and the distance YA–YB, being the distance the specimen was from the dentino-enamel junction, was determined and recorded. Each specimen was numbered and placed in 50% alcohol for processing after being marked to facilitate correct orientation in the microscope. Subsequently, the original block was refractured and remeasured to give separate, additional specimens at different known levels. Three or four specimens were obtained from each separate block. Several blocks were obtained from each tooth. These were assigned in order according to the distance from the dentino-enamel junction and then divided by level into six groups:

Group 1. Less than 0.5 mm.
Group 2. From 0.5 mm to less than 0.9 mm.
Group 3. From 1.0 mm to less than 1.4 mm.
Group 4. From 1.5 mm to less than 1.9 mm.
Group 5. From 2.0 mm to less than 2.4 mm.
Group 6. From 2.5 mm to less than 3.0 mm.

**SEM preparation**

Ten specimens from each group were used in the study. Each specimen was routinely dehydrated through graded alcohols (50% to 100%) for 3 days in each, then immersed in small amounts of absolute alcohol in a Petri dish and placed in a silica gel desiccator for 3 days until all the alcohol had evaporated from the specimen. Each specimen was mounted on a 7 mm aluminium stub, with the fractured surface as close to the parallel as possible to the surface of the stub, gold-coated and examined in a Jeol 35C Scanning Electron Microscope (Tokyo, Japan) operated at an accelerating voltage of 10 kV. Five separate photographs were taken of different areas within each specimen, making a total of 300 photographs (six groups × 10 specimens × five fields). The negatives were printed at exactly four times magnification to facilitate the area measurement. The prints were made with black borders to ensure that the whole of the negative was printed. Before each session on the microscope the recording magnification was criterion referenced by using standard-sized plastic spheres (0.48 μm) diameter. To determine the final magnification of the photographic prints, the diameter of the standard-sized plastic spheres were measured on the photographic prints with a travelling microscope and these diameters were compared with the original diameter of the plastic spheres.

**Morphometry**

To calculate the number of dentinal tubules per mm$^{-2}$, all the tubules contained in one photograph were counted. Where tubules intersected the edges of the photograph, only those that intersected the top and right-hand margins were included in the total. The number of tubules per square millimetre was then calculated by the formula:

$$\text{Number of tubules mm}^{-2} = \frac{\text{Number of tubules per photograph} \times \text{magnification}^2}{\text{Area of photograph in mm}^2}$$

To calculate the fraction of the area occupied by dentinal tubules a regular point grid was applied (Saunders & Kahles, 1942). The grid was constructed such that there were 100 regularly spaced points on a 13.5 × 25 cm rectangular area on a sheet of clear...
acetate film, points being arranged in rows 1.5 cm apart. Along each row the points were 2.5 cm apart. Each second row was staggered 1.25 cm to the left of the row above. The grid was enclosed in a solid border (16.8 × 26 cm) which corresponded to the size of the photographic print being examined. To ensure that all the points were located on some part of the photograph, the clear acetate sheet was overlayed on the enlarged photograph so that the lower solid border and the right-hand solid borders corresponded to the edges of the photograph. When a point fell on the edge of a tubule it was recorded as being within the tubule. The number of points which intersected dentinal tubules were counted. Each photograph was measured twice and the mean of the two readings was recorded.

The percentage of the area of the photograph that was occupied by dentinal tubules was calculated by the formula:

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\text{Percent of the area} = \frac{\text{Number of points intersecting dentinal tubules} \times 100}{\text{Total number of points}}
\]

Results

In transverse section, the dentinal tubules were typically found to have well-defined intertubular areas of dentine and variable amounts of peritubular dentine. Both the number of the dentinal tubules and the area occupied by the tubules increased from the dentino-enamel junction to the pulp (Fig. 5).

The number of tubules mm\(^{-2}\) found at each depth is contained in Table 1. The mean number of tubules varied from 22 000 mm\(^{-2}\) at the dentino-enamel junction to 49 000 mm\(^{-2}\) closer to the pulp. Midway between the pulp and the dentino-enamel junction, the mean number of tubules varied from 34 000 to 37 000 mm\(^{-2}\). When statistical comparisons were made between one group and another, the actual numbers of tubules per enlarged photograph were used rather than the number of tubules mm\(^{-2}\). Comparisons were made using the Students \(t\)-test. Values were calculated for N1 + N2-2 degrees of freedom. No significant difference was established between groups 1 and 2, groups 3 and 4, and groups 5 and 6. There were statistical differences at least at the \(P<0.05\) level between all other pairs of groups. Thus, tubule number varies significantly between three depths: less than 0.5–0.9 mm, 1–1.9 mm and 2–3 mm.

The percentage area of dentine occupied by dentinal tubules increased progressively from the dentino-enamel junction to the pulp. Values ranged from 3.63% near the dentino-enamel junction to 10.2% near the pulp. The mean values from each group, together with the standard deviation and the range of values obtained are

![Fig. 5. Scanning electron micrographs of fractured cross-sections of dentine illustrating the varied morphology of the tubular pattern at various levels in the tooth. A, 0.3 mm from the dentino-enamel junction; at this level tubules are narrow and are surrounded by a distinct border of peritubular dentine. (× 3400) B, 1.4 mm from the dentino-enamel junction; at this level tubules are wider and more numerous than at the dentino-enamel junction. A broad zone of peritubular dentine is visible (× 3400) C, 2.6 mm from the dentino-enamel junction; at this level, tubules were large, numerous, and more irregular in outline. Intertubular dentine is less and little if any peritubular dentine can be seen. (× 3400)
illustrated in Table 2. The range of the results within each group was large. There were significant differences at the \( P < 0.05 \) level between all pairs of groups except between groups 1 and 2, groups 3 and 4, groups 5 and 6. Thus, as with tubule number, dentinal tubule area per cent varied significantly at three depths.

### Discussion

In substantial agreement with previous studies, the number of dentinal tubules was found to increase significantly between three levels from the dentino-enamel junction towards the pulp. While methodologies may have differed between the manner of reporting in this and previous studies, nonetheless 22–24,000 tubules mm\(^{-2}\) near the enamel falls within the range of 9,000–24,000 given by Ketterel (1961) and is close to the 20,000 tubule mm\(^{-2}\) approximation determined by Brannstrom & Garberoglio (1972) for this zone. At 1–1.9 mm from the dentino-enamel junction, 34,000–37,000 tubules mm\(^{-2}\) agrees with the approximate figure given by Ketterel for the intermediate area. At 2–3 mm from the dentino-enamel junction, 49,000–48,000 mm\(^{-2}\) compares favourably with 45,000 mm\(^{-2}\) found close to the pulp by Brannstrom & Garberoglio (1972) but is not as high as the 64–70,000 mm\(^{-2}\) found by Ketterel (1961) less than 1 mm from the pulp. This clearly reinforces the clinically important finding that over twice as many tubules mm\(^{-2}\) are found nearer the pulp than nearer the enamel. This finding can be explained by the convergence of tubules towards one another as they approach the pulp. The three-dimensional morphology of the coronal dentine, and of that near the pulp, reflects the changing spatial relationship of odontoblasts approaching one another as they retreat from the dentino-enamel junction.

An almost threefold difference existed between the area occupied by the dentinal tubules close to the dentino-enamel junction compared with the area close to the pulp. Garberoglio & Brannstrom (1976) reported a value of 4% at the dentino-enamel junction, which compares favourably with the 3.63% found in this study. The values reported in this study (10.2%) close to the pulp is lower than that reported by both Hoppe & Stuben (1965) (27.7%) and by Garberoglio & Brannstrom (1976) (28%). The selection of mixed tooth types by Garberoglio & Brannstrom (1976) could account for variations in differences between their study and this present one. Ketterel (1961) estimated that the dentinal tubules accounted for 4% of the dentine at the dentino-enamel junction for all age groups studied (15 to 30 years, 30 to 50 year and 50 to 75 years), but that towards the pulp, values ranged from 79% in the younger age groups to 42% in the older age groups. Hoppe & Stuben (1965) reported much higher values (19%) close to the dentino-enamel junction. However, as they used decalcified material and as decalcification removes peritubular dentine, it would be expected that values would be higher than in undecalcified dentine.

As the rate of diffusion of materials through dentine is directly proportional to the percentage area of the dentinal tubules (Pashley 1988), it can be surmised that there will be a threefold increase in the rate of diffusion of materials through the dentine as the pulp is approached. Diffusion of material though dentine in a shallow cavity preparation would thus be substantially less than diffusion of the same material placed in a deeper cavity preparation. Studies that describe the sequelae of isolated enamel-dentine fractures in anterior teeth indicate that pulpal complications are rare. A greater incidence of pulpal involvement occurs in teeth with deeper corner fractures, and when deep crown fractures are left untreated (Ravn, 1981). It can be extrapolated from this study that, in deeper fractures, not only would the distance from the fractured surface to the pulp be decreased directly
resulting in a greater chance of diffusion of irritants into the pulp, but also a greater number of tubules of wider diameter would be involved in the fracture. The combination of these two factors would result in a markedly greater chance of increased inflammation, increased nerve excitability, and increased perception of pain in the pulp as the fractured surface approached the pulp.

The implication of the changing diameter of dental tubules at different tooth levels on the understanding of the hydrodynamic theory of pain perception in dentine is difficult to postulate. It would be expected however that pain transmission effects would vary from the dentino-enamel junction toward the pulp. At the dentino-enamel junction the tubules are narrower and hence capillary action would be greater, but fewer tubules would be affected. As the pulp is approached, tubule diameter increases. Hence, capillary action would be diminished but a large number of tubules would be affected. Which of these two facts is the more important in the transmission of a painful stimulus is hard to determine.

Values for the number of tubules and for area determinations in this study varied widely from one tooth to another and from one area of the tooth to another. This is consistent with previous studies as similar ranges of values can be seen in the data reported by Brannstrom & Garberoglio (1972). In all discussions on the morphology of dentine, attention must be drawn to the variation that occurs in normal mature teeth and also from changes that may occur from a developmental point of view as teeth mature. While an attempt was made to restrict variation as far as possible in this study by using only one type of tooth, namely third molars from a restricted age group of patients, it is acknowledged by the authors that maturation and eruption times do vary for third molar teeth and that this may explain some of the range of variation in results in this study. It would follow from the above that studies in which the sample size is small, or which view only a limited area of a given specimen, may be of little scientific value and their results need to be viewed with caution. Nevertheless, despite the variation in and/or the range of values obtained within the groups in this study, the statistical difference found both in the number of tubules mm\(^{-2}\), and the cross-sectional area of tubules between the outer (close to the dentino-enamel junction), middle, and inner (pulpal) layers of dentine must be considered of clinical importance.

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