Scientific Article

Microstructure of primary tooth dentin

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Abstract

Purpose: This study was performed to determine variations in dentin microstructure from primary anterior teeth at specific areas and depths in relation to the dentin enamal junction, (DEJ).

Methods: Ten freshly extracted, non-carious primary maxillary anterior teeth were sectioned to provide two 1.0 mm x 1.0 mm matchsticks extending from the DEJ to the pulp chamber—one each from the central and distal regions of each tooth. Slices were prepared at distances of 0.15, 0.8, and 1.45 mm from the DEJ. Following polishing, each slice was examined in a wet scanning election microscope, (SEM) and tubule density, tubular diameter, and peritubular width were determined at nine grid locations. Statistical analyses were carried out using multi-factor ANOVA, Tukey's multiple comparisons, and linear regression to compare rates of change for each parameter.

Results: Tubule numerical density consistently decreased with distance from the DEJ. Decreases of 11,800 mm^2/mm for canine distal matchsticks were significantly greater ($P<0.05$) than the rate of 4,400 mm^2/mm for canine central matchsticks. Rates for the lateral incisors were not significantly different. Tubule diameters increased with distance from the DEJ at rates of 0.28 µm/mm and 0.39 µm/mm for canines and lateral incisors, respectively, and there was a corresponding decrease in peritubular width. Microcanals or giant dentin tubules, 5-10 µm in diameter were incidently found in varying numbers in the midline of 4 of 20 teeth examined, including central and lateral incisors, but not in canines.

Conclusions: This work shows substantial differences in the microstructure of primary dentin as compared to permanent dentin, substantial differences with location, and the relatively common occurrence of microcanals. Therefore, the area of solid dentin that is available for dentin bonding is significantly reduced, accounting for reported differences in bond strength. Such differences may be important factors in tooth sensitivity, susceptibility to trauma, and caries progression.(Pediatr Dent 21:439-444, 1999)

Dentin in permanent and primary teeth has similar morphology and composition and it has been assumed that both kinds of teeth are similar in histologic structure. The findings obtained from permanent teeth have been assumed to apply to primary teeth, but some evidence suggests significant chemical and morphological differences between them.[1-3]

Restoration of primary teeth, particularly anterior teeth, is often difficult because of their small size, thinness of enamel, enamel morphology, pulpal anatomy, and rapid spread and extent of decay.[4]

Dentin bond strength comparisons between primary and permanent teeth have shown mixed results. Salama and Tao[5] found lower bond strength to primary dentin, Bordin-Aykroyd et al.[1] found higher bond strengths, while others.[6,7] found no significant differences.

Tubule diameters and tubule numerical density increase from the dentinoenamel junction (DEJ), towards the pulp, with peritubular dentin width displaying the inverse trend.[8] However, few studies have reported findings on tubule diameter and numerical density in terms of specific distances from an anatomic landmark[9] Koutsi et al.[10] found that the numerical tubule densities for primary dentin were lower than for permanent dentin in the same general locations. Studies of peritubular dentin thickness have been more limited, with Allred[11] reporting values from 1.0—2.5 µm. While information regarding permanent dentin is lacking, general information regarding primary tooth dentin is available.[8,9,12] The purpose of this study was to characterize the microstructure of anterior primary tooth dentin at specific areas and known depths in relation to the DEJ.

Materials & Methods

Ten freshly extracted, non-carious primary maxillary anterior teeth (three left canines, three left lateral incisors, three right lateral incisors, and one right central incisor) with full root development and with no visible signs of root resorption, were collected and stored in a 10% buffered formalin solution. The teeth were recovered from healthy, non-related children between the ages of three and five years. After sterilization by gamma-irradiation,[13] a 1.0 mm thick slice of tooth from the facio-lingual was cut using a water cooled saw (Buehler Isomet Low Speed saw, model#11-1180, Buehler Ltd., Lake Bluff, IL) with a 0.15 mm thick diamond blade. The slice was then sectioned to yield two matchstick-shaped pieces of dentin, 1.0 mm square and running from the DEJ to the pulp along the tubule direction. One stick came from the distal area and one came from the central region, but attempts to obtain a third mesial matchstick were not successful because of the small size of the teeth. The dentin matchsticks were imbedded in epoxy (Sty-cast, Grace Specialty Polymers, Emerson and Cuming Inc.,

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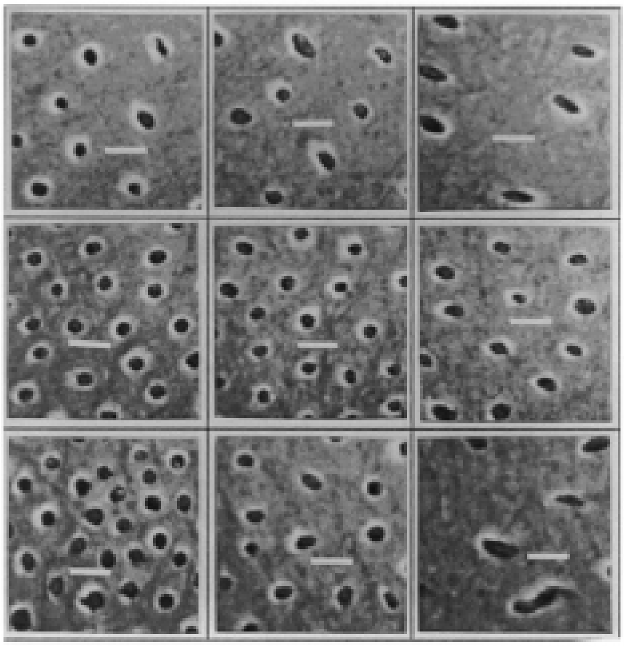

Fig 1. SEM photomicrographs depicting considerable variation of tubule number density and orientation at the centers of the nine grid squares from a single 1 mm x 1 mm square slice or disk. Bar=5 µm.

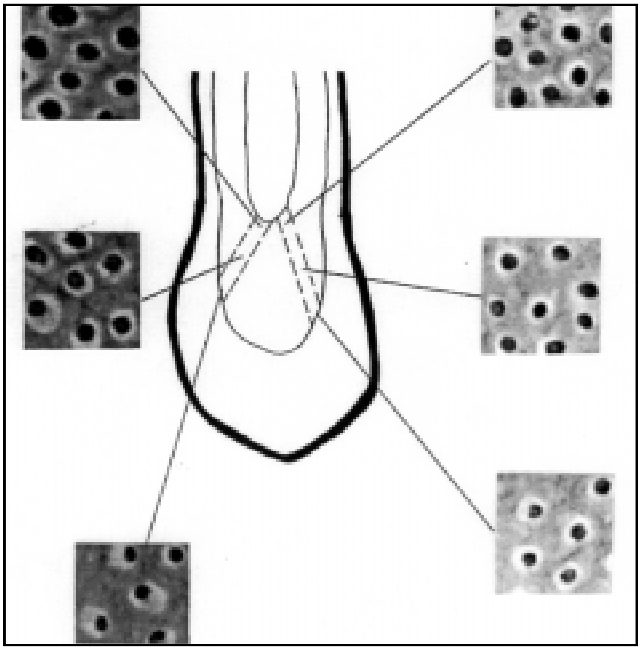

Fig 2. Photomicrographs depicting the increase of tubule number density at various dentin depths in various directions from the central and distal matchsticks.

Woburn, MA) and polished through 0.05 µm alumina slurry while maintaining the proper mesio-distal and facio-lingual orientations. A cut was made 0.5 mm from the DEJ end of the sample, producing a disk containing two squares of dentin 1.0 mm x 1.0 mm imbedded in an epoxy matrix. Subsequent disks were serially sectioned giving dentin slices at 0.65 mm intervals. Due to the short distance from the DEJ to the pulp, only three or four samples were produced for each matchstick.

The polished disks were studied in the scanning electron microscope (SEM) (ISI SX-40A modified with a CFAS system, Topcon Instruments, Pleasanton, CA) in the wet mode[14] at 20 kV. Images at 2000X were taken from nine areas in a grid for each square and labeled A-I, with A-C representing the labial aspect of the sample (Fig 1). The image analysis system (Advanced Imaging, Kevex Corp., San Carlos, CA) was optimized by manipulating the brightness and contrast to maximize the range of grey scale. SEM magnification accuracy was evaluated using a standard containing 5 µm x 5 µm squares, and was determined to be accurate within 1%. The digitized images were processed (Features II, Kevex Corp., San Carlos, CA) to maximize the grey scale range and average out the random noise. Desired features were pseudo-colored based on grey level, processed by the computer and the software automatically eliminated any remaining features containing only a few pixels.

Analyses of tubule density (number of tubules/mm^2), tubule diameters, and the tubule areas were made, and using a similar approach the areas occupied by the peritubular and intertubular dentin were colored and measured independently. Raw data from the computer-produced statistics for each image included: the number of features, and the percent field areas occupied by the features, which were normalized to 100% for the sum of tubule, peritubular, and intertubular dentin areas. Prior to normalization, the sum of the areas of all the features had to be within 5% of 100% to be acceptable. If they were not, an image analysis error was assumed due to grey scale discrepancies or poor sample preparation and the image was re-evaluated. Feature areas were calculated based on the assumptions that the tubules were circular, the peritubular dentin formed an annulus around the tubules and the intertubular dentin comprised the remainder of the images. Based on the known magnification and pixel size, image areas were converted to true areas.

The mean feature area per tubule (tubule area or peritubular area) was calculated for each image by dividing the total feature area of the tubules by the number of tubules after correction for partial tubules. The mean tubule diameter and peritubular width were calculated from these values.

Within the nine images from each square, there was a high variability of the features (Fig 1). Although there was a suggestion that characteristics were similar in some rows or columns, statistical analysis using multi-factor ANOVA (Gen-

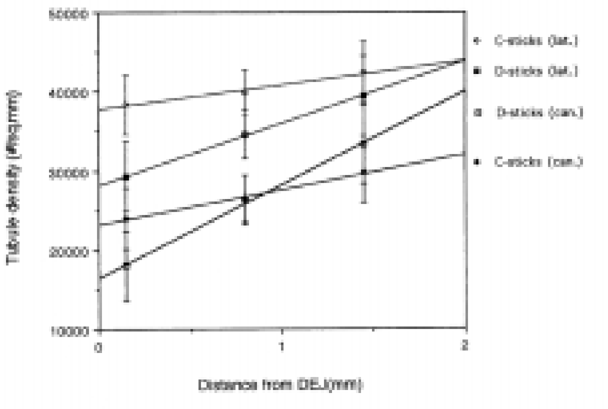

Fig 3. Tubule numerical density as a function of location for primary canines and lateral incisors separated according to location in the distal or central matchsticks. Error bars represent robust standard errors.

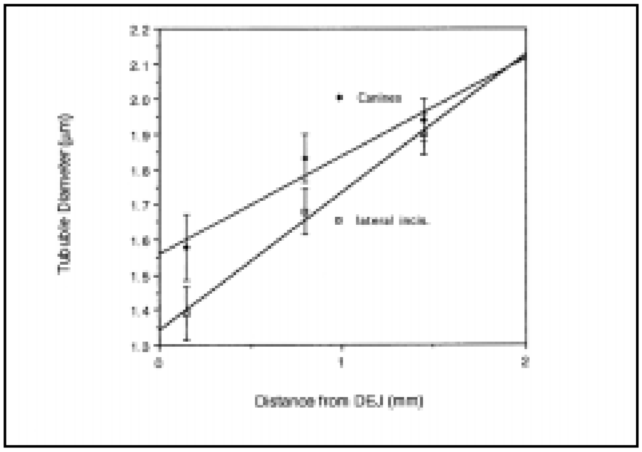

Fig 4. Tubule diameter as a function of location for the primary canines and lateral incisors. Error bars represent robust standard errors.

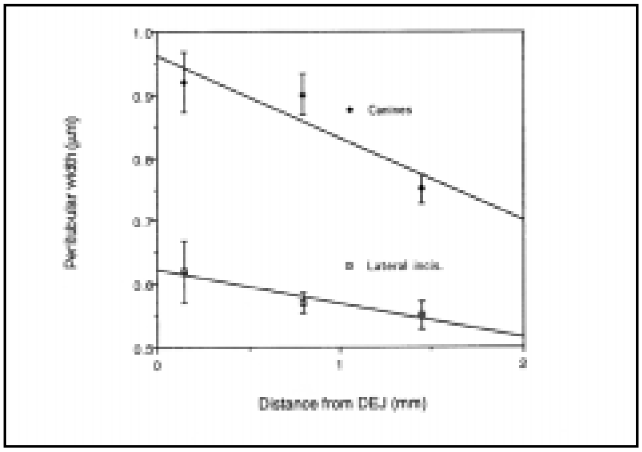

Fig 5. Peritubular width as a function of location for the primary canines and lateral incsiors. Error bars represent robust standard errors.

eral Linear Model, The SAS system) found that differences between rows and columns were not significant; therefore the measurements from the nine images at each level were averaged. Statistical analyses using a multi-factor ANOVA, Tukey's Standardized Range Test, and linear regression analysis were performed on each of the dentin characteristics separately. For the purpose of the statistical analyses, the individual central incisor was excluded as it represented a different tooth type.

Results

The numerical tubule density data are shown as a function of position in Fig 2. For both the canines and the lateral incisors, the average numerical tubule density for either direction increased as the distance from the DEJ increased. The large range of values reflects the wide variation found in all locations of all teeth. For statistical purposes, robust standard errors were used because they account for the effect of clustering. While it appeared that the numerical tubule densities in both directions within the lateral incisors were greater than those found in the canines, the only significant differences were between the central matchsticks of the canines and lateral incisors ($P<.05$). For the central matchsticks, the difference between levels 1 and 3 was statistically significant while in the distal matchsticks, the difference between each of the levels was statistically significant ($P<.05$). Due to individual tooth variation, extrapolating information from the y-intercept of the regressions is not as reliable as the results of the linear regressions would suggest. It was difficult to precisely locate the position of the DEJ because it is not anatomically flat. However, the samples were cut at specific intervals, allowing the use of slopes (rate of change between points) for extrapolating trends.

The numerical tubule densities of the different directions within the canines and lateral incisors are plotted versus distance from the DEJ (Fig 3). The numerical density increased at a rate of 11,800 tubules/ mm^2/mm ($R^2=0.997$). That was significantly greater ($P<.05$) than the rate of increase for the canine central matchstick, which was 4,400 tubules/ mm^2/mm ($R^2=0.997$). For the lateral incisor distal matchsticks, the numerical tubule density increased at a rate of 7,800 tubules/mm^2/mm ($R^2=0.999$), which was not significantly different from the lateral incisor central matchstick, 3,100 tubules/ mm^2/mm ($R^2=0.977$). The numerical tubule densities of the different tooth types were then separated by direction and the rates of change between different tooth types for the same direction were compared. Although the slopes appeared to be greater for the canines in both directions, the differences were not statistically significant.

The tubule diameters are plotted versus distance from the DEJ in Fig 4. For both the canines and lateral incisors, the tubule diameter increased with distance from the DEJ ($P<.05$). At each depth, the tubule diameter variation between the tooth types was not significant. Within the canines, there was no significant difference between the tubule diameters of the central and distal directions. However, in the lateral incisors, the tubule diameters of the central direction were significantly greater than those of the distal direction ($P<.05$). The tubule diameter of the canines increased 0.28 μm/mm with respect to distance from the DEJ ($R^2=0.952$). For the lateral incisors, the tubule diameters increased by 0.39 μm/mm ($R^2=0.994$), which was significantly ($P<.05$) greater than for the canines. A test for slopes for the same characteristics for direction within the tooth types revealed no statistically significant difference.

The peritubular widths for both tooth types are plotted versus distance from the DEJ in Fig 5. For both the canines and the lateral incisors, the width appeared to decrease as the distance from the DEJ increased. The peritubular widths in individual teeth, tooth types, and levels were significantly different ($P<.05$). Tukey's Studentized Range Test indicated that only the peritubular width at level 1 was significantly greater than level 3 ($P<.05$). The peritubular width for the canines was significantly greater than for the lateral incisors ($P<.05$). The peritubular widths in the canines and lateral incisors decreased by 0.13 μm/mm ($R^2=0.837$) and 0.05 μm/mm ($R^2=0.942$), respectively, with distance from the DEJ, which was not significantly different. The slope for the combined canines and lateral incisors was a decrease of 0.08 μm/mm.

The presence of microcanals was incidentally recorded in the course of this investigation. The location of the microcanals was constant, appearing in the midpoint facio-lingually of the dentin, as seen in Figure 6a. When more than one microcanal was present, they were arranged in a mesio-distal line, again near the midpoint facio-lingually. These features were approximately five to 10 times the size of the normal dentin tubules (Fig 6b). Microcanals presented in four out of 20 teeth (two

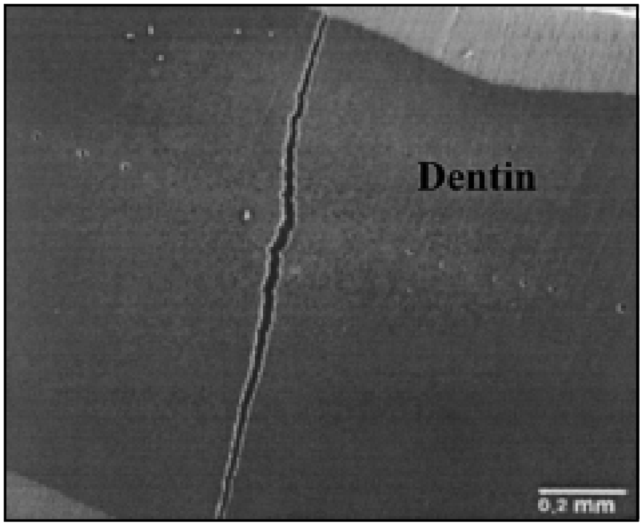

Fig 6a. SEM photomicrograph showing microcanals lying in a mesiodistal direction and centered faciolingually. Low magnification showing a series of microcanals as viewed from the incisal in a polished section. Crack is an artifact introduced during dessication in the SEM. Bar=0.2 mm.

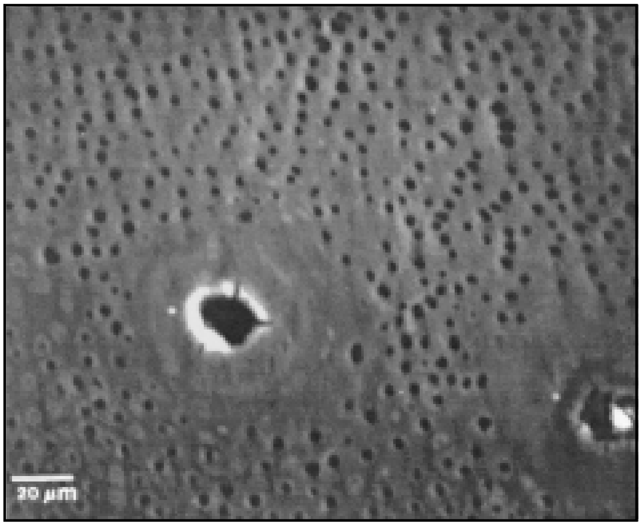

Fig 6b. SEM photomicrograph showing microcanals lying in a mesiodistal direction and centered faciolingually. Higher magnification showing characteristic features including the enlarged lumen surrounded by a thick region resembling peritubular dentin. Bar=20 μm.

of five central incisors; two of 11 lateral incisors; 0 of four canines) in this study (the additional 10 teeth were used in a preliminary study). Within an individual tooth, as many as 15 microcanals and as few as one microcanal were detected. Serial sectioning demonstrated that the microcanals extended from the DEJ to the pulp, similar to the course of normal tubules.

Discussion

The analysis of the data showed that near the DEJ, the tubule density of primary dentin appears to be greater than that for permanent dentin. However, most previous studies did not define the distance from the DEJ at which measurements were made. General descriptions such as "outer", "middle", and "inner" dentin probably do not reflect the same intervals at which the measurements in this study were made. As primary teeth are smaller than permanent teeth, the thickness of permanent dentin from the DEJ to the pulp is greater.

Garberoglio and Brannstrom[9] reported the numerical tubule density of permanent teeth at 0.5 mm intervals as distance from the pulp increased and found a rate of change that was greater than in this study for the lateral incisors (3,l00 central and 7,800 distal tubules/mm^2/mm) and central matchsticks of the canines (4,400 tubules/mm^2/mm), but less than that for the distal matchsticks of the canines (11,800 tubules/mm^2/mm). Our findings are similar to the data on occlusal and buccal locations in third molars of permanent teeth from Olsson et al.[15] Koutsi et al.[10] characterized the tubule density in terms of general locations in relation to the pulp for primary molar teeth. In comparing the rates of change from that study, the current work showed a larger rate of change in tubule density for the distal matchsticks of the canines and a smaller rate for the lateral incisors and the central matchsticks in the canines.

The increase in numerical tubule density as the distance from the DEJ increased resulted in significant differences at each depth only within the distal matchsticks. Between tooth types, the numerical tubule density of the central matchsticks of the lateral incisors were significantly higher than for the canines. It is likely that other differences were not found due to a sample size that was too small. Comparing the basic coronal anatomy of canines versus lateral incisors provides insight into these findings. The incisal edge of the lateral incisor is relatively flat and unchanging, while the incisal edge of the canine is angled, with gradual apical sloping as it approaches the proximal angle. The outer enamel shape roughly reflects the shape of the DEJ, and thus the dentin profile. Overall, in the distal of the canines, the tooth shape changes more rapidly and the odontoblasts are forced to migrate within a more constricted volume during dentinogenesis, which probably leads to the increased rate of change in tubule density seen there. The numerical tubule density in the primary anterior teeth was greater than that found previously for primary molars, suggesting a trend of decreasing numerical tubule density with posterior tooth position. The tubule density of primary anterior teeth appears to be greater than that of permanent teeth, possibly reflecting a larger number and size of odontoblasts that are needed for the relatively short development time available for anterior primary teeth, since they develop much faster than any other teeth in either dentition.[12,16] It might also be hypothesized that microcanals may be associated with the relatively rapid tooth development of primary anterior teeth.

The tubule diameter in primary dentin appears to be greater than that in permanent dentin. Near the pulp, the tubule diameter of primary dentin appears to approach that of permanent dentin. Garberoglio and Brannstrom[9] found that tubule diameter varied from 0.8 μm to 1.6 μm, while the values for primary canines and lateral incisors in this study varied from 1.39 μm to 1.94 μm. The slope of change of tubule diameter with distance in the primary lateral incisors (0.39 μm/mm) was similar to that found for premolars (0.37 μm/mm),[9] and both were larger than that found for primary canines (0.28 μm/mm). The tubule diameters for primary posterior teeth[10] were reported to be less than those of anterior primary teeth found in this study, but the rates of increase with distance were similar. In this study, the rates of change of tubule diameter between the different directions within teeth were not significantly different, but the rate of increase for the lateral incisors was significantly greater than for the canines. The relatively flat incisal edge of incisors compared to canines suggests that as the odontoblasts migrate to the pulp they are less crowded than in canines. This might also reflect a decrease in the amount of peritubular dentin deposition since the peritubular dentin is deposited within the dentin tubule,[12] thus decreasing the tubule diameter.

Peritubular width has not been studied as extensively as numerical tubule density or tubule diameter. Allred[11] reported that peritubular width ranged from 1.0 µm to 2.0 µm in molar and premolar teeth, and ten Cate[12] reported that it decreased from 0.75 µm near the DEJ toward the pulp, whereas the thickest widths found in this study were 0.92 µm and 0.62 µm for canines and lateral incisors, respectively. Hirayama et al.[3] reported that the peritubular dentin found in primary teeth was two to five times thicker than that found in permanent dentin.

The increase in tubule diameter and the decrease in peritubular thickness in relation to depth that were found in this study are probably related, since increasing peritubular dentin deposition might reduce tubule diameter. We compared the slopes of the tubule radii and peritubular widths, and found the canine slope decreases for the radius (0.14 µm/mm) seem to approach the increasing values for peritubular width (0.13 µm/mm). For the lateral incisors, the tubule radius slope value (0.20 µm/mm) was slightly larger than the corresponding value for peritubular width (0.05 µm/mm). Thus, as the tubule diameters increase, the peritubular dentin width decreases at a similar rate. This finding supports the theory that the dentin tubules start out at the same diameter and that the increase in tubule diameter with distance from the DEJ can be accounted for by a decrease in the amount of peritubular dentin deposition within the tubule.

Microcanals or giant dentin tubules have been described minimally in the literature.[2,17-20] In agreement with these studies, our study found very large tubules located within the intertubular matrix surrounded by a cuff of hypermineralized matrix resembling peritubular dentin. They are thought to be the result of odontoblast crowding and necrosis[20] or a disturbance during dentinogenesis and subsequent imperfection.[2] The position of the microcanals was consistently in the labiolingual midline. When more than one microcanal was present, they were always in a line mesio-distally. Thus, while their morphological appearance would seem to suggest that they resemble normal dentin tubules of an enlarged nature, their odd, yet specific, positioning suggests their origin might be due to an interference or disruption during dentinogenesis as odontoblasts migrate apically, rather than as simply an imperfection (which could occur anywhere). Although current literature suggests that they might be more prevalent in primary anterior teeth, the prevalence of microcanals has not been established nor has a definitive explanation for their origin been provided.

Understanding the dentin substrate may lead to improved bonding techniques. Since the numerical tubule density in primary teeth is greater than that of permanent teeth, the decrease in solid dentin may cause the significant difference in bond strengths usually observed. Furthermore, peritubular dentin etches rapidly during bonding treatments and leaves the etched intertubular dentin matrix with enlarged tubule lumens.[8] Since primary tooth dentin has larger tubule diameters with peritubular dentin at least as thick as permanent dentin, acid etching would give larger tubule lumens, further decreasing the solid dentin available for bonding. The presence of microcanals and the possibility that they have a higher prevalence in primary teeth would further reduce bond strength.

The structural differences between primary and permanent dentin suggest that primary teeth might be more susceptible to sensitivity, trauma, and noxious substance transmission through an increased number of larger tubules. Microcanals may have important implications, in addition to decreasing the area of solid dentin, inducing higher wetness, and lowering bond strength. Their large size may be the source of idiopathic tooth sensitivity, and might be expected to increase the rate of carious attack. Finally, exposure of microcanals due to trauma may result in significant pulpal pathology.

In conclusion, this study shows that there are important structural differences in primary dentin as compared to permanent dentin. These differences may have important implications for bonding characteristics. Clinical treatments and further study are needed to specifically define the relationships and ascertain the origin of the structural differences.

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Abstracts of the Scientific Literature

Sedating Uncooperative, Stable Children for Post Traumatic Head CT

The purpose of this study was to characterize variations among pediatric emergency physicians and their hospitals regarding sedation of the uncooperative, stable child for head CT following closed head injury. Although painless the CT scan requires that a child hold relatively still. An analysis of 304 (51%) returned usable mail surveys revealed that one or more published guidelines were followed by 74%; ten percent were unaware of the existence of relevant published guidelines for sedation. Twenty-six percent of the respondents were very or somewhat dissatisfied with their sedation practices. Over 20 different sedation regimens were offered for each of 3 different clinical scenarios. Midazolam and chloral hydrate were the most commonly chosen individual drugs chosen for the scenarios involving children age 3 and under.

Comments: It is interesting to note the variation in ED Sedation practices of the same age children that we sedate in pediatric dentistry. The desired result, having the child hold their head relatively still is our intent in sedation, as well. It was also interesting that that such a low percentage of respondents were somewhat or very dissatisfied with the effects of their sedation practices. LPN

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Variations in sedating uncooperative, stable children for post traumatic head CT. Connors GP, Sacks WK, Leahey NF: Ped Emerg Care 15:241-244, 1999.

24 references

Association of Dental Caries and Blood Lead Levels

Experiments show that dental caries rates are higher among lead exposed animals, but this association has not been established in humans. This survey of 24,901 persons was conducted to examine the relationship between blood lead levels and dental caries and included a dental examination and venipuncture blood lead assay. The study concluded that environmental lead exposure is associated with an increased prevalence of dental caries in the US population.

Comments: There is a paucity of published dental topics in the medical literature. This article appeared in a recent issue of the Journal of the American Medical Association. The results of this study suggest that lead exposure may contribute to the association between poverty and dental caries. On the other hand, the decline in environmental lead exposure may also have contributed to the decline in the prevalence of dental caries. Three mechanisms of the role of lead and dental caries are suggested: 1) a negative effect on salivary gland function, 2) lead incorporation into enamel resulting in defective enamel, and 3) inteference with bioavailability of salivary fluoride. AK

Association of Dental Caries and Blood Levels. Moss ME, Lanphear BP, Auinger P. JAMA 281:2294-2298, 1999.

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