Laser light transmission in teeth: a study of the patterns in different species

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Abstract

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Aim The aim of this study was to investigate the pattern of light transmission through teeth of different species and to examine laser light propagation within enamel from various animal sources.

Methodology Sectioned teeth from five species – pig, horse, sheep, cat and rat – were evaluated. Samples were illuminated individually by a helium–neon laser light from the buccal surface using a probe 0.5 mm in diameter placed at varying angles between 60 and 120°C. The pattern of light transmission was observed macroscopically. Further evaluation of laser light transmission in enamel was achieved using confocal microscopy.

Results In each species, light was transmitted through the tooth to the pulp, but in the cat and the rat, light was also transmitted to the far side of the tooth. Despite the different patterns of enamel in the species, light was transmitted through enamel to dentine.

Conclusion Light from a laser Doppler probe appeared to reach the dental pulp in all the species; however, in the mammals with smaller teeth, light may also have been able to reach the periodontium and thus the reflected signal may not be entirely of pulpal origin.

Keywords: dentine, enamel, light transmission, teeth.

Introduction

Laser Doppler flowmetry (LDF) is a non-invasive method of recording blood flow, and its use in human teeth was first described by Gazelius *et al.* (1986). Since then, the technique has found application in monitoring dynamic changes in pulpal blood flow following administration of local anaesthesia (Pitt Ford *et al.* 1993, Odor *et al.* 1994) and also in response to various stimuli (Aars *et al.* 1992, McDonald & Pitt Ford 1994). All these studies involved human subjects with a similar method of recording from teeth; a light probe was stabilized against the buccal surface of the tooth under investigation using an elastomeric splint. In addition, the technique has been used extensively for monitoring blood flow in pulp physiology experiments on a number of different species: dogs (Kim *et al.* 1990), cats (Olgart *et al.* 1991), rats (Kerezoudis *et al.* 1992) and pigs (Vongsavan & Matthews 1993).

The successful use of laser Doppler flowmetry in teeth appears surprising as the depth of penetration of laser light in skin has been shown to be in the region of only 1–2 mm (Ramsay *et al.* 1991); the thickness of enamel and dentine in human teeth is normally much greater than this (Shillingburg & Grace 1973). The ability to record pulpal blood flow using this method is due to the optical properties of enamel and dentine (Odor *et al.* 1996).

Enamel is formed of crystallites, slightly flattened hexagonal rods, which are gathered together into structural subunits, enamel prisms (Boyde 1989,

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Eisenmann 1994); these run from the amelodentinal junction (ADJ) to just beneath the outer surface of the enamel, and do not follow a straight course but have an undulating path in the horizontal plane as well as displacement in the vertical plane. This undulation results in the appearance of alternate bright and dark bands (HunterSchreger bands), which extend through the inner two-thirds of enamel (Eisenmann 1994). Superimposed on this are the incremental lines extending obliquely from the ADJ towards the enamel surface. They represent changes in the rate of enamel formation, similar to the annual rings of a tree. Where these lines intersect the enamel surface, they produce a pattern of wavy bands separated by fine grooves known as perikymata or imbrication lines; the ADJ also has a wavy course. Enamel tufts, resembling tufts of grass, arise near the ADJ; their courses follow changes in direction of the enamel prisms. In different species of animal, enamel prisms are packed differently within the tissue, giving rise to distinct patterns of enamel structure (Fig. 1) (Boyde 1989). In rats, the enamel is only found on the buccal surface of the tooth and is often pigmented by iron oxides which impart a brownish colour.

Dentine, which forms the greatest part of the tooth, has a tubular structure, with the tubules following a sinuous course from the pulp surface to the ADJ, where they branch. The tubules are surrounded by a dense uniformly mineralized tissue, known as peritubular dentine. Intertubular dentine, containing a large amount of collagen, lies between the dentinal tubules. The chief difference in the structure of mammalian dentines occurs in the deposition of peritubular dentine. It is not found at all in rodents but is present in primates and carnivores (Bradford 1967). In ungulates, it commonly forms on only one side of each tubule (Boyde 1969).

Human dentine has been reported to produce a magnified image as a result of the dentinal tubules acting as optical fibres (Walton *et al.* 1976). More recently, it has been demonstrated that in human and canine teeth, enamel and dentine are able to act together as a light gathering and transmitting device (Altshuler *et al.* 1991, Odor *et al.* 1996). It is conceivable that if the structure of the tissues is different, the light-transmitting properties may be altered.

The aim of this study was (i) to investigate the pattern of light transmission through teeth of different species, and (ii) to use confocal microscopy to examine laser light propagation within the enamel of the different species.

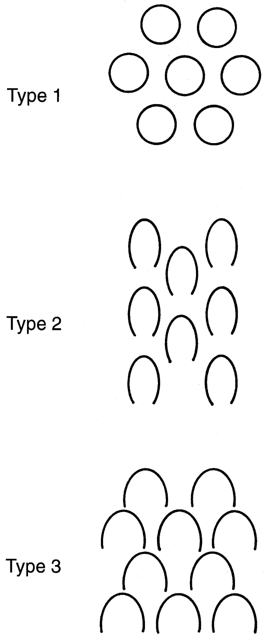

Figure 1 Arrangement of enamel prisms in different types of enamel (after Boyde 1989).

Materials and methods

The method is similar to that used for examination of human and canine teeth (Odor *et al.* 1996). The teeth investigated in the study were primary incisors of pigs, permanent incisors of horses, sheep and rats, and canine teeth of cats. Ten samples of each type were obtained; five of each type were sectioned longitudinally through the pulp chamber using a diamond wafering saw. Each of the remaining samples was sectioned transversely.

The samples were individually illuminated by a 633-nm helium-neon laser light from the buccal surface using a fibre-optic probe 0.5 mm in diameter placed at varying angles between 60 and 120°. The maximum power output was 2.8 mW. The pattern of light transmission was initially observed macroscopically and some specimens were photographed.

The sectioned teeth were then mounted with the cut surface parallel to the stage of a tandem scanning reflected light microscope (TSM). The fibre-optic probe was placed perpendicular to the buccal surface of the tooth and immediately below the cut surface of the specimen. The probe was connected to the same laser light source to illuminate the sample from the side (Fig. 2). Using the TSM, a confocal image of the tissue adjacent to the laser light probe was initially obtained

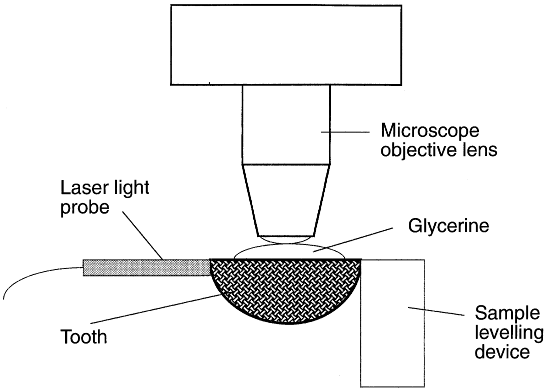

Figure 2 Diagram of the method used to examine the teeth.

without side illumination by laser light. Two objectives, ×10 and ×20, and two pass filters, a 541–549 nm bandpass filter with a peak transmission at 546 nm (green light) and a 450–490 nm bandpass filter with a peak transmission at 470 nm (blue light), were used in turn. Glycerine was used as the immersion medium. These confocal images were examined. The sample was then side illuminated using the laser light source, and the combined confocal/laser light source image examined. Finally, the same field was observed solely with side illumination, by occluding the light source of the microscope. This process was repeated for each sample.

Results

The pattern of light transmission observed macroscopically was similar in all samples from each species. In pig, horse and sheep teeth, the red laser light appeared to be transmitted through the enamel and then follow the line of the dentinal tubules to the pulp space irrespective of the angle of the probe. A bright area appeared at the ADJ where there was some lateral spread of light (Fig. 3). Light and dark areas were visible in the enamel of horse teeth, which had pronounced decussation (Fig. 3b). In the smaller teeth (cat and rat), light was transmitted right through the tooth, exiting on the opposite surface; in common with pig and horse teeth, there was a bright area at the ADJ (Fig. 3c). Longitudinal and transverse sections of sheep teeth are shown in Figs 3(d) and (e); in both planes light passed to the pulp space.

A confocal image of a pig primary incisor is shown in Fig. 4(a). The structure of the enamel was type 3 with pronounced incremental lines, similar to human enamel. The laser image (Fig. 4b) shows a bright area just below the enamel surface, corresponding to an area of aprismatic enamel. A bright area can also be seen at the ADJ, where there is much branching of both the enamel prisms and dentinal tubules. In the combined image (Fig. 4c), the darker areas of the confocal image are brightly illuminated by the laser light.

The horse incisor had type 2 enamel with some decussation. Laser images showed distinct light and dark areas in the enamel, with a very bright area at the ADJ. A combined image (Fig. 5) showed correlation between the bright areas of the laser image and the darker areas of the confocal image. The course of the dentinal tubules was readily visible in this species as the light was reflected by the highly mineralized peritubular dentine.

A similar pattern of light distribution was observed in the cat canine tooth (Fig. 6), although the enamel was much thinner than in the pig or horse. This species also featured type 3 enamel, but the pattern of decussation was not very pronounced. Because of the small size and brittleness of the rat incisors, preparation of specimens for microscopy was difficult; however, microscopic examination revealed similar transmission patterns to other species.

A combined image of the ADJ of a sheep incisor is shown in Fig. 7. There is pronounced decussation of the enamel with alternate light and dark zones, the latter appearing red from the laser light. There is a bright area in the enamel immediately under the ADJ, whilst the adjacent dentine has a dark band.

Discussion

Microscopy

Preparation of dental hard tissues for microscopic examination often involves extensive grinding of the sample to allow transmission of light through the tissues. Such techniques may result in disruption of the structure. Confocal microscopy is able to produce high-resolution optical sections within tissues, thus negating the need for extensive preparation. The images produced are of high quality, as only light from the plane in focus is transmitted. The transmission of reflected and scattered light from all other planes is prevented (Watson 1991). The brightness of the image may be improved by the use of an immersion liquid; commonly, immersion oil is used for this purpose. Whilst producing bright images, the oil may be absorbed by the sample, resulting in an alteration of its

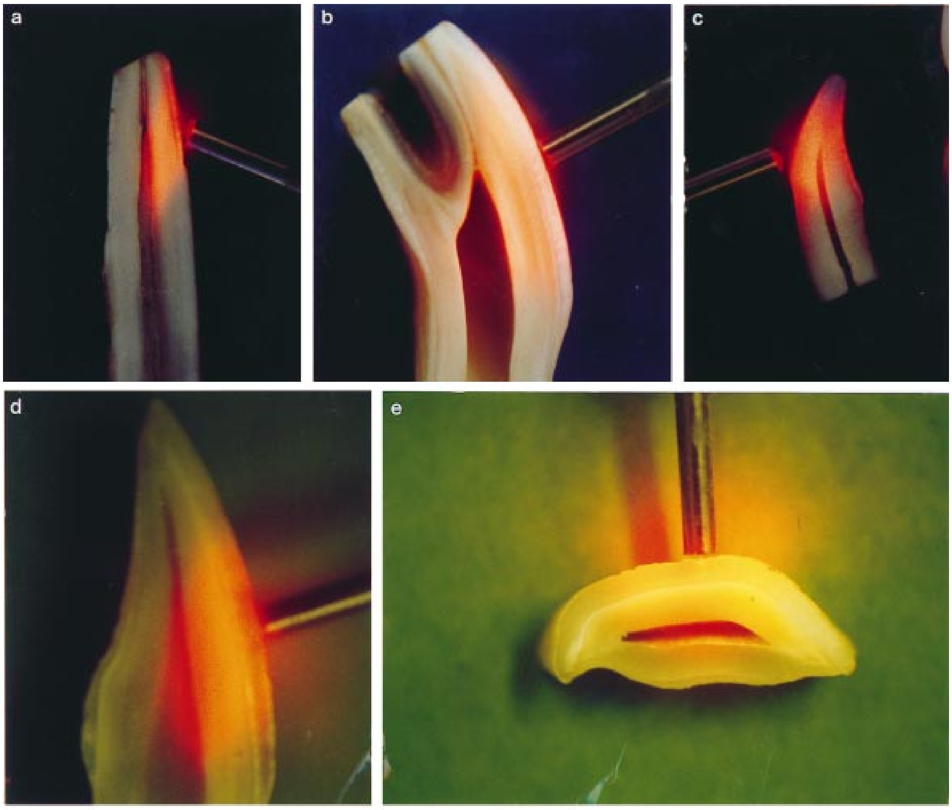

Figure 3 Photographs of He–Ne laser light transmission through longitudinal sections of (a) pig primary incisor, (b) horse incisor, (c) cat canine, (d) sheep incisor, and (e) a transverse section of a sheep incisor.

optical properties. Moreover, loss of fluid from teeth can produce changes in optical properties (Spitzer & ten Bosch 1975, Brodbelt *et al.* 1981). In this study, glycerine, which has a higher refractive index than water, was used to maintain the samples in as near natural a state of hydration as possible whilst producing bright images.

Enamel

There is great variation in the appearance of enamel in the different species studied. The enamel prisms do not run straight through the enamel but follow a sinuous course. Thus enamel has a woven crystalline structure, known as prism decussation, which is due to complex movements of ameloblasts during enamel formation. Decussation presents a characteristic appearance when seen in microscopic sections: a series of light and dark zones may be distinguished depending on where the prisms have been cut. In some parts of the section, the prisms have been cut more or less parallel to their length; these appear bright in a reflected confocal image (parazones). In other parts the prisms may have been cut transversely or obliquely as they are bending away from the plane of the section; such areas will appear dark in a confocal image as light is transmitted into the body of the sample (diazones). Some species have very conspicuous decussation. In carnivores, the prisms are aligned almost parallel to the long axis of the parazones, with zones appearing straight along their length and perpendicular to the ADJ (Boyde 1969). Ungulates, however, have thicker enamel with prisms aligned obliquely to the long axis of the parazones which are at an angle to the ADJ, where there is a band of enamel without decussation. The horse incisor was investigated because of its unusual crown form and, as it is a domesticated animal, its potential for physiological research. In the enamel of pig teeth, the prisms also lie at an angle to the long axis of the parazones, but less so than in ungulates; there is a narrow band of aprismatic enamel both at the crown surface and adjacent to the ADJ.

In spite of this wide variation in the structure of enamel from the different species, the basic unit of the

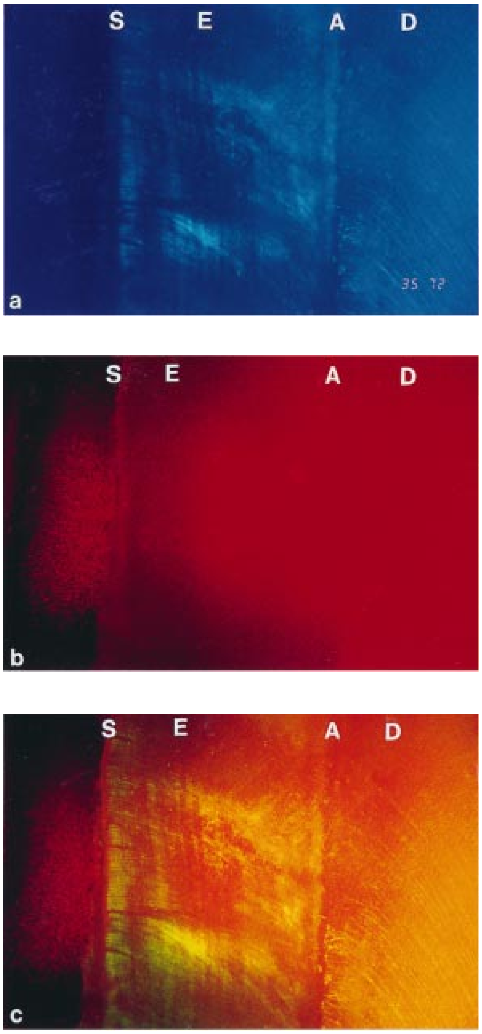

Figure 4 (a) Confocal image of a longitudinal section of pig primary incisor; (b) side illumination with 633 nm He–Ne light source; (c) combined confocal and laser light image (546 nm green pass filter). S, enamel surface; E, enamel; A, amelodentinal junction; D, dentine. Field width = 600 μm.

enamel, the prism, appears to transmit and reflect light in a similar way in each case. When the prisms are parallel to the cut surface, light is reflected, giving rise to the bright areas in the confocal image, and when they are cut obliquely they give rise to darker areas in the confocal image. In the case of the laser image, the light source is at right angles to that of the confocal image. Thus, those prisms that were parallel to the light source are now at right angles to it. They transmit light into the sample and give a dark appearance in the laser image to areas that were bright in the confocal image. Similarly, those prisms that were obliquely placed to the confocal light source are now able to transmit laser light into the plane of observation and appear bright. Thus it appears that enamel prisms may also be able to act as optical fibres and transmit light to the dentine regardless of the pattern of prism packing.

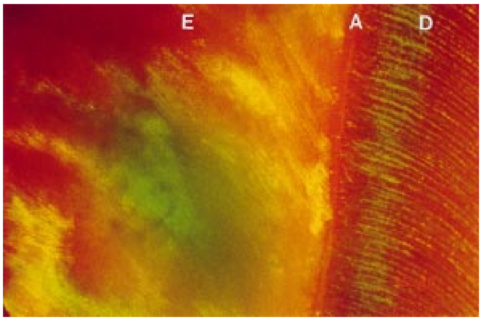

Figure 5 Combined confocal and laser light image (546 nm green pass filter) of a longitudinal section of horse incisor amelodentinal junction. E, enamel; A, amelodentinal junction; D, dentine. Field width = 600 μm.

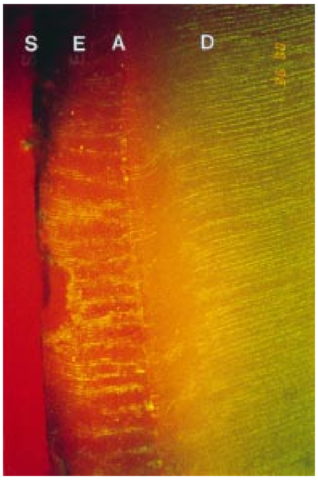

Figure 6 Combined confocal and laser light image (546 nm green pass filter) of a longitudinal section of cat canine. S, enamel surface; E, enamel; A, amelodentinal junction; D, dentine. Field width = 600 μm.

Dentine

It has long been accepted that dentine tubules are able to act as optical fibres (Walton *et al.* 1976). The conditions for total internal reflection, namely a material of one refractive index with a coating of another with a different refractive index, are met by the dentinal tubules, where there is a difference in

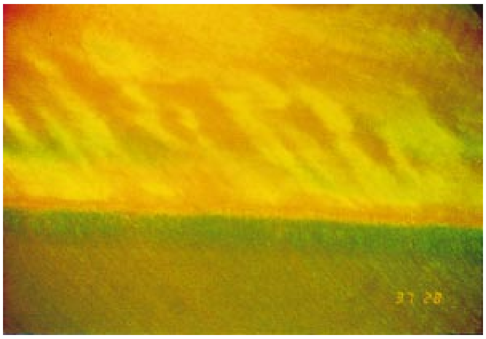

Figure 7 A combined confocal and laser light image (546 nm green pass filter) of a longitudinal section of the amelodentinal junction of a sheep incisor. Field width = 600 μm.

mineralization, and hence refractive index, between peritubular and intertubular dentine. This difference was most pronounced in the horse. In all species, the dentinal tubules followed an S-shaped course through the dentine; thus when observed in the longitudinal plane, that part of the pulp illuminated by the laser light was not opposite the probe. When the results of laser Doppler experiments using different species are interpreted, it is important to consider the anatomy of the teeth under scrutiny, since the path of light transmission is affected. This determines the site from which blood flow readings are taken and could affect the readings themselves since the vasculature of the dental pulp varies greatly between different areas (Kim 1985).

Laser Doppler flowmetry

The significance of the present findings for research using laser Doppler flowmetry is twofold. First, in the teeth of the species investigated, laser light was shown to reach the dental pulp, and therefore the reflected Doppler-shifted signal was likely to be from the pulp. Secondly, with very small teeth (as in the cat or rat), the standard fibre-optic probe of 0.5 mm diameter did allow light to reach the far side, and thus the reflected signal could contain a Doppler-shifted component from the vessels of the periodontium. This is an area in need of further investigation; a smaller probe might well prevent light passing to the far side of the tooth.

Conclusion

This research group has now examined the pattern of light transmission in teeth of species which have been used for pulp physiology experiments employing laser Doppler flowmetry. Sheep were also investigated as they are used for medical research in New Zealand. There are potential veterinary applications in sheep and horses.

Acknowledgements

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