represents the conditions in a normal tooth. Brownian motion of red cells in the replaced pulp (Vongsavan and Matthews, 1996) could also have made a small contribution to the difference.

The data obtained in the experiments in which the pulp was replaced therefore give the best estimate of the proportion of the signal recorded with opaque rubber dam from an intact tooth in an adult human subject that can be attributed to blood flow in the pulp. This value is 43%. Without dam, the figure is approximately 10%.

These conclusions contrast with those of other studies in which it has been suggested that most of the signal record with a laser Doppler flowmeter from intact human teeth (Gazelius *et al*.,1986, 1988 and Olgart *et al.*, 1988) or from pig teeth (Vongsavan and Matthews, 1996) is from the pulp. The recordings from human teeth were made without dam. The probe was supported by a large, green, silicone rubber bite block that would have provided some screening of the surrounding tissues. This type of bite-block has the advantage that it is simple to construct but, apart from providing less satisfactory screening of the surrounding tissues than opaque rubber dam, it does not support the probe as rigidly as a solid, plastic splint of the type used in the present experiments. Any movement of the probe in relation to the tooth causes artifacts that may be synchronous with the heartbeat and resemble a blood-flow record (Vongsavan and Matthews 1993b). Hartmann *et al.* (1996) found that even a well adapted, silicone bite-block gave much less satisfactory results than a rigid plastic splint used with rubber dam.

One of the factors that would be expected to affect the contamination by non-pulpal tissues of blood flow signals from teeth is the proportion of the crown that is occupied by pulp. This would account for the fact that, in contrast to the present results, at least 85% of the signal recorded from intact deciduous incisors in pigs was shown to be derived from pulp (Vongsavan and Matthews, 1996). The pig teeth were similar in size to human incisors but the pulps were much larger. Records from permanent canine teeth in young adult cats are also due mainly to blood flow in the pulp, since cutting the pulp at the root apex almost abolishes the signal (unpublished observations). Since the pulps of human teeth decrease in size with age (Morse *et al.*, 1993), records from young teeth should include a larger pulpal component than those from teeth of older subjects. The data reported by Gazelius *et al*. (1986, 1988) and Olgart *et al.* (1988) were obtained from young subjects aged 7-16 years.

The wavelength of the light will also affect the extent to which the signal is affected by tissues outside the tooth. This is because light of long wave-length such as infra-red penetrates tissues to a greater depth than shorter wavelengths such as red and particularly green (see Vongsavan and Matthews, 1993 a & b). Gazelius *et al*. (1986, 1988) and Olgart *et al.* (1988) used red light in their studies. Ingófsson *et al*. (1994) also used red light to record from human teeth and obtained data that suggest that, even with this wavelength, up to 60% of the blood flow recorded from human teeth is from non-pulpal tissue. In this and other studies, non-vital teeth have been used as controls. This is not ideal since the optical properties of pulp and dentine will change after death of the pulp, and such changes will affect the amount of light transmitted to tissues outside the tooth (Vongsavan and Matthews,

1993a & b). This is also apparent from the results of the present experiments, in which it was shown that the record from a tooth in which the pulp and been removed and replaced was different from that obtained from the same tooth when the pulp camber was left empty. Estimates of the contribution made by the pulpal circulation to records of blood flow from intact teeth that are based on data from dead teeth are therefore unreliable.

A further problem in using laser Doppler flow meters to record pulpal blood flow is that once contamination from non-pulpal tissues has been reduced to a minimum by using opaque rubber dam, the signal remaining is often close to the maximum sensitivity of the instrument. The blood flow signal may appear to be greater than it really is unless allowance is made for any offset due to noise in the recording system. This offset can be measured by recording from a stationary reflector with the same level of back-scattered light as present while recording from the tooth (Vongsavan and Matthews, 1993b). The signal-to-noise ratio in the record can be improved by reducing the bandpass of the flow meter, but this will bias the recording to the slow flow rates of blood present in capillaries and near the walls of arterioles (Vongsavan and Matthews, 1993a).

These experiments demonstrate that, at their present state of development, laser Doppler flow meters are not a reliable way of assessing the vitality of teeth in adults.

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Series 2 experiments

The use of the replica technique to record fluid emerging from exposed dentine

Introduction

There is normally a continuous outward flow of fluid through exposed dentine *in vivo*. This has been demonstrated both in the cat (Vongsavan and Matthews, 1992a) and in man (Ciucchi *et al*., 1996). The flow rate of fluid through dentine is likely to be affected by the tissue fluid pressure of the pulp which, in turn, will depend upon the state of the microcirculation of the pulp. In the cat, stimulation of cervical sympathetic trunk (CST) caused a fall in pulpal blood flow and this was associated with either a marked reduction in the rate of outward flow of fluid or even reversal of this flow. A similar effect would presumably occur following infiltration of a local anesthetic solution containing a vasoconstrictor over the apex of a tooth, which causes a profound fall in pulpal blood flow. Electrical stimulation of peripheral cut end of inferior alveolar nerve (IAN) in the cat produced an increase in pulpal blood flow and the rate of fluid flow through dentine (Vongsavan and Matthews, 1992b; Matthews and Vongsavan,1994; Vongsavan *et al*., 2000).

In the experiments referred to above, fluid flow through dentine *in vivo* was measured by recording the movement of fluid in a capillary sealed to the dentine surface. The method used by Vongsavan and Matthews (Vongsavan and Matthews, 1992a; Vongsavan and Matthews, 1992b) in cat teeth is the most sensitive. However, this technique requires a very high level of mechanical and thermal stability and is not suitable to use in human experiments. For the larger human teeth, Ciucchi *et al.*, (1996) were able to use a method that is less sensitive to thermal effects and movement of the subject. However, this technique is still complicated to set up, requires a large

area of exposed dentine, and is not convenient for use in most clinical conditions.

We have investigated an alternative approach based on the method used by Bharali *et al.*, (1988) to record sweat secretion from the hind paw of the rat. They recorded the emergence of sweat droplets from the skin with a dental impression material. Brännström (1963) and, more recently, Sasazaki and Okuda (1994, 1995) and Itthagarun and Tay (2000), used a similar technique in an attempt to record droplets of fluid on exposed human dentine. Itthagarun and Tay (2000) also used this technique to record the emerging fluid droplets from the exposed deep dentine surface.

We have carried out experiments to validate this technique by determining the effect of changes in pulpal tissue fluid pressure on the presence of droplets recorded on exposed human dentine *in vitro.*

Materials and methods

The experiments were carried out on 24 healthy premolars from 24 subjects. The teeth were scheduled for extraction for orthodontic reasons. All the teeth were fully erupted, vital, free of caries and without restorations. The subjects were healthy non-smokers, aged 15-20 yrs. (mean 17.2 yrs.).

Dentine was exposed at the tip of the buccal cusp by cutting a cavity (diam. 3 mm, depth 3 mm) with diamond burs (Intensiv® 201, round and 204, fissure; Viganello-Lugano, Switzerland) under a constant stream of water. In 12 of the teeth, the cavity was etched with 35% phosphoric acid (3M Dental

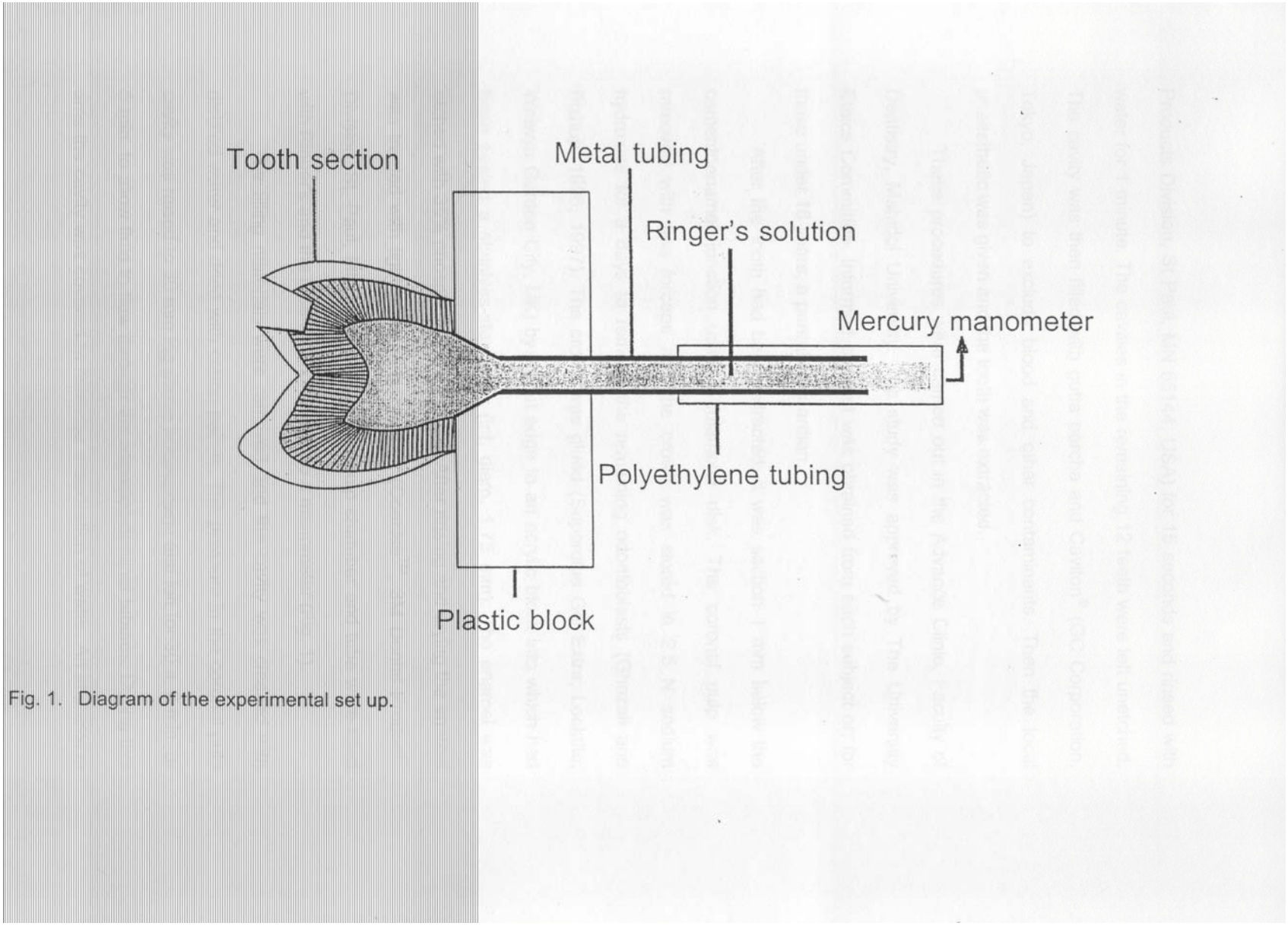

Fig. 1. Diagram of the experimental set up.

Products Division, St Paul, MN 55144, USA) for 15 seconds and rinsed with water for 1 minute. The cavities in the remaining 12 teeth were left unetched. The cavity was then filled with gutta percha and Caviton® (GC Corporation, Tokyo, Japan) to exclude blood and other contaminants. Then the local anaesthetic was given and the tooth was extracted.

These procedures were carried out in the Advance Clinic, Faculty of Dentistry, Mahidol University. The study was approved by The University Ethics Committee. Informed consent was obtained from each subject or, for those under 18 years, a parent or guardian.

After the tooth had been extracted, it was section 1 mm below the cementoenamel junction using a diamond disk. The coronal pulp was removed with fine forceps and the crown was stored in 2.5 N sodium hydroxide for 3 days to remove the remaining odontoblasts (Ghazali and Bishop, 1996, 1997). The crown was glued (Superglue Gel Extra; Locktite, Welwyn Garden City, UK) by its cut edge to an acrylic block into which had been sealed a stainless-steel tube (int. diam. 1.75 mm). The enamel was etched with 35% phosphoric acid for 15 s. After rinsing and drying the enamel was treated with light cured white sealant (Concise™; 3M Dental Products Division, St. Paul, MN 55144, USA). The pulp chamber and tube were filled with Ringer's and the tube was connected to a manometer (Fig. 1).

The filling materials were removed and the cavity was cleaned with distilled water and dried with cotton pellets. The pressure in the coronal pulp cavity was raised to 30 mm Hg above atmospheric and left for 30 s, 2 min. or 5 min. to allow fluid to flow through the exposed dentinal tubules. During this time the cavity was covered to minimise evaporation of water. An impression

was then taken of the cavity floor with a hydrophobic, silicone-rubber material (Xantopren® VL; Heraeus, Kulzer, Germany). The impression material was flowed onto the dentine surface and no additional pressure was applied. These procedures were repeated in exactly the same way with the pulp cavity pressure at atmospheric and at 5 mm Hg below atmospheric. The teeth were then stored at 4°C in 0.9% saline containing 0.1% w/v thymol to prevent bacterial growth.

Replicas of the exposed dentine surface were made from the impressions with epoxy resin (Stycast® 1266; Hitek Electric Materials Ltd., South Humberside, UK). These were fixed to stubs with conductive adhesive tape (SPI Supplies, USA), sputter-coated with gold-palladium under vacuum (SPI-Module™; SPI Supplies Division of Structure Probe, Inc., USA), and examined in a scanning electron microscope (SEM; Jeol® JSM–5410LV; Jeol Ltd., Tokyo, Japan). Photomicrographs were taken at a magnification of X1000 on Kodak® Tmax100 film (Eastman Kodak Company, Rochester, NY 14650, USA).

At the end of the experiment, each crown was dehydrated and prepared as described above for examination in the SEM. Finally, the crown was sectioned longitudinally and the remaining dentine thickness (RDT) between the highest pulp horn and the floor of the cavity surface was measured in the SEM.

Results

Fig. 2A shows a scanning electron micrograph of a replica of an exposed, unetched dentine. The impression that was used to make the replica was taken when the pulp cavity pressure was at atmospheric pressure. For comparison, Fig. 2B shows a scanning electron micrograph of the same dentine surface that was obtained directly from the tooth. The similarity between the two images demonstrates that the replica technique can be used to obtain an accurate record of a dentine surface. There was no evidence in the replica of water droplets on the exposed dentine surface. Similar evidence of the accuracy of the replica technique to reproduce the surface of dentine was obtained for etched dentine under conditions when there would be expected to be no outward flow of fluid through the dentinal tubules.

Evidence of flow through etched dentine

When impressions were taken from etched dentine after the pulp cavity pressure had been raised to 30 mm Hg above atmospheric for 30 s or 2 mins., the replicas showed the open ends of the dentinal tubules but no evidence of fluid on the exposed dentine surface. Only when the same pressure was maintained for 5 minutes was evidence of the accumulation of fluid droplets obtained (Fig. 3A). The droplets were flattened and tended to coalesce. Similar droplets were found in all 12 teeth examined under these conditions.

Corresponding scanning electron micrographs of replicas of the exposed dentine surface with the pulp cavity pressure at atmospheric and 5 mm Hg below atmospheric for 5 mins. are shown in Figs. 3B and 3C

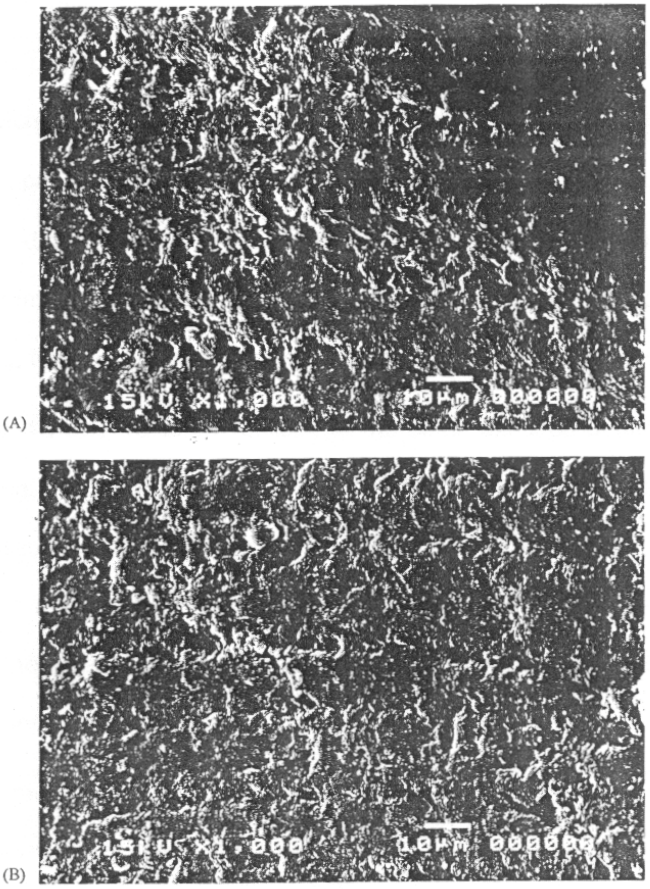

Figure 2 Scanning electron micrographs of (A) a replica of an exposed, unetched, dentine surface in an extracted premolar, and (B) the dentine surface from which the replica was made.

respectively. Under these conditions, no fluid droplets were recorded; the replicas just show the opened dentinal tubules at the exposed dentine surface.

Evidence of flow through unetched dentine

Surprisingly, replicas of unetched dentine showed fluid droplets as soon as 30s after raising the pulp cavity pressure to 30 mm Hg above atmospheric pressure. The droplets were dome-shaped and not flattened like those on etched dentine. Their diameters ranged from approximately 5 to 10 μm. The spacing between the droplets was of the same order and corresponded that with that of the dentinal tubules at this level in human teeth (13). An example is shown in Fig. 4A. With the longer periods of application of +30 mm Hg, the droplets were larger but generally still did not coalesce.

Figs. 4B and 4C show replicas of the same exposed dentine surface shown in Fig. 4A, but they were made from impressions taken when the pulp cavity pressure was at atmospheric pressure and at 5 mm Hg below atmospheric respectively. Under these conditions, the replicas showed the surface of the smear layer with no fluid droplets. Similar results were obtained from all 12 teeth in which unetched dentine was studied.

The mean RDTs of the etched and unetched dentine group were 1.06 ± 0.40 mm (range 0.60-1.10) and 1.10 ± 0.24 mm (range 0.97-1.42) respectively.

In preliminary experiments, it was found that treatment with sodium hydroxide to remove the odontoblasts was necessary to prevent droplets

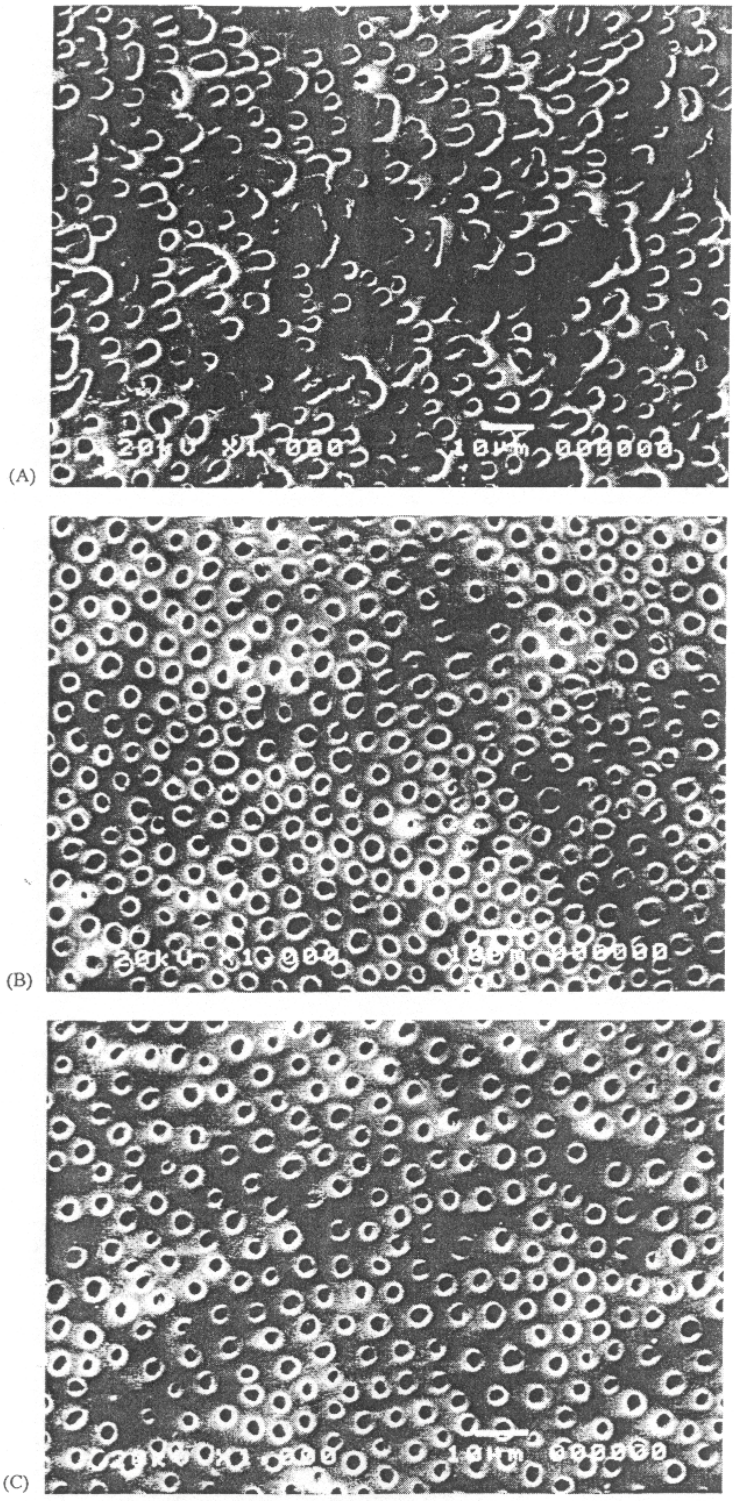

Figure 3 Scanning electron micrographs of replicas of exposed, etched, dentine surfaces in vitro when the pulp cavity pressure was set at (A) 30 mmHg above the atmospheric, (B) atmospheric, and (C) 5 mmHg below atmospheric.

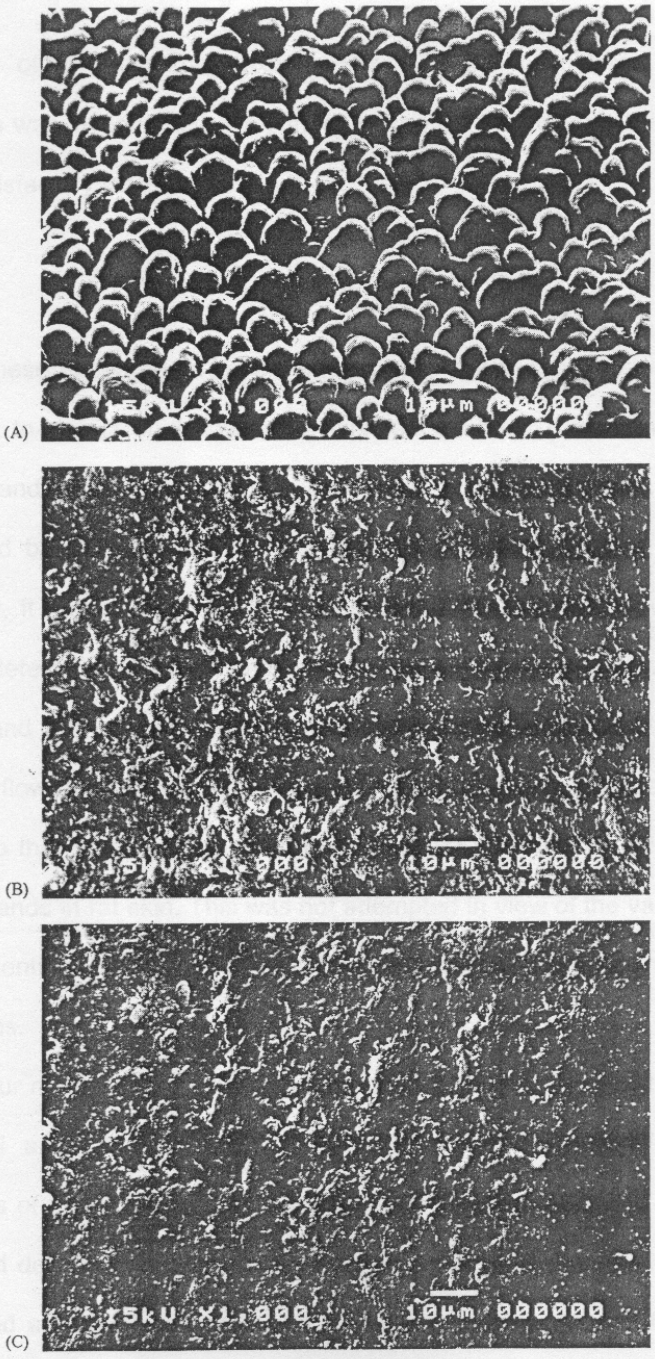

Figure 4 Scanning electron micrographs of replicas of exposed, unetched, dentine surfaces in vitro when the pulp cavity pressure was set at (A) 30 mmHg above the atmospheric, (B) atmospheric, and (C) 5 mmHg below atmospheric.

forming on unetched dentine at the ends of the dentinal tubules, even when the pulp cavity was at atmospheric pressures.

In other preliminary experiments a range of different impression materials was investigated. Only those based on hydrophobic silicone rubber gave satisfactory results.

Discussion

These experiments have demonstrated that the replica technique can be used to detect fluid flow from exposed dentine. The method is much simpler and more convenient to use under clinical conditions than those employed by Vongsavan and Matthews (1992) and Ciucchi *et al.*, (1996). However, it has several disadvantages compared with those methods: it is able to detect only outward and not inward flow, does not give a continuous record, and is at best semi-quantitative. We had hoped to estimate the rate of outward flow by measuring the volume and number of the droplets, in a way similar to that employed by Bharali *et al*., (1988) to estimate secretion from sweat glands in rat skin. This was not attempted in view of the variable shape of the dentinal fluid droplets and their tendency to coalesce under some conditions.

Our results confirm that the data obtained using a similar technique by Sasazaki and Okuda (1995) represent fluid flow from dentinal tubules. Bevenius *et al.*, (1994) noted what appeared to be fluid droplets on exposed, unetched dentine on the floor of a cervical cavity in human teeth *in vivo* but they used a hydrophilic impression material. This may account for the small size of the droplets.

The results reported by Sasazaki and Okuda (1995) correspond in several respects with those of the present study. In both studies, droplets were observed on unetched dentine in freshly extracted human teeth when the odontoblasts were left *in situ* and the pulp cavity was at atmospheric pressure. This result is difficult to explain. Without a hydrostatic pressure gradient across the ends of the tubules, flow could only be due to osmotic forces. Maybe autolysis of the odontoblast processes resulted in an osmotic gradient across a membrane formed by the remnants of the odontoblast cell bodies and this gradient caused water to move from the pulp into the dentinal tubules. But this would not explain the droplets observed by Sasazaki and Okuda (1995) *in vivo*. In cat teeth *in vivo* there was a small inward flow through exposed dentine after the blood circulation had been stopped, and this was attributed to an osmotic effect of protein in the pulpal tissue fluid (Vongsavan and Matthews, 1992a).

The shape and distribution of the droplets recorded by Sasazaki and Okuda (1995) from unetched dentine were very similar to those found in the present study. Both studies also indicate that droplets form less rapidly on etched than on unetched dentine, even when the odontoblasts have been removed. This is not what would be expected if etching simply increased the hydraulic conductance of the tubules. The results could be explained if the organic matrix of the etched dentine acted as a sponge which collapsed on being dried and then, over the next few minutes, absorbed the fluid emerging from the tubules, thus preventing it accumulating as droplets on the dentine surface (Pashley, et al., 1995). However, Itthagarun and Tay (2000) used the similar technique on the very deep dentine, they obtained a lot of emerging

fluid droplets. This emerging fluid droplets after etching in the very deep cavity might interfered sealibility of dentine bonding agent and restorative material (Pashley *et al*., 2002).

Our preliminary experiments showed that hydrophobic silicone impression materials must be used to record the presence of fluid on exposed dentine. The impression material we used had the highest water contact angle (approx. 108°) of those tested by Boening *et al*., (1998). This property is a disadvantage for taking impressions in restorative dentistry, and for this reason hydrophilic materials have been developed that are less affected by surface moisture and hydrophobic impression materials may be difficult to obtain. The method used by Absi *et al.*, (1989) to record the micro-morphology of the surface of hypersensitive dentine in man *in vivo* was very similar to the method we have employed, but they found no fluid droplets. The absence of droplets in their replicas could have been due to several factors. They dried the dentine surface very thoroughly and applied the impression material immediately, although not under pressure (Absi, EG, personal communication). They also used local anaesthetic with a vasoconstrictor. Particularly with infiltration anesthesia of upper teeth, this would have reduced pulpal blood flow and pulpal tissue fluid pressure, and hence outward flow through the dentine.

Acknowledgements

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

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