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Effect of specimen block orientation on separation artefact in pulpal sections.

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Keywords: histology; pulp

SUMMARY

In an earlier study the effect of the end point of decalcification on separation artefact at the pulp-dentine interface in monkey teeth was investigated. This showed that there was significantly less separation on the labial compared to the palatal side. Since all sections had been cut longitudinally through the short axis in a labial to palatal direction, it was decided to investigate the effect of orientation of the specimen block to the knife edge on pulp-dentine separation. Five vervet monkey incisor teeth were decalcified in a formic-citric acid mixture, monitored by flame photometry, and histological sections were prepared at 4 different block orientations for each specimen. The sections were evaluated under the light microscope for separation at the pulp-dentine interface on both the labial and palatal using a calibrated graticule. The measurements were analysed using SAS (1985). A 4-way ANOVA revealed a significant variation of separation between the labial and the palatal sides, irrespective of the orientation of the block and irrespective of the direction from which the cut was made. This observation suggests that there is a differential quality of attachment between pulp and pulpal wall on the labial and palatal surfaces: the labial surface having a stronger attachment than the palatal surface.

OPSOMMING

Tydens 'n vorige studie is die effek van eindpunt ontkalking op die artefak-skeiding van die aansluiting tussen die pulpa en die dentien ondersoek. Aansienlik minder skeiding het voorgekom op die labiale kant as palataal. Aangesien alle seksies in die vorige studie in the lengte van die tand vanaf die labiale na die palatale kant gesny is, is besluit om die uitwerking van die oriëntasie van die monsterblok ten opsigte van die mes op die skeiding tussen die pulpa en die dentien te ondersoek. Ontkalking van vyf blou-aap snytande is in 'n mieresuur-sitroensuurmengsel deur middel van 'n vlamfotometer gemonitor. Hierna is vier verskillende blokoriëntasies gebruik om histologiese seksies te verkry. Die mate van skeiding wat by die aansluiting tussen die pulpa en die dentien op beide die labiale en palatale kante van die tand voorgekom het, is onder 'n ligmikroskoop met behulp van 'n gekalibreerde graadnet geëvalueer. Die SAS (1985) rekenaarprogram is gebruik om die afmetings te ontleed. 'n Vierrigting ANOVA dui op 'n betekenisvolle verskil in die skeiding wat op die labiale kant en op die palatale kant voorgekom het, ongeag blokoriëntasie of rigting van beweging van die mes. Hierdie bevindinge dui op 'n verskil in hegsterkte tussen die pulpa en pulpawand op die labiale en palatale oppervlaktes: 'n Stewiger hegting bestaan op die labiale oppervlakte van die pulpawand as op die palatale oppervlakte.

INTRODUCTION

Before restorative materials can be used clinically they must be biocompatible with dental tissues, in particular with the pulp. At present, to evaluate the biological response of the dental pulp to restorative materials, histological sections must be examined. These must be of a high quality, details of which are outlined in the specifications of the American Dental Association (ADA) (1979) and the Federation Dentaire Internationale (FDI) (1978). A particular problem encountered in preparation of histological sections of teeth is that the soft delicate pulpal tissue is surrounded by hard dentine. When a tooth is sectioned, therefore, the knife first cuts through dense dentine and then into soft delicate tissue and back into dense dentine.

In a previous study on determining the end point of decalcification by means of radiographs and flame photometry in order to find which method would produce superior histological sections of teeth (Van Wyk, 1986), the sections were cut longitudinally through the short axis of each tooth, as recommended by Brain (1966). The sections were cut in a labial to palatial direction with the block orientated at 90° to the knife edge. The sections were then evaluated on the labial and palatial sides according to the longitudinal extent of separation at the pulp-dentine interface. It was noted that there was significantly less separation on the labial side compared to the palatial side. I therefore suggested that the direction of cut was important in that there might be less separation when the cut went from dentine to pulp (hard to soft tissue) than in the reverse direction. However, Luna (1968) has indicated that dense capsuled, or tough surfaces of tissue should be at the top when sectioning, that is, the direction of cut should be from soft to hard tissue.

The objective of this investigation was to assess the effect of specimen block orientation on separation artefact at the pulp-dentine interface in order to find optimum orientation of the block associated with the least separation artefact.

MATERIALS AND METHODS

Three vervet monkeys (*Cercopithecus aethiops pygerythrus*) were perfused retrogradely using a modification of the technique described by Retief and Austin (1973). One litre of physiological saline containing 10g of procaine hydrochloride, was introduced via a canula into the descending aorta, and heparin (5 000 i.u./ml; 0,2 ml/Kg body weight) was injected into the canula at the beginning of perfusion. The procaine hydrochloride was used to dilate blood vessels and the heparine to prevent clotting. This was followed by one litre of buffered 10 per cent formalin. After perfusion, the monkeys were decapitated and by using a bandsaw, the mandible was severed from the skull, between the occlusal surfaces of the teeth. Thereafter blocks of bone containing the maxillary incisors were excised by cutting posterior to the canines up to the bridge of the nose. This ensured that there was no damage caused to incisor crowns and roots. The block specimens were held in the fingertips to avoid pressure damage to the teeth and the central incisors were cut out by using bone cutters to nibble away small portions of bone and adherent soft tissue until no bone or mucosa was visible. Five teeth were then replaced into the fixative, blotted on tissue paper and weighed to the nearest 0,001g on a Sartorius analytical balance (Zeiss, West Germany), after which they were placed directly back into fixative. This procedure took only approximately 35 sec which ensured that no damage was caused by allowing the specimen to dry out.

The decalcification procedure was carried out at room temperature (approximately 20°C), using 100 ml of formic-citric acid mixture per tooth (formic acid 1 200 ml 87 percent, plus citric acid 450 g, plus water 2 400 ml), (Collins, 1960). This decalcification fluid was replaced with fresh fluid every 24h at which intervals, the decalcification solution had flame photometry readings taken to determine the end point of decalcification (Van Wyk, 1987).

A flame photometer M4 QIII (Zeiss, West Germany) was calibrated using the following 5 standard solutions made up in the stock decalcification fluid: 6 mgCa/100 ml, 4 mgCa/10 ml, 3 mgCa/100 ml, 2 mgCa/100 ml and 1 mgCa/100 ml. They contain low concentration of calcium because at high concentrations there is an error of self-absorption in the flame. Six individual calibration tests were performed in which the standard solution of 6 mgCa/100 ml was used to set the galvanometer to read 80,0. The mean proportional values were recorded in an IBM 3058 J24 computer via a terminal and a standard calibration curve was produced using SAS (1981). The test readings were then read off this curve (Fig. 1). Test readings over 80,0 were diluted with the stock decalcification fluid and adjusted by the dilution factor. A reading of 9,0 on the galvanometer indicated that there was 0 mgCa/100 ml present in the decalcification fluid, thus the end point had been reached.

When the end point of decalification had been determined, the specimens were blotted on tissue paper to remove the excess decalcifying fluid and were processed up graded alcohols, cleared in xylene and embedded in Histosec (E. Merck, West Germany). An orientation device (Fig. 2), was designed to allow reproducable orientation of the block in a horizontal plane to the knife edge. The round stub at the top is the base on which the specimen is mounted. It fits tightly into the central hole below, thus reducing any movement and the peripheral holes engage a stop on the underside of the stub. The peripheral holes allow orientation at 45° intervals and the screws on either side allow the stub to be firmly clamped. In Fig. 3, one can see the specimen mounted on the orientation device as well as the multi-directional spirit level which was used, firstly, to ensure that the wax block was mounted horizontally and secondly to adjust to microtome so that when the orientation device was clamped in, it was also horizontal.

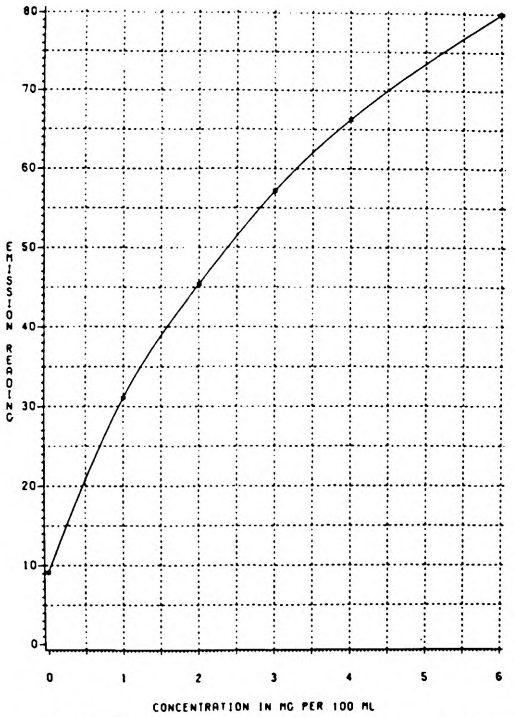


Fig. 1: **Computer plotted standard calibration curve of the flame photometer.**

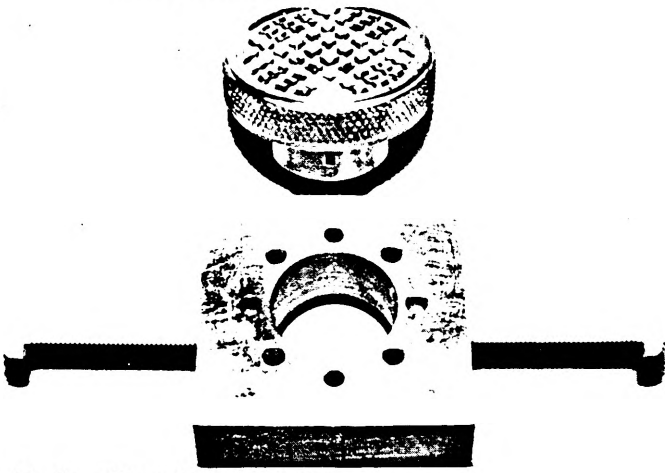


Fig. 2: **The orientation device.**

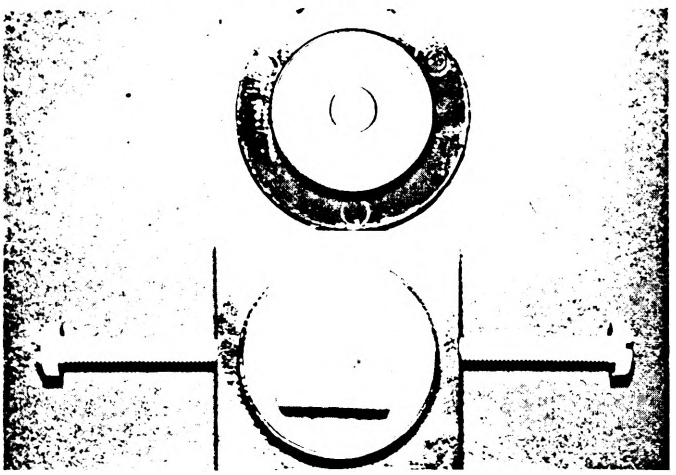


Fig. 3: **The orientation device showing a mounted specimen and the multi-directional spirit level.**

The block and orientation device was then clamped into a standard base sledge 1400 microtome (Leitz, West Germany) and the teeth were sectioned manually using a wedge-shaped (Profile C) knife, with a cutting angle of 20° and a clearance angle of 5 deg. The knife was sharpened on a Shandon Autosharp 5 knife sharpener (Shandon Southern Products Ltd., Cheshire) and then hand stropped using a leather strop (Lipshaw, Detroit) before cutting each block.

Initial sections were discarded until the pulp was reached, thereafter longitudinal serial sections were cut at 5 µm. Each tooth was sectioned at 4 orientations, each of these were coded 1 to 4 (Fig. 4). The sequence of sectioning was different for each tooth and was allocated using a table of random numbers. At code 1 sections were cut in the palatial to labial direction with the block orientation at 90° to the knife edge; at code 2 sections were also cut in the palatial and labial direction but the block was orientated at 45° to the knife edge; at code 3 the block orientation was the same as in 2, except the sections were cut in a labial to palatial direction and at code 4 the block orientation was the same as in 1, except the sections were cut in a labial to palatial direction. Four sections were cut at each code and 2 sections were placed on each slide. A total of 8 slides per block were stained with a modified Mayer's haematoxylin and eosin method (Molnar, 1976). The first and last sections of the stained slides for each code were evaluated for separation at the pulp-dentine interface using a Univar research microscope (Reichert, Austria) with built in calibrated graticule.

To evaluate the sections, an imaginary line was drawn through the centre of the pulp, dividing it into a labial side and a palatial side. The longitudinal extent of separation on both sides was assessed at a magnification of ×100. The total length of the pulp was measured at a magnification of ×25 (Fig. 5). The percentage separation was then calculated for each pulp. This was done for each of the 80 measurements made, subdivided into 20 measurements for each of the 4 orientation groups so that 10 were labial side and 10 palatial side. The measurements were recorded in a computer and statistically analysed using a 4-way analysis of variance (SAS, 1985).

RESULTS

The mean percentage separation of the 5 blocks was calculated. For block 1 the percentage separation was similar on the labial and palatial sides, but for the remaining 4 blocks, there was approximately 3 times more separation on the palatial side (Fig. 6). The mean percentage separation for the 4

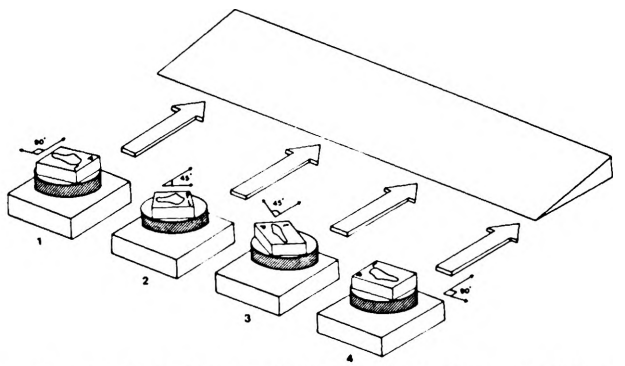


Fig. 4: **Diagram showing the block orientations to the knife edge and direction of cuts.**

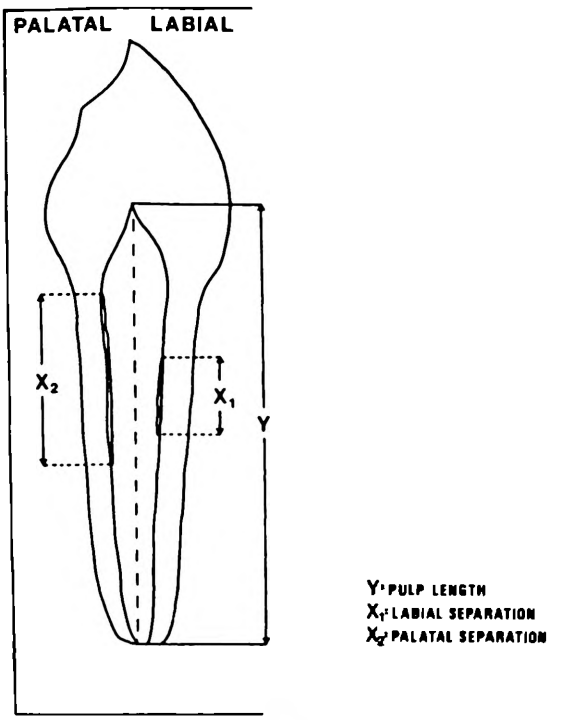


Fig. 5: **Diagram showing how the separation measurements were made.**

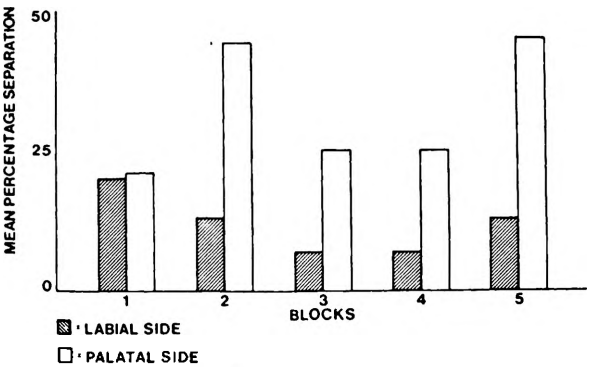


Fig. 6: **Bar chart showing the mean percentage separation for the 5 blocks.**

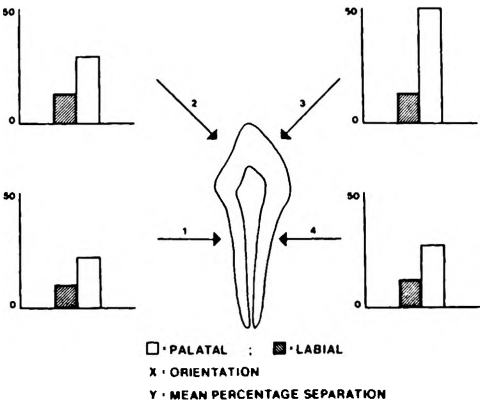


Fig. 7: **Diagram showing the mean percentage separation for the 4 orientation groups.**

orientation groups was then calculated. It was found that less separation occurred on the labial side, irrespective of the orientation of the block and irrespective of the direction from which the cut was made. It also appears that both the 45° orientation give the least favourable results (Fig. 7). The 4-way analysis of variance was then applied (Table I). This analysis revealed a significant variation in separation between the labial and palatial sides, irrespective of the orientation of the block and irrespective of the direction from which the cut was made. The angle of cut had some effect on the results but the direction of cut and block did not significantly influence the results.

Table I: **Results of 4-way analysis of variance**

FACTOR	F	P VALUE
Side of separation	31.87	0.0001
Angle of cut	5.67	0.02
Direction of cut	3.78	0.06
Block	2.45	0.0·

DISCUSSION

This investigation has shown that there is a difference in attachment between the pulpal tissue and the dentine on the labial and palatial sides, the labial side having less separation than the palatial side, irrespective of block orientation. This is an observation not previously made, and for which there is not yet an explanation. It could suggest that the labial surface has a stronger attachment than the palatial surface, but this requires further investigation. This study has confirmed the suggestion made by Brain (1966), that the short axis of a rectangular block be orientated at 90° to the knife edge for superior sections.

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