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Penetration of resin into experimentally formed infractions in porcine tooth crowns

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Abstract Tooth infractions are regarded as possible pathways for micro-organisms and other pulpal irritants in gaining access to the dental pulp; hence, these defects must be sealed to prevent subsequent pulpal involvement. This study aims to examine the structure of experimentally formed visible infractions and evaluate the penetration of resin into infractions after the application of resin onto the tooth surface that contains such defects. In experiment 1, porcine premolars were subjected to impaction procedures to create infractions and transverse ground sections were prepared to study the structure of the defect. In experiment 2, penetration of resin was evaluated. Another set of specimens were subjected to the same impaction procedure. These teeth received the application of 4 META/MMA-TBB resin and sectioned horizontally. The exposed surfaces were initially immersed in HCl then stained with Toluidine blue and subsequently with Fuchsin. For SEM examination, another tooth specimen was subjected to impaction procedure and prepared similarly with experiment 2. The results show that microscopically, infractions were wider than enamel lamellae, and some reached deep into the dentine. The penetrated resin was identified as a structure that stained with both Toluidine blue and Fuchsin in all infractions. The average penetration depth was $338.83 \pm 138 \mu$m and is not related to enamel layer thickness. SEM examination revealed that the outlines of surrounding enamel rods were visible as imprinted on the surface of the penetrated resin. In conclusion, the present results suggest that resin may adequately seal the infractions, thus prevent the ingress of pulpal irritants via these defects. Therefore, it is recommended that the tooth should be covered with dental adhesive resin as soon as possible after trauma.

Key words
Lamella,
Resin,
Tooth infractions,
Trauma

Introduction

The term of infraction has been used to describe an incomplete fracture (crack) of the enamel without loss of tooth substance[1)]. However, such a crack can involve the dentine layer as well as enamel layer[2,3)]. The acute dental trauma may be a major cause of infractions, but in certain circumstances infractions can also occur spontaneously as the result of biting stress[4)]. In some population, the incidence of the infractions in the incisors has been reported as high as 35–100%[4,5)]. The infractions were assumed to be the cause of subsequent caries damage and complete fractures along those lines[6,7)]. The presence of infraction lines should draw attention to the possible presence of associated injuries, especially to the supporting structures such as dentine, pulp and periodontal ligament[1)]. Ravn[8,9)] reported that, pulpal necrosis was observed in 1.7–3.5% of traumatized teeth with enamel infractions in combination with supportive tissue damage. In a study concerning the

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exposed dentine by attrition, it was demonstrated that bacteria were seen in dentinal cracks, exposed dentinal tubules and necrotic pulp of the teeth[10]. Once the microorganisms gain access to the pulp system through enamel-dentine cracks, pulpal infection can be established in 2–3 weeks[11]. After trauma, dental pulp of a tooth with infractions should be considered to have injury and be potentially susceptible to further damage from oral bacteria[12]. Love[13] reported that bacteria could invade the root canal system via enamel/dentine infractions of simulated traumatic teeth. After coating with resin, however, those teeth didn't demonstrate bacterial invasion over 7 days even though the resin was worn away *in situ*. However, Love didn't examine the penetration of resin into the infractions.

If resin can adequately penetrate into infractions and seal these tooth defects properly from the oral environment, dental pulp can probably be protected by preventing the ingress of pulpal irritants such as chemicals and bacteria. The purposes of this study are to investigate the structure of the experimentally formed tooth infractions that are visible on the outer tooth surface, and to determine penetration of resin into those infractions after the application of dental adhesive resin on the tooth surfaces.

Materials and Methods

Mandibular permanent first premolars from 6 months old porcines were used in this study. Teeth were extracted by elevator cautiously to prevent imparting any damage to the enamel. The soft tissues that remain around the premolars were removed with a scaler. The teeth were cleaned thoroughly with pumice-water slurry using a rotary brush, and rinsed with water. The specimens were stored in 0.1% thymol solution at 4°C. All teeth were inspected for the presence of any defects caused by the tooth extraction procedures under a stereo microscopy. One hundred teeth were measured, and the average dimensions were established from the measurements gathered, and all the teeth that have the standard crown size were selected and included in the study. Each chosen specimen has the following measurements: 6.1–6.9 mm mesiodistally, 3.2–3.8 mm buccolingually and 5.8–7 mm occlusocervically.

Experiment 1

Ten teeth were used for the Experiment 1. The teeth were randomly divided into 2 groups; 5 teeth served as the control group and the rest formed the experimental group. Each tooth was mounted into self-curing acrylic resin (Unifast II™, GC Corporation, Tokyo, Japan) using the holding device (Fig. 1-H). The tooth was embedded with a portion of the crown exposed 6 mm occlusocervically from the top of the cusp. Upon curing, the teeth were removed from the resin block, and stored in distilled water for 24 hours at room temperature. Subsequently, the crown surface was stained with 0.5% Fuchsin solution for 5 minutes. During this procedure, the surface was rubbed with cotton pellets, moistened with Fuchsin solution. Excess solution was removed by wiping with water-moistened tissue paper. The resin block was then fixed in holding device and the crowns of teeth were examined under a stereo zoom microscope (SZH-ILLB, Olympus, Tokyo, Japan) at $\times 20$ and $\times 40$ magnifications. Photomicrographs of teeth were recorded from buccal, lingual, mesial, distal and occlusal directions with a digital camera (DP 70, Olympus).

In the experimental group, the tooth fixed in the holding device (Fig. 1-T) was stabilized on the metal block (Fig. 1-M). A steel rod weighing 495 grams (Fig. 1-W) was plunged through the tube with a 20 mm vertical distance from the occlusal tip of the tooth specimen. During the preliminary experiments, tooth fractures occurred when the steel rod was dropped from more than 20 mm height. Hence, from this result, the steel rod dropping height was standardized to 20 mm. After impaction, the tooth surfaces set in holding device were examined with a

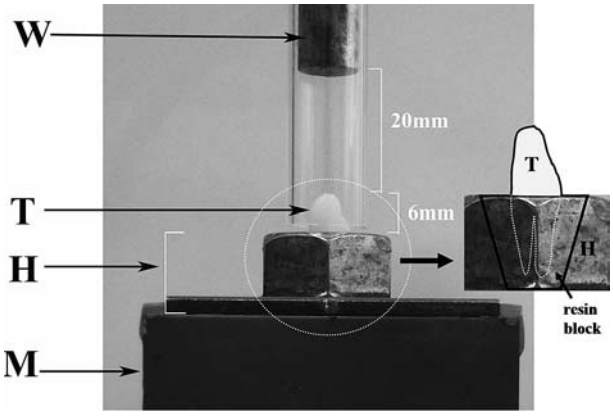

Fig. 1 Set-up and arrangement of apparatus for creating infractions

W: steel rod of 495 g weight, raised on a dropping height of 20 mm, T: tooth mounted on a resin block, 6 mm of crown exposed occlusocervically, H: holding device, M: metal block.

stereo zoom microscope at ×20 and ×40 magnifications. The experimentally formed infraction lines were recorded photographically from buccal, lingual, mesial, distal and occlusal directions.

All the specimens from both groups were removed from resin blocks and fixed in 10% neutrally buffered formalin solution for 3 days. The specimens were dehydrated through immersion in a series of ascending concentrations of ethanol solutions, and repositioned in corresponding resin blocks and embedded in polyester resin (Rigolic; Oaken Co., Tokyo, Japan). Specimens were sectioned at the distance of 700μm from cusp tip perpendicular to the long axis of the tooth with a rotary diamond saw (Leica SP 1600 saw microtome; Leica Microsystems Nussloch Gmbh, Nussloch, Germany). The cut surfaces of the cusp tip specimens were adhered on plastic plate with adhesive (Aron Alpha A, Sankyo, Tokyo, Japan). Ground sections approximately 100μm in thickness were prepared from the procured cusp tip specimens by manual grinding using emery papers under running water. These transverse ground sections were examined accordingly by conventional microscopy (BX50F4, Olympus) at the magnifications of ×40, ×100, ×200 and ×400, respectively.

Experiment 2

Twenty teeth were used in this experiment. They were randomly divided into 2 groups; 10 teeth served as the control group and the rest formed the

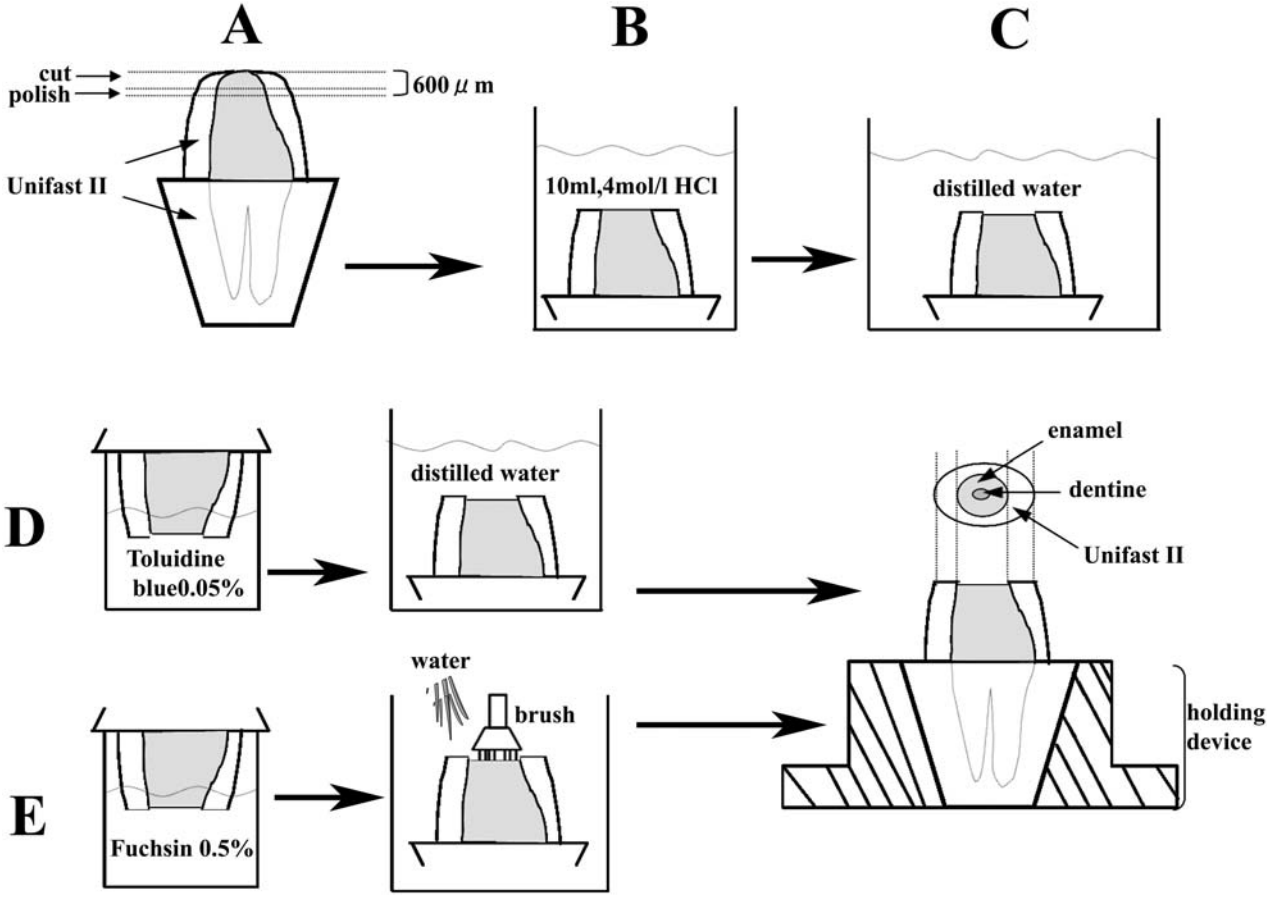

Fig. 2 Schematic illustration of the experimental procedures

(A) Resin coated tooth was cut with a diamond saw (Leica 1600) and polished (cut surface is about 600μm from cusp tip).
(B) The sectioned tooth specimen is immersed in 10 m*l* 4 mol/*l* HCl for 2 minutes.
(C) The sectioned tooth specimen is gently placed in distilled water for 2 minutes.
(D) Cut surface is dipped in 0.05% Toluidine blue solution for 1 minute and placed in distilled water for 10 seconds. Tooth specimen is fixed in holding device for examination of stained cut surface.
(E) Specimen was dipped in 0.5% Fuchsin solution for 5 minutes and rubbed with cotton pellets. Stained surface is cleaned by brushing manually under running water for 160 times. Tooth specimen is fixed in holding device for examination of stained cut surface.

experimental group.

Each tooth was mounted, stained with Fuchsin and recorded photographically, as with the same procedure in experiment 1.

In experimental group, teeth were impacted to make infractions, and formed infractions were recorded photographically as with the procedures for experiment 1. Crowns of all teeth were coated with Super bond C&B™ system, 4 META/MMA-TBB resin (Sun Medical Co., Ltd., Shiga, Japan). Prior to the application of resin material, teeth were initially acid etched with 65% phosphoric acid gel (Red activator™; Sun Medical Co., Ltd.) for 30 seconds, then washed with water spray for 20 seconds and air dried. After the single application of activated liquid (the mixture of the monomer and Catalyst S), each tooth was coated with Super-Bond C&B™ by using brush-dip technique. Afterward, tooth crowns were covered with Unifast II™ (GC Corporation) with more than 2 mm resin thickness. The resin coated teeth were stored in distilled water for 24 hours at 37°C.

Schematic illustration for the experimental procedures was described in Fig. 2. The specimens were sectioned 500μm from cusp tip perpendicular to long axis of teeth with a rotary diamond saw (Leica SP 1600 saw microtome). The cusp tip fragments were discarded. The cut surface of the remaining

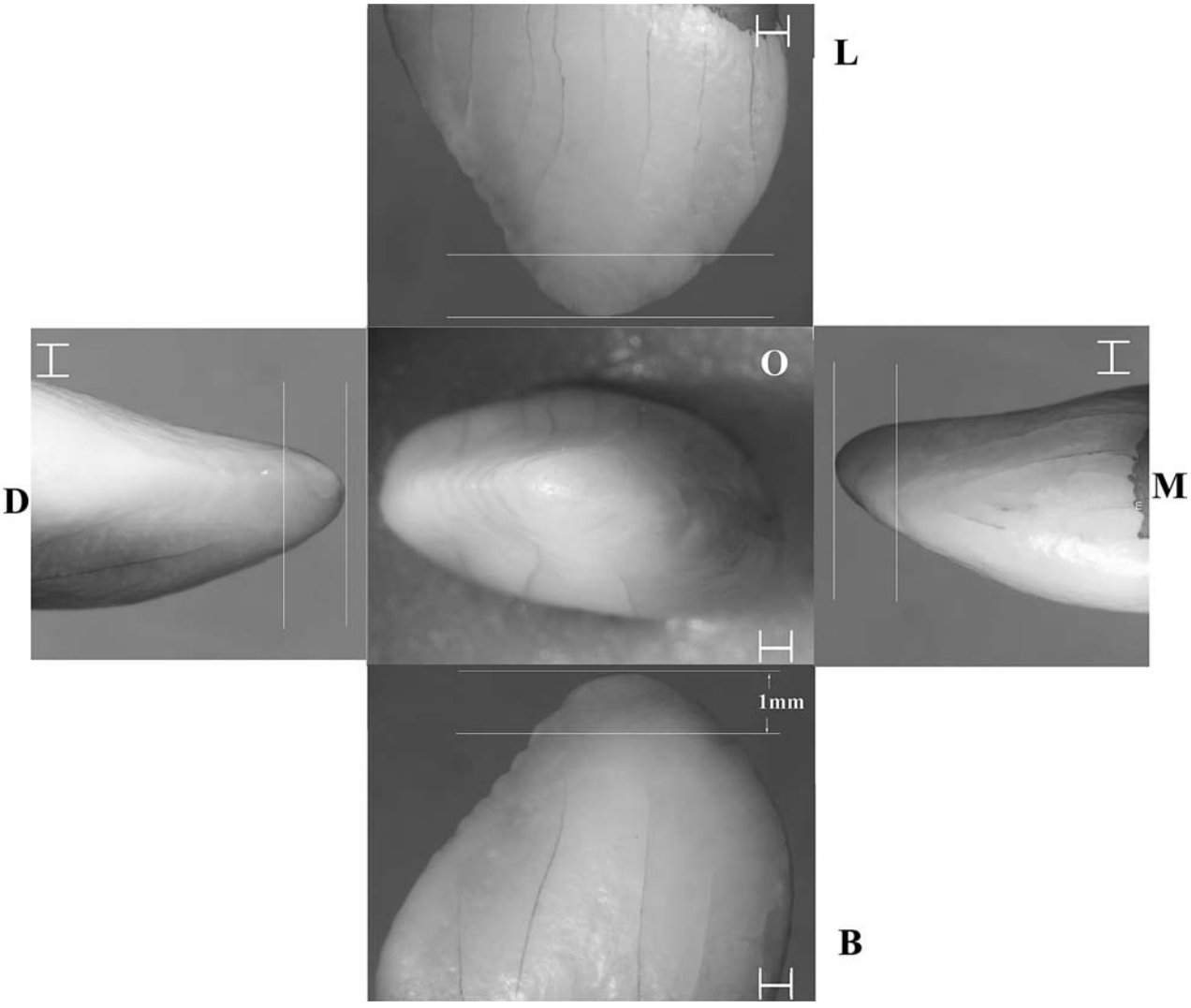

Fig. 3 Stereomicroscopic photographs of mandibular porcine first premolar from 5 aspects

The red lines are seen after staining with 0.5% Fuchsin solution for 5 minutes. These lines appeared numerously on the cervical third than on the occlusal third. B: buccal view, L: lingual view, M: mesial view, D: distal view, O: occlusal view, Scale bars 500μm. Stereomicroscope $\times$ 20.

tooth was polished under a water stream using emery papers to attain a smooth surface finish. The cut surface was approximately 600μm from the cusp tip of the original sample tooth (Fig. 2A). After polishing the exposed surface, each tooth specimen was immersed in 10 m*l* of 4 mol/*l* HCl solution for 2 minutes to dissolve the superficial layer of the cut surface (Fig. 2B), exposing further the penetrated resin for study. To inactivate this process, specimens were carefully placed in distilled water for 2 minutes (Fig. 2C).

1. Staining with Toluidine blue

After etching with 4 mol/*l* HCL solution, each tooth specimen was stained with Toluidine blue to examine the penetrated resin. Each tooth specimen was dipped in 0.05% Toluidine blue solution for 1 minute and gently placed in distilled water for 10 seconds (Fig. 2D). It was again fixed in holding device and examined under a stereo zoom microscope at $\times$40 and $\times$64 magnifications. Photomicrograph of the stained cut surface was compared with those of the tooth surfaces of the corresponding tooth to determine whether the stained structures were identical to the infraction lines.

2. Staining with Fuchsin

The tooth specimens were further stained with 0.5% Fuchsin solution for 5 minutes to detect the minute space in between enamel and resin. Each cut surface was rubbed with cotton pellets moistened with the Fuchsin solution. Under running water, the surface was brushed 160 times manually with Merssage brush™ (Shofu Inc., Kyoto, Japan) (Fig. 2E), and examined with a stereo zoom microscope. Photomicrograph of the stained cut surface was compared

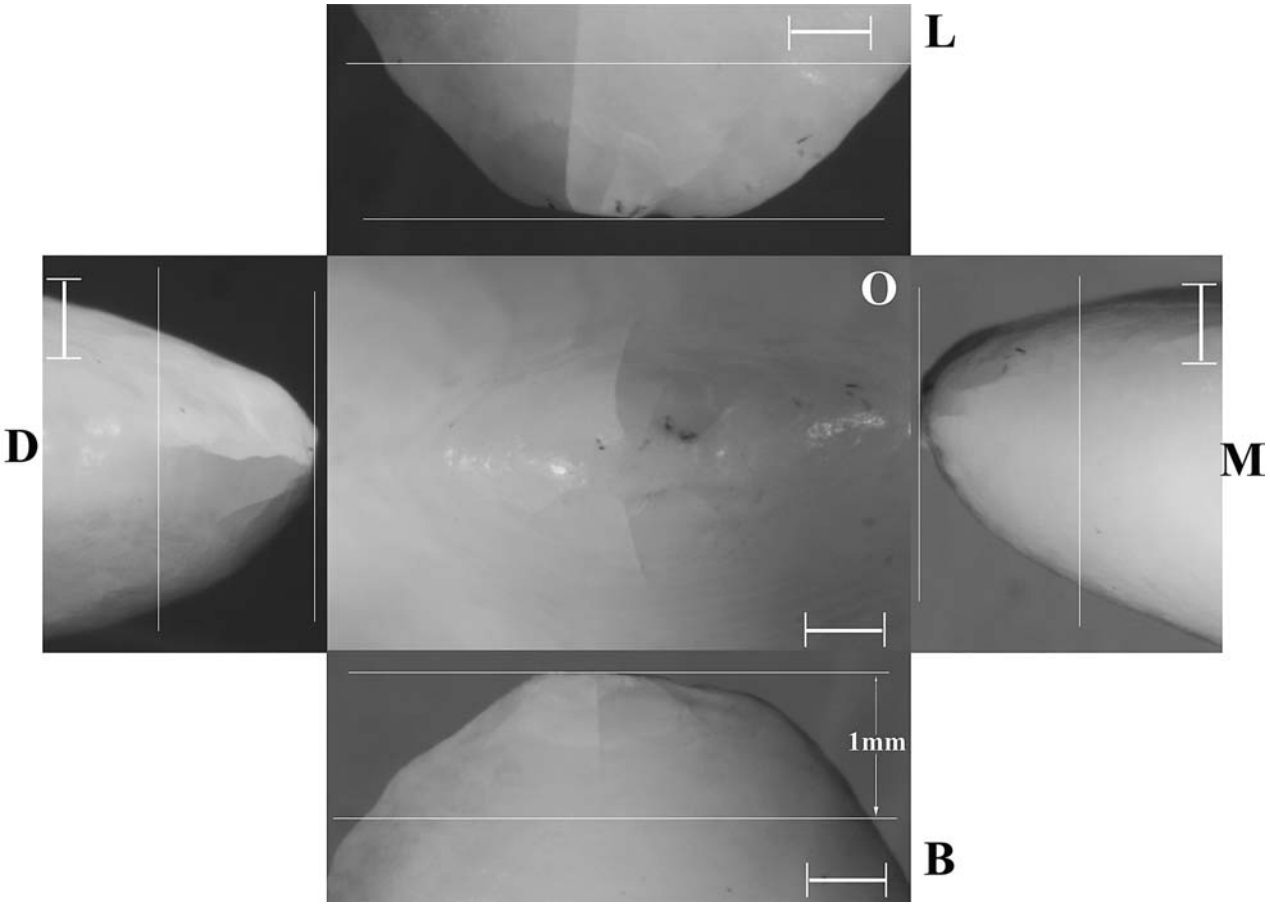

Fig. 4 Experimentally formed infraction lines (IL) on the surfaces of tooth viewed from 5 aspects

IL run as clear lines from occlusal tip towards cervical third, after staining with 0.5% Fuchsin solution for 5 minutes and impacted with 495 g weight. Note IL were more numerous within 1 mm from cusp tip. B: buccal view, L: lingual view, M: mesial view, D: distal view, O: occlusal view, Scale bars 500μm. Stereomicroscope $\times$40.

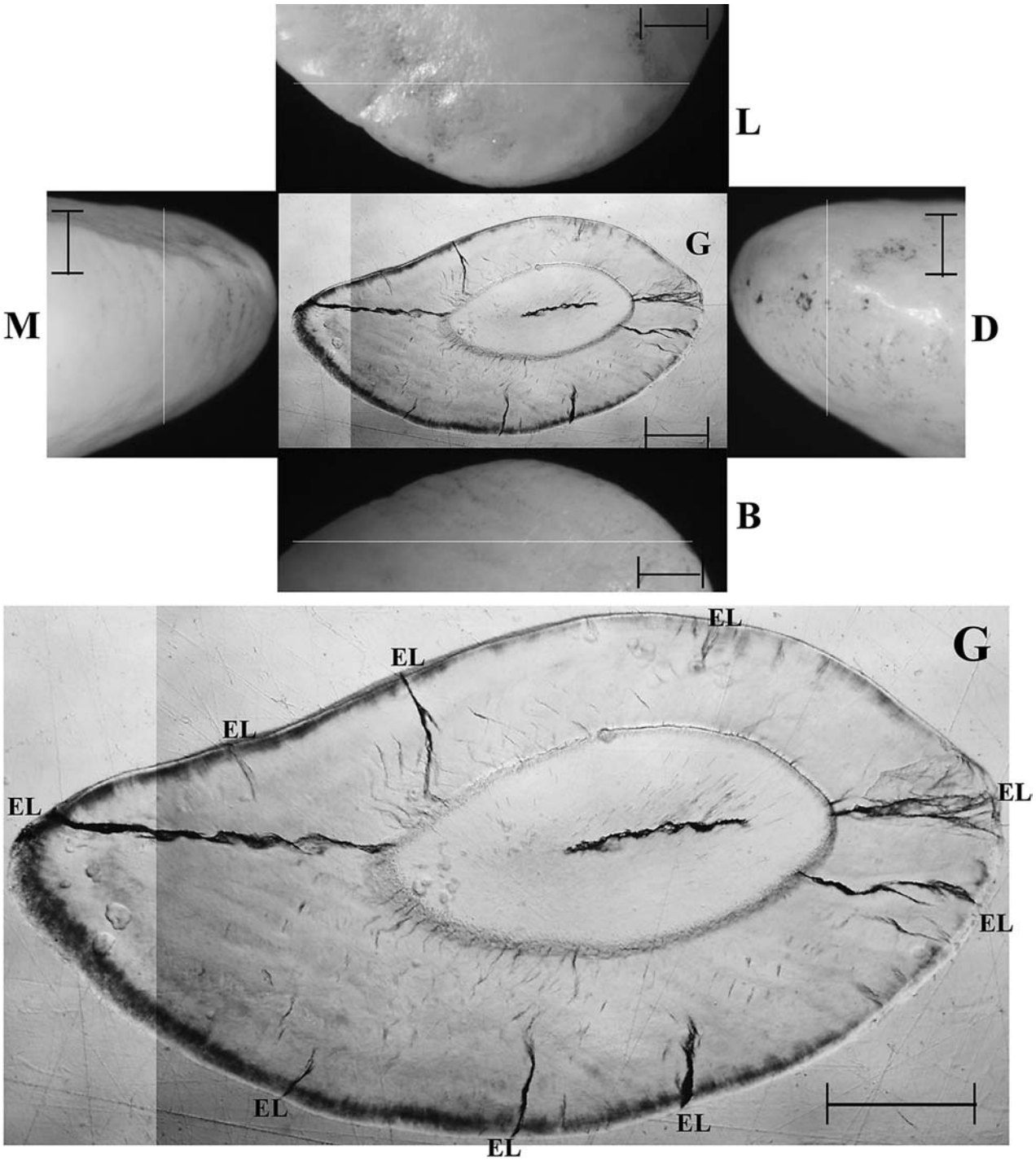

Fig. 5 Transverse ground section and corresponding tooth surfaces as viewed from 4 aspects in control group

B: buccal view, L: lingual view, M: mesial view, D: distal view, G: ground section. In ground section (G), several enamel lamella-like structures (EL) and enamel tufts are seen in enamel. EL at mesial and distal sides are wider and thicker than buccal and lingual sides. The position of ground section is indicated by straight lines on tooth surfaces, after staining with 0.5% Fuchsin solution for 5 minutes. B, L, M, D: stereomicroscope $\times 40$. G: conventional microscope, original magnification $\times 40$. Scale bars 500μm.

with those of the surfaces of the corresponding tooth.

Lengths of Toluidine blue stained lines and Fuchsin stained lines on micrographs were measured using the measuring software. The measurements were repeated 3 times for each line.

SEM observation of cut surface

Another tooth specimen was subjected to impaction procedure to make infractions using the same procedures for experiment 1 and the obtained cut surface was prepared with the same method as with experiment 2. The sectioned surface was treated with 2 mol/*l* HCl for 20 seconds to dissolve the superficial layer of the cut surface. The specimen was carefully placed in distilled water for 2 minutes and stained with 0.05% Toluidine blue solution for 1 minute and observed stereo microscopically. After the specimen has dried, it was mounted on SEM specimen stub, gold-sputtered, and observed under SEM at ×37 to ×2,000 magnifications.

Results

Detection of infraction lines

1. Stereomicroscope appearance

In all teeth, the red lines stained with Fuchsin were more numerous in the cervical third of crown surface compared to the occlusal third (Fig. 3). At the occlusal third, within 1 mm from cusp tip, red lines appeared on the mesial and distal cuspal edges. These lines appeared on mesial and distal cuspal edges of 3 teeth in control group and 2 teeth in experimental group before impaction. After impaction, newly formed infraction lines (IL) were observed in all teeth on the experimental group. ILs were observed as clear lines that run from the cusp tip toward the cervical third, and occasionally occurred continuously with Fuchsin stained red lines. At the occlusal third, infraction lines were observed to be more numerous within 1 mm from the cusp tip (Fig. 4).

2. Ground section of the specimens

In the present study, the enamel lamella-like dark lines that appeared in the ground section of specimens belonging to the control group were termed as enamel lamella-like structures (EL) (Fig. 5). ELs extended from enamel surface toward dentine at various lengths. On the mesial and distal portions of all sections, ELs were found on the mesial and distal dentine corners; occasionally they interconnect with corresponding lines that extended from enamel surface toward dentine. ELs on mesial and distal sides were wider and thicker than those on the buccal and lingual sides. Table 1 shows distribution and length of EL from enamel surface toward dentine as examined by conventional microscope with a magnification of ×200.

In the experimental group, dark lines appeared on ground sections at the same position of IL, which were visible on the surfaces of the corresponding tooth (Fig. 6). These lines were found at the enamel surface and reached a short distance into the dentine. Other dark lines not visible on tooth surfaces were considered as EL. Most IL reached the dentine and were longer than EL. Three IL reached into the dentine and communicate with each other showing complicated structures. Researchers termed these structures as complicated infraction lines (CIL) (Fig. 7). Table 1 shows the distribution of EL and IL in transverse ground sections of the specimens.

3. Control group and experimental group

On the buccal and lingual portions of the teeth, the histological features of EL and IL were quite similar within the outer half of enamel layer (Fig. 8). In those regions, both run almost parallel to the surrounding enamel rods (Fig. 8C and D). Within the inner half of enamel layer, IL became wider than EL, and it showed thicker branches than EL. In higher magnification, IL exhibited more fine cracks (Fig. 8F) than EL (Fig. 8E), that branch into different directions.

Dental adhesive resin penetration

1. Control group

On the tooth surfaces, Fuchsin stained red lines appeared on mesial and distal cuspal edges at the position of prepared cut surface (Fig. 9). These lines appeared in 9 out of 10 teeth on the control group on the mesial and distal cuspal edges.

On the cut surface, the internal enamel rim appeared to be blue fine fringe after staining with Toluidine blue (Fig. 10A). After fuchsin staining, red lines were observed on the mesial and distal enamel areas of the crown (Fig. 10B). The schematic illustration of the configuration of these lines was described in Fig. 10C. The center line was drawn by connecting the 2 points of the widest mesio-distal dentine diameter. All mesial and distal lines were within 200μm above and 200μm below the centre line.

2. Experimental group

Before teeth had impaction, Fuchsin stained red lines appeared on the surfaces of mesial and distal cuspal edges of 5 teeth at the position of cutting. After impaction, ILs were observed in all teeth as clear lines and that run from the cusp tip toward the cervical third (Fig. 11).

Table 1 Distribution of EL and IL in transverse ground tooth sections

Length	control	exp	
	EL	EL	IL
< Half of enamel layer	16	16	—
≥ Half of enamel layer	15	8	3
Reached into dentine	10	1	11
CIL	—	—	3
Total	41	25	17

EL: enamel lamella-like structures, IL: experimentally formed infraction lines, CIL: complicated infraction lines (Infraction lines which reached into dentine and communicate with each other forming complicated structures); examined by conventional microscope ×200. (Control = 5 sections, experimental = 5 sections)

After Toluidine blue staining, penetrated resin appeared as blue stained lines on the cut surface at the same position of ILs which were visible on the surfaces of the corresponding tooth (Fig. 11). Those lines were observed to be continuous with the outer resin cover of the enamel surface. These blue lines were found in the enamel as well as in the dentine; some are short, which terminated within the enamel layer of the teeth, while some lines traversed the entire thickness of enamel, with a few reaching the dentine. After Fuchsin staining, red lines (Fig. 12A) appeared in the enamel, at the same position of the corresponding blue lines (Fig. 12B). These lines were measured from the enamel surface to its dentinal termination as described in Fig. 12C and D.

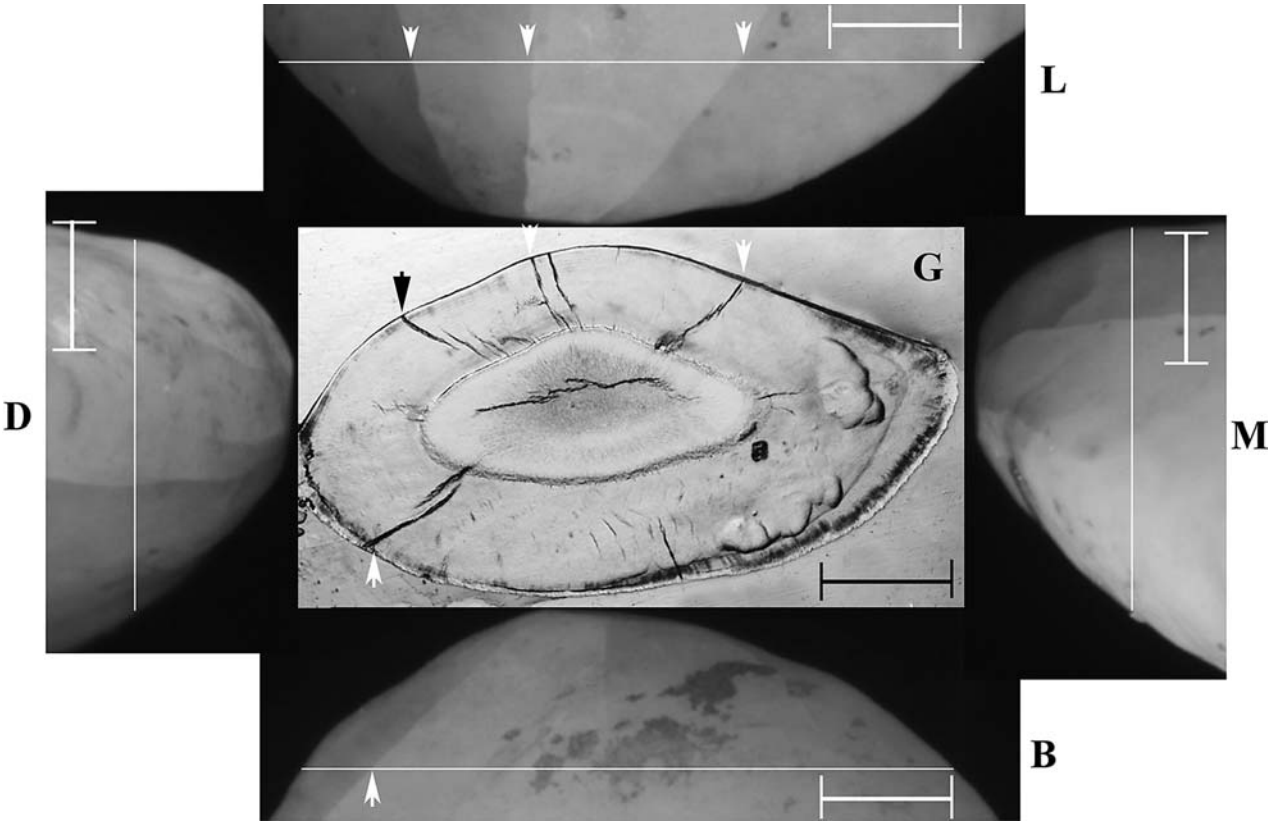

Fig. 6 Transverse ground section and corresponding tooth surfaces viewed from 4 aspects in experimental group

B: buccal view, L: lingual view, M: mesial view, D: distal view, G: ground section. IL are seen as clear lines on corresponding tooth surfaces, after staining with 0.5% Fuchsin solution for 5 minutes and impacted with 495 g weight. Straight lines on tooth surfaces indicate the position of ground section. Arrow heads indicate the positions of IL visible on tooth surfaces and ground section. In the ground section (G), IL, EL and enamel tufts are seen in enamel. B, L, M, D: stereomicroscope ×40, G: conventional microscope original magnification ×40, Scale bars 500 μm.

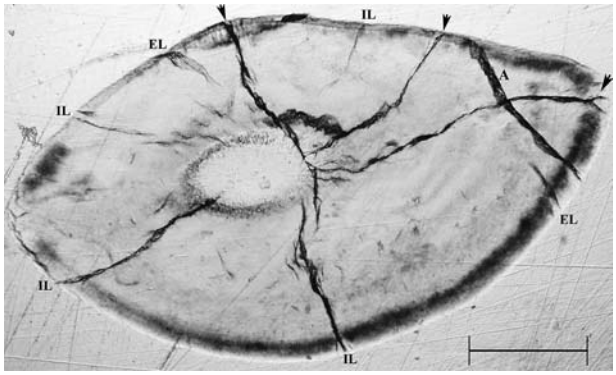

Fig. 7 Transverse ground section of experimental group

IL, EL and enamel tufts are seen in enamel. Arrows indicate complicated infraction lines (CIL) reaching into dentine and crossing each other, conventional microscope original magnification × 40. A: artifact line occurred during sectioning with a cutting machine. Scale bar 500μm.

Fifty blue lines were observed in experimental group (Table 2). All lines were verified with Fuchsin stain at the same position.

Figure 13 shows the relationship between the ratio of blue and red lines and enamel thickness at buccal and lingual area, (n = 27). In all infractions, blue stained penetrated resins were longer than red lines; however, this observation is not related to the thickness of enamel layer. The longest length of blue line was more than 600μm and the average length was 338.83 ± 138μm.

The 16 lines found on the mesial and distal areas of the crown and 7 lines that crossed (CIL) in buccal and lingual areas were excluded from the measurement.

SEM observation of penetrated resin

Under SEM observation, blue stained lines on the cut surface (Fig. 14A) were seen as continuous

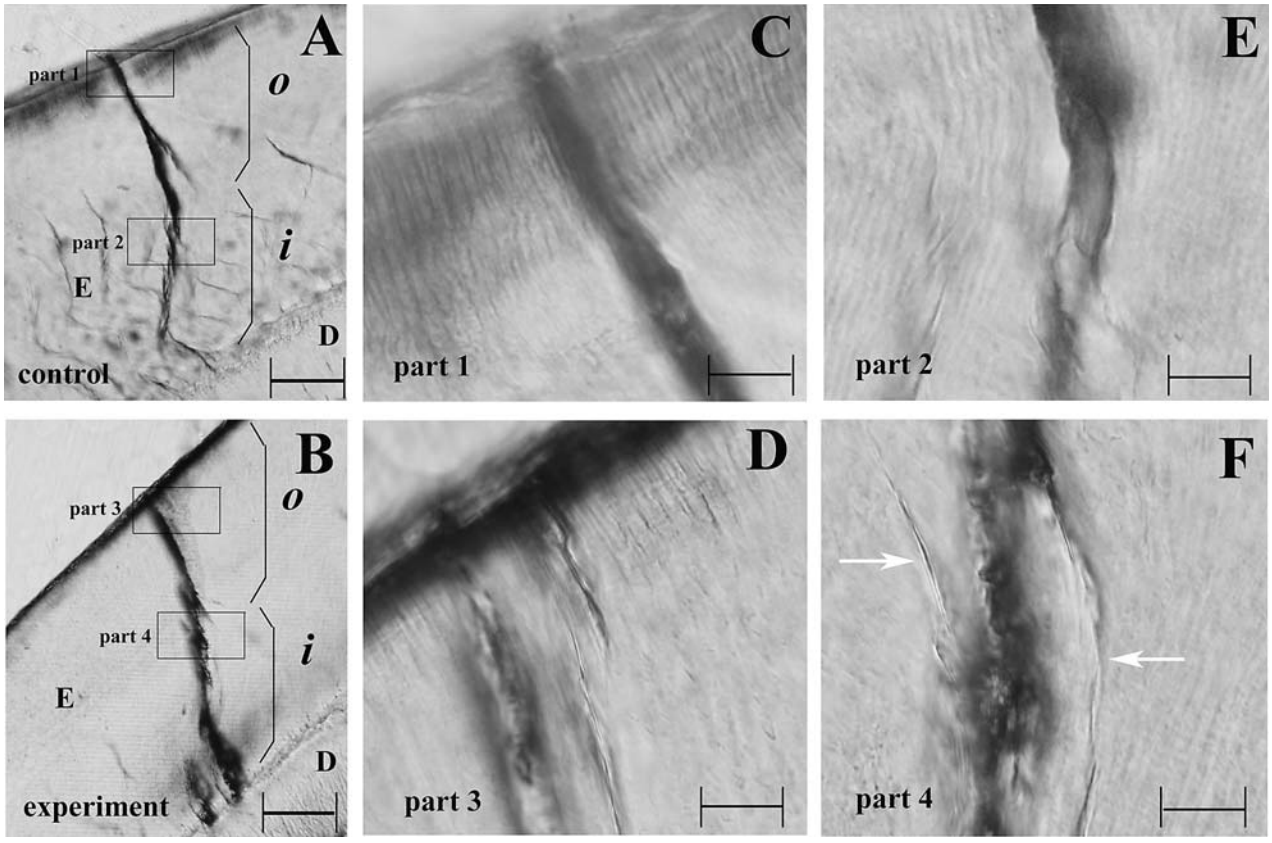

Fig. 8 EL and IL in transverse ground section examined by conventional microscope

(A) EL of control group. C, E are higher magnifications of the boxed area in A.
(B) IL of experimental group. D, F are higher magnifications of the boxed area in B.
E: enamel, D: dentine, arrows: fine cracks, Scale bar 100μm in A, B and 20μm in C, D, E, F. EL and IL are quite similar within outer half of enamel (*o*), they ran almost parallel to surrounding enamel rods (C and D). IL (B) is wider than EL (A) within inner half of enamel (*i*) and has thicker branches. It has more fine cracks (F) compared with EL (E).

raised oblique bands within partially demineralized enamel surface (Fig. 14B). In higher magnifications, the raised oblique band was continuous with the surrounding resin cover of Unifast II at the resin-enamel interface (Fig. 15A) and enamel rod outlines were visible on the surface of the raised bands (Fig. 15B).

Discussion

Since porcine teeth have been reported to have similarities to the human teeth in terms of the resin bonding strength values[14], and the morphological structures of enamel after phosphoric acid etching[15], they were used as the experimental samples in this study. Furthermore, since the present study required a large sample size of teeth that have the same morphological and physical characteristics, such as size and dimension, to make reproducible infractions, porcine teeth were used instead of human teeth.

It is reported that Fuchsin can stain enamel lamellae in teeth without any pretreatment procedures[16]. Since only a few EL and all of IL were present in occlusal region, 1 mm from the cusp tip in porcine teeth; tooth sections were made at this point. Before tooth impaction, Fuchsin-stained ELs were found on the mesial and distal cuspal edges of most teeth. In ground sections and cut surfaces, wide ELs (in control and experimental groups) were present in mesial and distal areas of the crown. Therefore, comparison between ILs and ELs were done on other areas except on these regions.

In ground sections, enamel lamella-like structures (EL) and infractions (IL) showed features quite similar to each other in the outer half of enamel. But IL appeared wider than EL in the inner half of

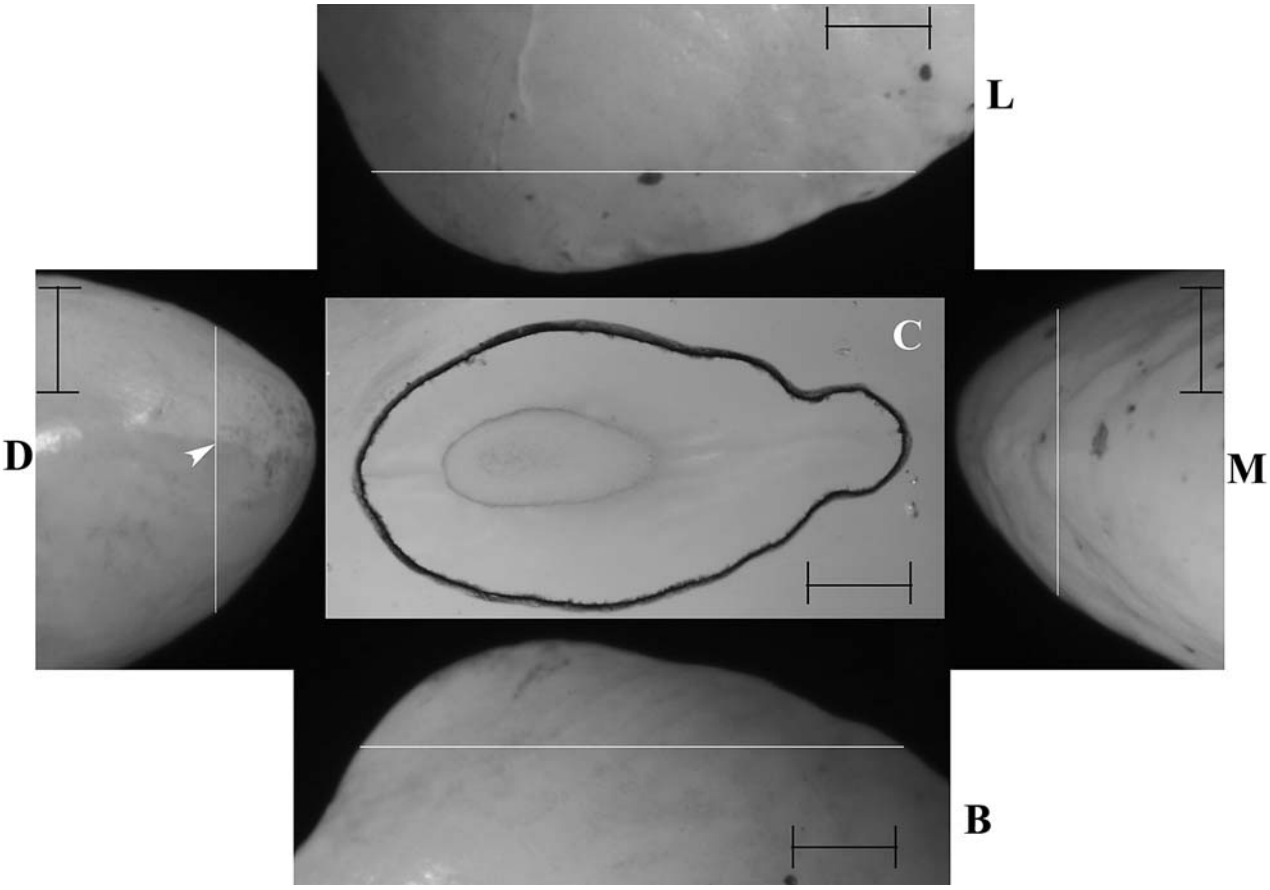

Fig. 9 The cut surface and corresponding surfaces of tooth from 4 aspects in control group

B: buccal view, L: lingual view, M: mesial view, D: distal view, C: cut surface. The cut surface (C), after immersion in 10 m*l* of 4 mol/*l* HCl for 2 minutes and stained with 0.05% Toluidine blue solution for 1 minute. Straight lines on tooth surfaces indicate the position of cut surface. Notes one stained line (arrow head) on distal cuspal edge, after staining with 0.5% Fuchsin solution for 5 minutes. Scale bars 500μm. Stereomicroscope $\times$ 40.

enamel and some IL have reached deep into dentine. Some of the newly formed IL is continuous with Fuchsin-stained EL on the outer surface of the tooth, suggesting that EL could serve as a forerunner for IL, and could develop into IL after the tooth suffers from impaction or trauma. In a report concerning a fractured crown fragment of human tooth, infraction line was seen within the enamel and some parts of infraction seemed to follow the course of enamel rods[1]. In the present study, IL also ran almost parallel to the surrounding enamel rods in the outer half of enamel layer. Moreover, in a histological study of cervical enamel margins after crown preparation for dental treatment, the micro-fracture of enamel margin was suggested to be related to enamel structures such as enamel lamellae, incremental lines of Retzius, non-rod enamel, electron microscopic rod sheath, hypocalcified zone, direction of enamel rod[17].

Enamel lamellae had been reported as a permeable pathway to gaining access of caries-producing bacteria to the dentine-enamel junction[18]. Present study indicates that ILs are wider than ELs and may reach deep into dentine, therefore, there is no doubt that ILs create a higher risk for bacterial and chemical invasion to dentine and pulp than enamel lamellae. In order to prevent this from occurring, tooth has to be sufficiently covered and sealed by a suitable material that possesses acceptable physical and chemical characteristics, which would adequately flow into the entire depth of the defect. In this study, 4 META/MMA-TBB resin was used to serve this purpose since it had been reported that this material has enough low flow rate to make deep resin tags into the enamel[19]. Furthermore, it possesses excellent tensile strength[20], biocompatibility[21], and

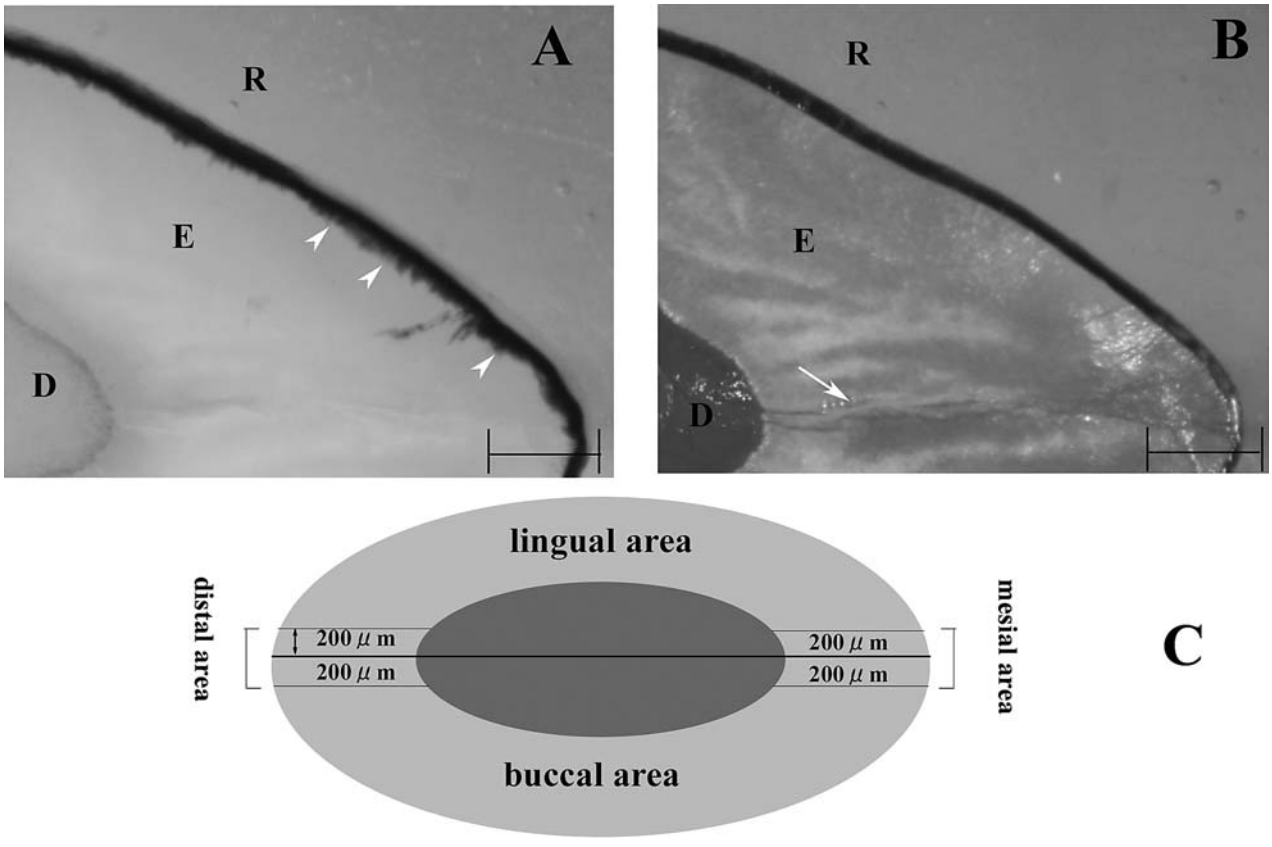

Fig. 10 The cut surface of tooth in control group

(A) Toluidine blue stained cut surface. After immersion in 10 m*l* of 4 mol/*l* HCl for 2 minutes, and stained with 0.05% Toluidine blue solution for 1 minute. The internal enamel rim (arrow heads) appears to be blue fine fringe. E: enamel, D: dentine, R: Unifast II resin, Scale bar 200μm. Stereomicroscope $\times$64.

(B) Same surface was stained with 0.5% Fuchsin solution for 5 minutes and brushed. Arrow indicates Fuchsin stained mesial line.

(C) Schematic illustration of the area of all mesial and distal lines. The centre line is drawn by connecting the widest mesiodistal diameter of dentine. All lines are within 200μm above and below of the centre line.

minimal cytotoxicity[22], making it a desirable material for sealing traumatized tooth. In the preliminary study, 2 types of resins were used, the 4 META/MMA-TBB resin and a light curing dentin bonding resin, Fluorobond. The results gathered from this study showed that 4 META/MMA-TBB resin was able to penetrate the infraction lines deeper than Fluorobond.

With the observations done on specimens that received resin application, red lines were seen along with IL in the cut surface stained with Fuchsin solution, indicating the basal IL. Since Fuchsin solution had infiltrated into IL along the surface of penetrated resin, this resin probably failed to seal the IL spaces completely even though it was able to penetrate into ILs. Furthermore, with Fuchsin staining solution, the dentine layer of the cut surface was stained darkly red. Consequently, the dentinal part of the red stained IL cannot be distinguished from the adjacent Fuchsin-stained dentine; however, red lines can be easily detected in the enamel layer.

HCl treatment facilitated the removal of enamel layer of about 100μm thickness from the cut surface, thereby resulting to the partial dissolution of the enamel surrounding the penetrated resin, and enhanced the visibility of penetrated resin for microscopic study. As evidenced by the pilot study done by the researchers, immersion of tooth specimen in 4 mol/l HCl for 2 minutes resulted to the optimal partial dissolution of cut surface of about 100 microns. The blue stained structures are assumed to be the penetrated resins. Toluidine blue solution is capable of staining rough surfaces of the resin which were exposed by the partial dissolution

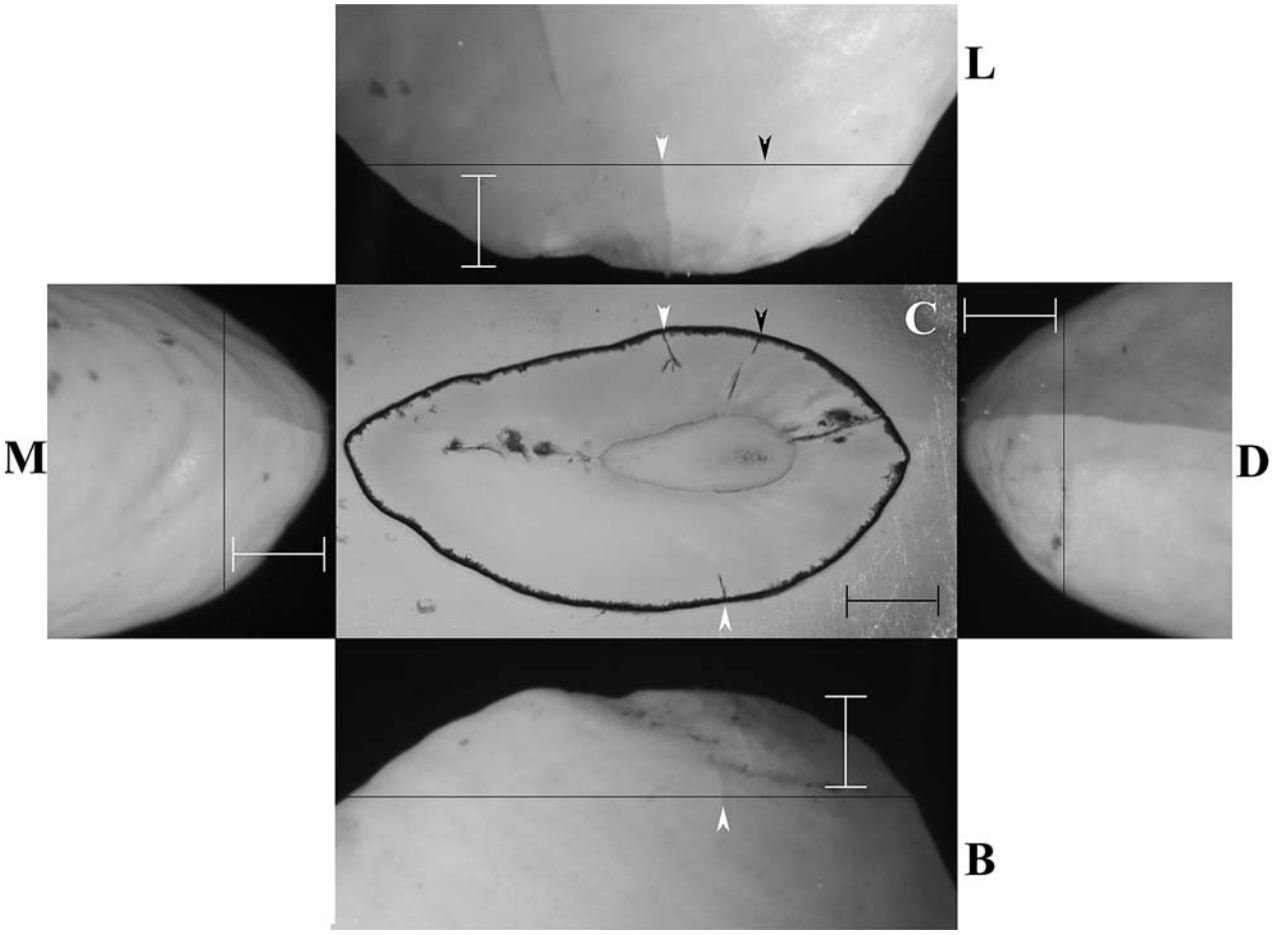

Fig. 11 The cut surface and corresponding tooth surfaces viewed from 4 aspects in experimental group

B: buccal view, L: lingual view, M: mesial view, D: distal view, C: cut surface. Blue stained lines on the cut surface (C), after immersion in 10 ml 4 mol/l HCl for 2 minutes and stained with 0.05% Toluidine blue solution for 1 minute. The position of cut surface is indicated by straight lines. Arrow heads indicate same position of IL in cross section and tooth surfaces. Stereomicroscope $\times$ 40. Scale bars 500μm.

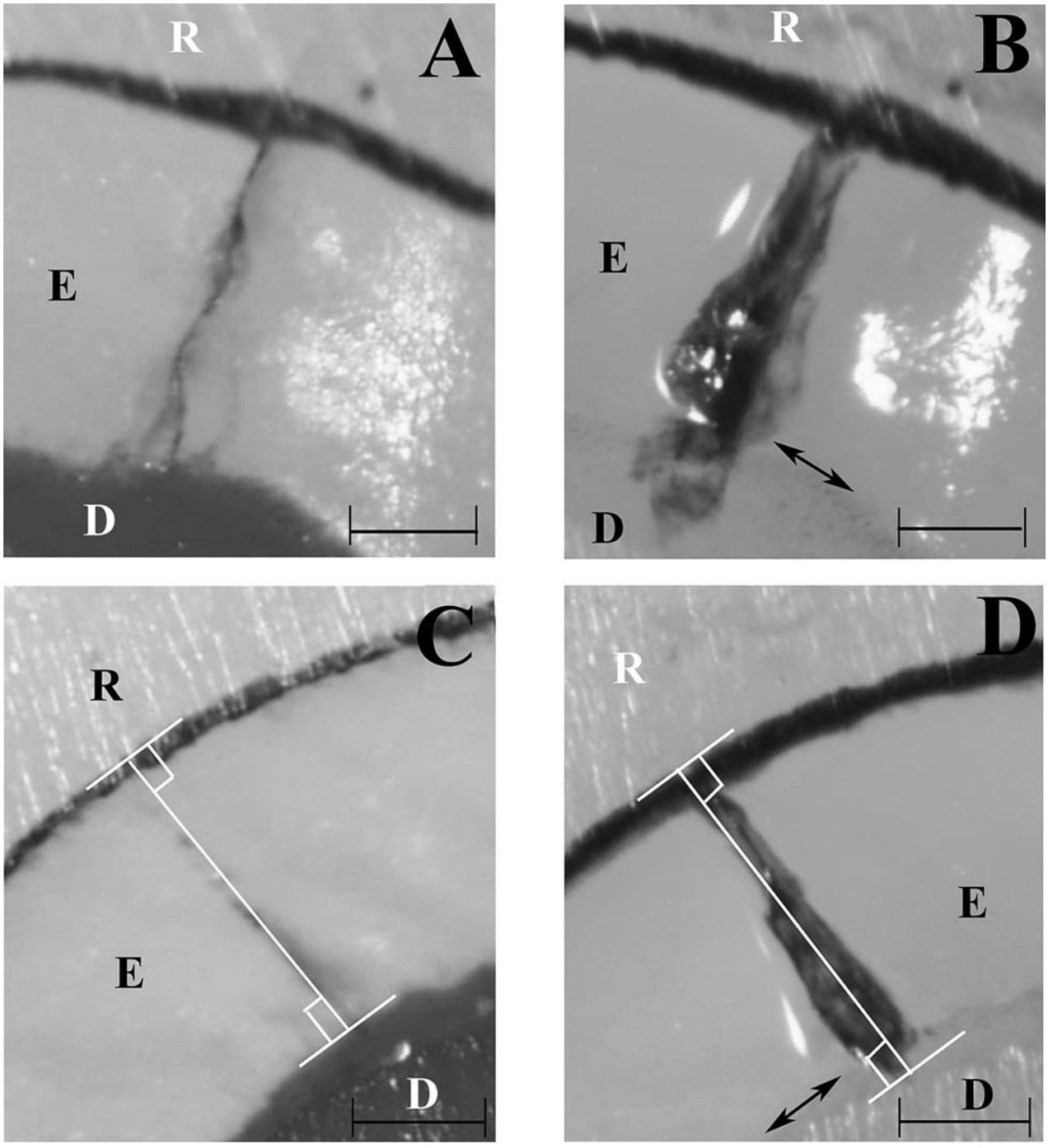

Fig. 12 The cut surface of teeth in experimental group showing red and blue lines

(A) Red line is seen at the same position of blue line after staining with 0.5% Fuchsin solution and brushing. Dentine is stained deeply by Fuchsin.

(B) Blue line after stained with 0.05% Toluidine blue solution.

(C and D) Measurement of red and blue lines. A straight line, corresponding to IL was drawn from the enamel surface to its dentinal termination. E: enamel, D: dentine, R: Unifast II resin, double head arrows: dentinoenamel junction, Scale bars 100μm. Stereomicroscope $\times$ 64.

of the enamel crystals, but not the dentine layer. Therefore, the dentinal portions of the Toluidine blue-stained resin were distinct in the cut surface.

As the cut surface was stained with Toluidine blue or Fuchsin solution, the blue lines could be seen in deeper area of the tooth than the red lines; it therefore suggests that resin was able to penetrate the entire depth of enamel IL and even deeper into dentine IL.

Table 2 Distribution of the blue lines in cut surface of experimental group

	B	L	M	D	Total
Blue line	15	12	3	9	39
Blue CIL	3	4	2	2	11
Total	18	16	5	11	50

B: buccal, L: lingual, M: mesial, D: distal, CIL: complicated infraction lines, examined by stereomicroscope × 40.

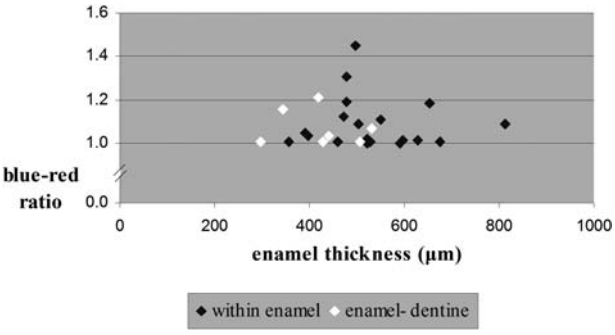

Fig. 13 The relation of blue-red ratio and thickness of enamel layer (n = 27)

$$\text{blue-red ratio} = \frac{\text{the depth of resin penetration (blue staining) from tooth surface } (\mu\text{m})}{\text{the depth of the minute space in between enamel and resin from the tooth surface (red staining) } (\mu\text{m})}$$

In all infractions, blue lines are longer than red one, but the ratio is not related to thickness of enamel layer. The blue-red ratio signifies the ability of the resin to penetrate into the infraction lines.

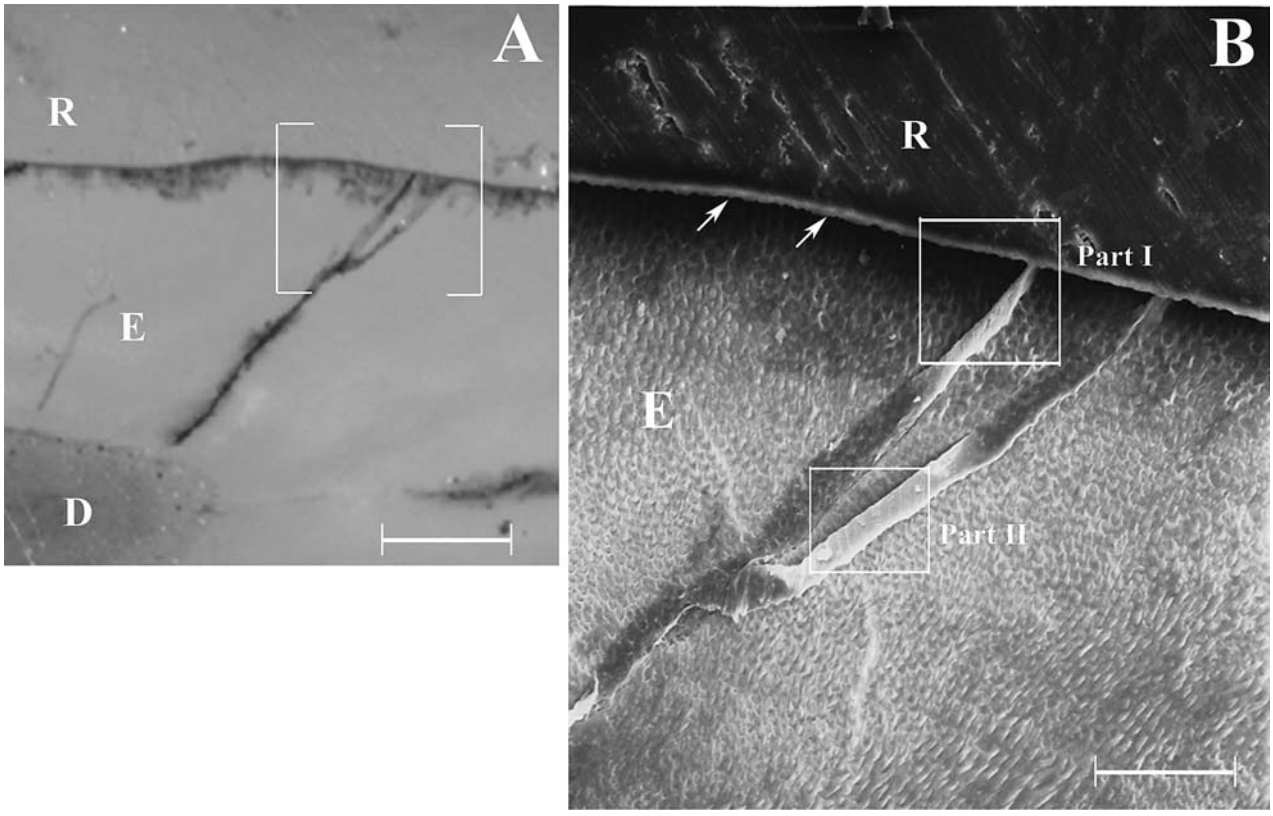

Fig. 14 Stereomicroscopic and SEM images of cut surface of impacted tooth specimen

(A) Blue lines on the cross section of impacted tooth; after 20-second application of 2 mol/*l* HCl and 0.05% Toluidine blue staining for 1 minute. Stereomicroscope × 64. E: partially demineralized enamel surface, D: dentine, R: Unifast II resin cover, Scale bar 200μm.

(B) SEM image of boxed area in A. The continuous raised oblique bands are seen in partially demineralized enamel, at a magnification of × 300. Scale bar 60μm, arrows: resin-enamel interface.

SEM examination of the cut surface showed that the outlines of the enamel rods were visibly imprinted on the surface of the penetrated resin in the SEM micrographs. It is assumed that the resin was able to adequately penetrate into the surrounding enamel rods spaces occupied by enamel crystals which were removed by HCl treatment. Moreover, the micropore penetrations of resin or tags into acid-etched enamel contribute to the mechanical bond to the enamel surface[23,24]. The bulk of penetrated resin in IL may possibly enhance the retention of the resin to the tooth.

In conclusion, results gathered in the present study indicate that the histological structure of visible infractions showed that most of these lines were able to reach dentine, suggesting that these structures may serve as potential risky pathways for bacterial and chemical irritants in gaining access to the pulp system. Hence, it is recommended that the tooth must be covered with resin as soon as possible after clinical detection of the presence of crown infractions.

Resin can sufficiently penetrate into experimentally formed visible infraction lines when it is applied onto the tooth surface. It was also suggested that the resin could penetrate nearly the entire depth of infraction lines. These findings suggest that resin may possibly protect the dental pulp to some extent from microbial or chemical irritations by preventing the entry of pulpal irritants into the pulp system. However, the durability of penetrated resin, and coronal stiffness of resin coated tooth during function warrant further investigation to confirm the effectiveness of this treatment. Moreover, further studies that would test other types of resin using the same method employed in the present study should be conducted.

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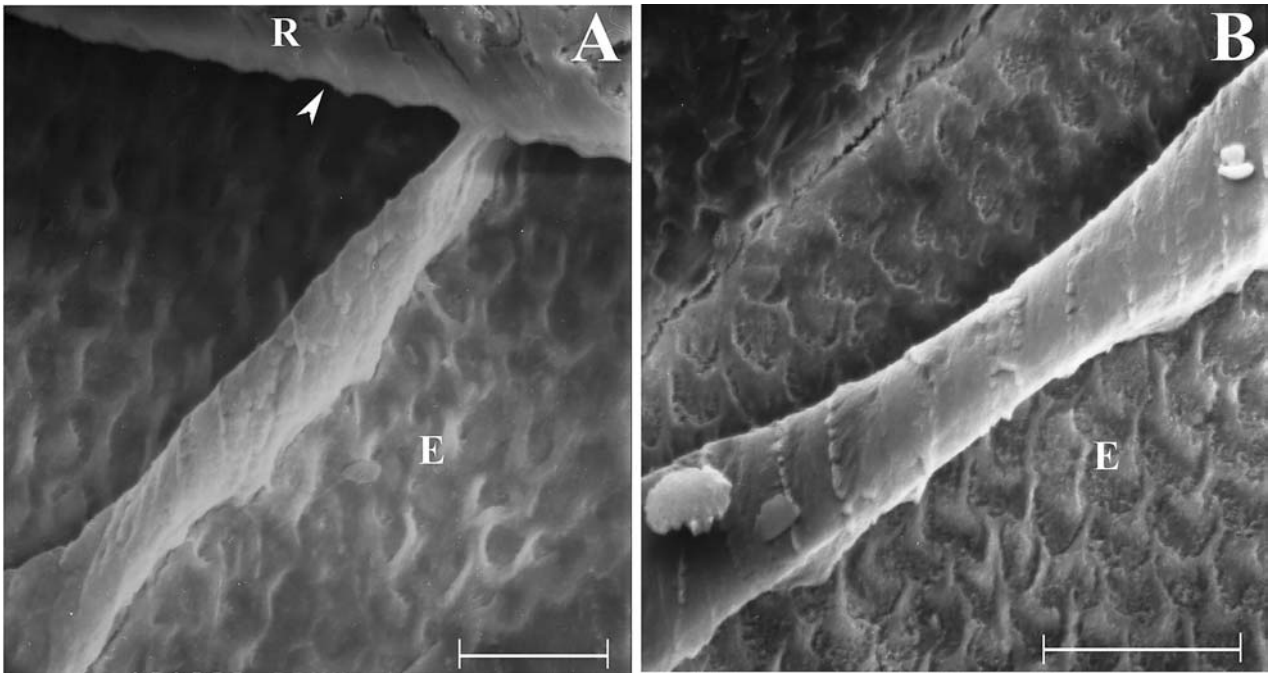

Fig. 15 SEM image of higher magnifications of boxed areas in Fig. 14B

R: Unifast II resin cover, E: partially demineralized enamel surface, Arrow head: resin-enamel interface, Scale bars 14.6μm.
(A) Part I of (Fig. 14B). The raised oblique band is continuous with the surrounding Unifast II resin cover and the enamel rod outlines are visible on the surface, at $\times$1,500.
(B) Part II of (Fig. 14B). The enamel rod outlines are visible on the surface of raised oblique band at $\times$2,000.

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