The amelocemental junction in young premolar teeth

A replica study by scanning electron microscopy

Joan Bevenius, Sven Lindskog and Kjell Hultenby

Department of Cariology and Department of Oral Histology and Cell Biology, Karolinska Institutet, and Clinical Research Center, Huddinge Hospital, Stockholm, Sweden

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To investigate the validity of traditional data on the frequency of various relationships of root cementum to coronal enamel at the amelocemental junction, the relationship in 50 young premolar teeth extracted on orthodontic indications was documented, using replica techniques for scanning electron microscopy (SEM), combined with polarization microscopy of thin ground sections (<40 µm). Of 200 surfaces, 30 were excluded because of damage during specimen preparation. Replicas of the other 170 surfaces were photographed at 20× and 80× in SEM and classified as having an edge-to-edge relationship of enamel to cementum in 76% and an overlap of cementum onto the cervical enamel in 14%. What appeared to be a gap between enamel and cementum at low magnification (10%) was seen at high magnification to be a ditch or trough in the cementum: no gaps with exposed dentin were observed in SEM. Polarization microscopy of ground sections of the original specimens confirmed the SEM classification. In this material, the edge-to-edge relationship of enamel to cementum predominated: overlapping of cementum onto the enamel was less prevalent than previously reported. The methods applied in this study circumvented some major sources of artefact formation due to shrinkage during specimen preparation, which may previously have caused misinterpretation of 'gap' relationships. □ *Amelocemental junction; cementum; replica; scanning electron microscopy*

Joan Bevenius, Department of Cariology, Faculty of Odontology, Box 4064, S-141 04 Stockholm, Sweden

Macroscopically, the amelocemental junction delineates the anatomic crown, covered with enamel, and the anatomic root, covered with cementum, serving as a point of reference in clinical dentistry and in dental radiography.

Once exposed to the oral environment—for example, by gingival recession—the hard tissues of this region are vulnerable to pathologic changes such as root caries or cervical abrasion/erosion. Documentation of the morphology of the normal amelocemental junction is a prerequisite for interpreting events after exposure to the oral environment.

For many years, textbooks of oral histology (1) have described three possible variations of the amelocemental junction: in about 60% of cases the cementum overlaps a small part of the enamel cap; there is an edge-to-edge relationship in 30% of cases, and in 10% of cases enamel and cementum fail to meet, leaving a strip of exposed dentin of unspecified dimension or extent. The source of these data is somewhat obscure, but Hopewell-Smith (2) quoted them as being based on light microscopic studies of ground sections of about 65 teeth by Thorsen in 1917. This information has been quoted and requoted, unconfirmed but largely unchallenged, for 75 years.

Two recent studies of the amelocemental junction using exclusively light microscopy have been reported. Muller & van Wyk (3) examined 150-µm-thick sections from 152 teeth extracted from a South African Cape colored population and reported a significant frequency, 17.7%, of gap junctions, mainly on the buccal and lingual surfaces, and a 49.2% frequency of edge-to-edge relationships. The authors suggested that the differences in prevalence between their material from Cape coloreds and Thorsen's Scandinavian material might be race-related.

Mayhall & Rose (4) examined 67 permanent mandibular cuspid teeth from prehistoric American Indian skulls. Labiolingual sections 100–150 µm thick were examined: 31% had edge-to-edge relationships and 18% had gap junctions. This archaeologic material, although very limited, is of interest because the teeth would never have been subjected to the effects of modern dental or oral hygiene procedures implicated in cervical lesions in modern toothbrushing populations.

Schroeder & Scherle (5) showed that the relationship of enamel, cementum, and dentin at the amelocemental junction varied even over the width of a 150-µm ground section and that light microscopy alone is an inadequate means of documentation. Scanning electron microscopy (SEM) of extracted teeth correlated with light microscopy of tooth sections showed, for example, that in the eight young premolars in their study edge-to-edge contact predominated (ca. 70%) and that gap junctions were very rare (1%).

Recently, Grossman & Hargreaves (6) described the SEM appearance of the amelocemental junction in 18 teeth atraumatically removed from one young adult and concluded that, although the predominant arrangement of tissues was that of cementum overlapping the enamel, the relationship was irregular and unpredictable. Gaps 10–60 µm wide with exposed dentin occurred, but infrequently.

Many fundamental questions arise, not least the source of teeth for study and selection criteria. Methodologic errors may also have been incurred during preparation of tooth specimens for scanning electron and light microscopy.

In the present study a replica technique, specially designed for SEM study of the cervical region (7), was applied to examine the micromorphology of 50 young premolar teeth carefully removed on orthodontic indications. The SEM appearance was subsequently correlated with polarization microscopy of thin ground sections of the original teeth.

Materials and methods

The material comprised 50 young premolar teeth, extracted for orthodontic reasons and handled carefully to avoid damage to the amelocemental junction region. The teeth were stored individually in 10% buffered formaldehyde until the time of the investigation. They were then immersed in 5% sodium hypochlorite solution for 20 min and rinsed in running water for 5 min. Each tooth was carefully inspected under a light microscope to ensure that remnants of soft tissue were completely removed and that there was no obvious damage in the immediate vicinity of the amelocemental junction—for example, from elevators or extraction forceps. The buccal and lingual enamel surfaces were then marked differentially, using a small round bur in a high-speed handpiece: a cross to denote the buccal and a dot to denote the lingual surface.

The bulk of the crown and root of each tooth was then removed with a diamond disc under water coolant, leaving a specimen comprising the amelocemental junction with some adjacent coronal enamel and radicular cementum. The specimens were stored individually in 70% alcohol for 1 week before taking impressions.

To obtain as complete a view as possible of the entire circumference of each tooth, the specimens were supported on a small knob of heavy body elastomer impression material (President, Coltene, Switzerland), buccal aspect uppermost. Light body material (President, Coltene) was syringed onto the surface, and a copper band containing heavy body material was superimposed.

The specimen was then remounted with an approximal surface uppermost, and a new impression was taken, followed by an impression of the lingual surface and then the other approximal surface; that is, four impressions were taken for each specimen.

The impressions were allowed to bench-cure overnight before fabrication of replicas in epoxy die material (Epoxydie, Ivoclar, Lichtenstein); the specimens were stored in 70% alcohol. Detailed procedures for fabrication of the dies have been described previously (7).

The epoxy die replicas were carefully examined in the light microscope for any surface defects. They were then mounted on aluminum specimen holders, sputter-coated (Polaron, Watford, England) with 20 nm gold–palladium, and examined in the SEM (Philips, The Netherlands) at 30 kV, at magnifications of 20× and 80×.

Enlargements of the SEM micrographs to 160× were spliced and assembled to enable the circumference of the tooth to be followed. From these enlargements the morphology of the cementoenamel junction was assessed for buccal, lingual, and approximal aspects as an overlapping of cementum over the coronal enamel, an edge-to-edge junction, or a gap relationship.

Representative areas showing these relationships were relocated on the original specimens. The tooth specimens were then sectioned through these areas of interest: a 100-µm section was cut using a special saw (8). The sections were photographed in SEM (Jeol 820S, Tokyo, Japan) at 3 kV, without a conductive coating of metal, at tilt angles that showed the contour of the tooth surface and the sectioned surface. The sections were then attached face down on a glass microscope slide (Pertex), carefully ground and subsequently polished to a maximum thickness of 40 µm, and photographed under transmitted polarized light to enhance contrast between the different mineralized tissue components in the section.

Results

Two hundred replicas were documented by scanning electron microscopy. Thirty replicas, 17 approximal and 13 buccal or lingual surfaces, were discarded because examination in SEM showed preparational damage in the amelocemental region which had not been detected at light microscopic magnifications. The overlapping of the photographs of the replicas at 160× enabled reconstruction of the amelocemental junctions around the full periphery of each tooth.

At low magnification examination of the replicas in SEM, the border between crown and root followed a gently curving or undulating, smoothly scalloped course. At a magnification of 160×, the border on 24 (14.12%) of the replica surfaces was characterized mainly by a thin layer of acellular, afibrillar cementum covering the enamel (Fig. 1). This layer of cementum varied in thickness and extent. In Figs. 1a and 1b it is seen as a continuous, even band closely adapted to the coronal enamel. In Fig. 1c the overlapping cementum is less clearly defined, appearing as an irregular, mesh-like structure with uneven, fingerlike projections haphazardly extending onto the enamel. In Fig. 1d the cementum overlapping the enamel was irregular in width and characterized by a sharply demarcated border.

In 130 (76.47%) of the replicated surfaces examined at 160× the enamel and cementum appeared to have an edge-to-edge relationship (Fig. 2). Figs. 2a and 2b show clear demarcation between the crown and the root. In Figs. 2c and 2d the border has a more tortuous course, and the topography of the cementum nearest the enamel is more variable, but without overlap.

On 16 (9.41%) of the replicas examined at 160× the enamel and cementum did not appear to meet. At higher magnification, what appeared to be a gap between enamel and cementum was shown to be more like a trough or ditch in the cementum. Fig. 3 shows some examples of these atypical relationships between enamel and cementum. In Figs. 3a and 3b the cementum on the side of the trough sloped up to meet the enamel edge to edge. In Fig. 3c the relationship of the enamel to the cementum is edge to edge, but the gap appearance seen at low magnification is due to the atypical morphology of a band of cementum running parallel to the coronal border. Fig. 3d shows the troughed appearance of the cementum, with patches of cementum extending over the enamel.

Analysis of the results with regard to surface showed equal distributions for overlapping and gap relationships for approximal and buccal and lingual aspects of the teeth. The 16 surfaces on which the gap relationship was observed were from 8 teeth. More than

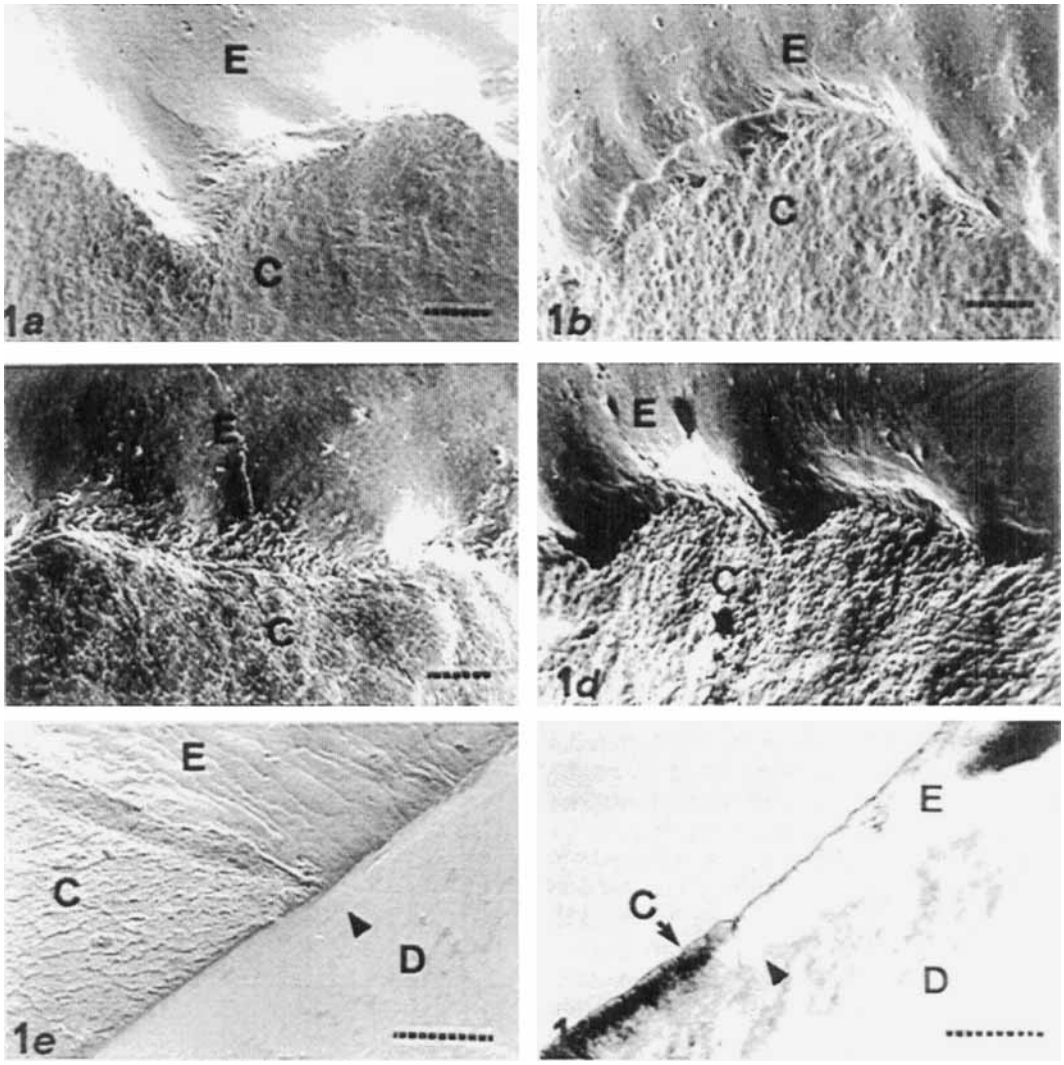

Fig. 1. Amelocemental junctions in which cementum overlaps enamel (14% of surfaces). E = enamel; C = cementum; D = dentin. Figs. 1a, 1b. Scanning electron micrographs (SEM) of replicas, showing relationship in which the cementum overlapping the enamel is seen as a thick, even band closely apposed to the underlying coronal enamel. (Bar = 25 μm.) Fig. 1c. SEM micrograph of replica in which the cementum overlapping the coronal enamel is irregular and mesh-like, with uneven, finger-like projections. (Bar = 25 μm.) Fig. 1d. SEM micrograph of replica showing cementum of irregular width and a sharply demarcated border. (Bar = 25 μm.) Fig. 1e. Low-voltage SEM of unsputtered section of original tooth, showing relationship of overlapping cementum to enamel and dentin on sectioned surface. Arrowhead indicates the coronal limit of cementum overlap. (Bar = 0.1 mm.) Fig. 1f. Specimen from Fig. 1e, section ground and polished to < 40 μm, and photographed in polarized light to enhance contrast between the components. The overlapping cementum is clearly discernible. As in 1e, the arrowhead indicates the coronal limit of the cementum overlap. (Bar = 0.1 mm.)

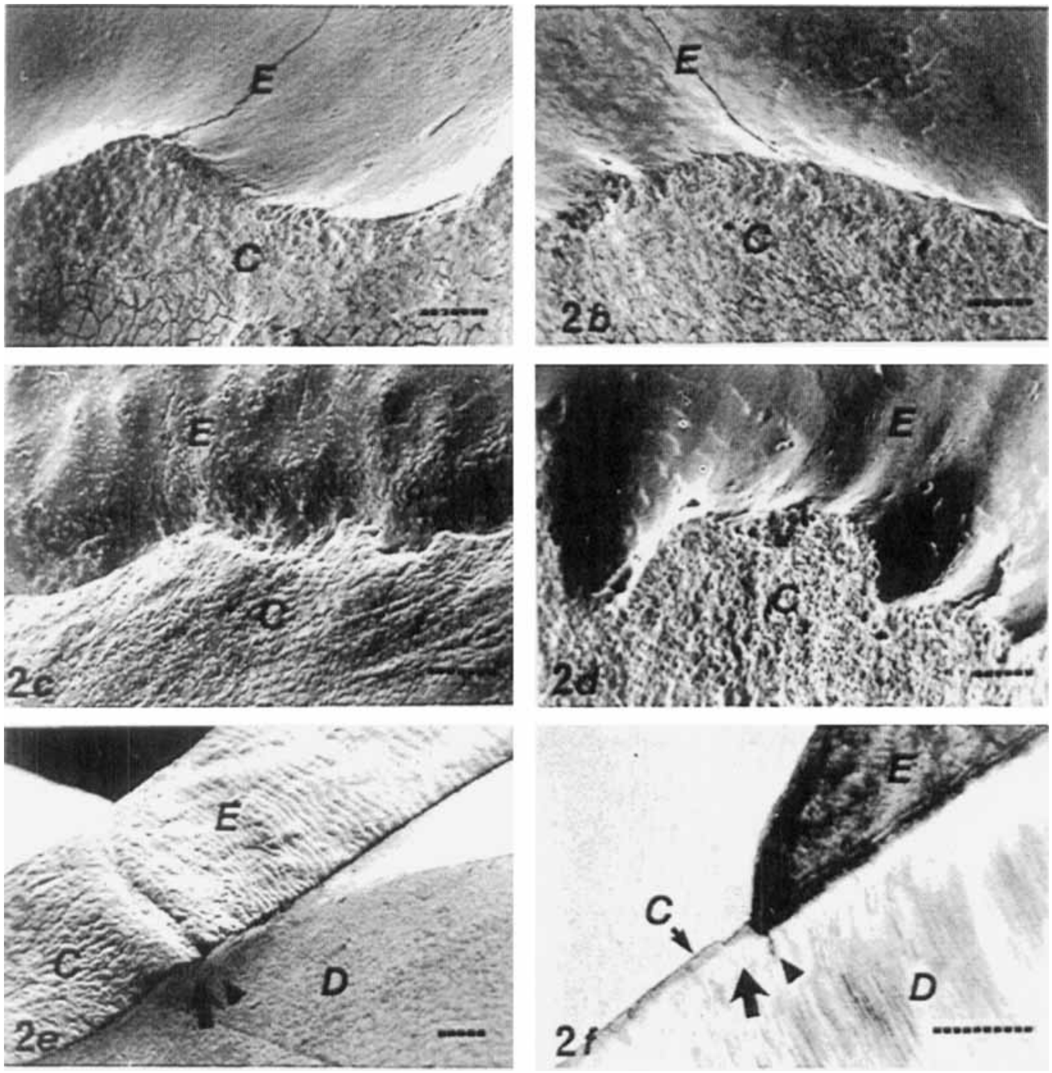

Fig. 2. Amelocemental junctions in which enamel and cementum meet edge-to-edge (76% of surfaces). E = enamel; C = cementum; D = dentin. Figs. 2a, 2b. Scanning electron micrographs (SEM) of replicas in which the coronal enamel and the radicular cementum are clearly demarcated by the amelocemental junction, which follows a gently undulating course. (Bar = 25 μm.) Figs. 2c, 2d. SEM micrographs of replicas in which the course of the amelocemental junction is more tortuous, and the cementum is more variable in topography, but without overlap. (Bar = 25 μm.) Fig. 2e. Low-voltage SEM of ground section of original tooth, replicated in 2c, showing relationship of enamel, cementum, and dentin at an edge-to-edge junction. The large arrow and the arrowhead indicate the depression and change in thickness of the cementum nearest the enamel (see 2f). (Bar = 0.1 mm.) Fig. 2f. Same specimen as in 2e, section-ground and polished to < 40 μm and photographed in polarized light to enhance contrast between the components. Compared with the cementum overlap shown in 1e, the surface of the cementum nearest the enamel is uneven, and the thickness varies. In polarized light the depression indicated by the arrow and the arrowhead in 2e appears as a sharply demarcated change in surface level and thickness of cementum nearest the enamel. (Bar = 0.1 mm.)

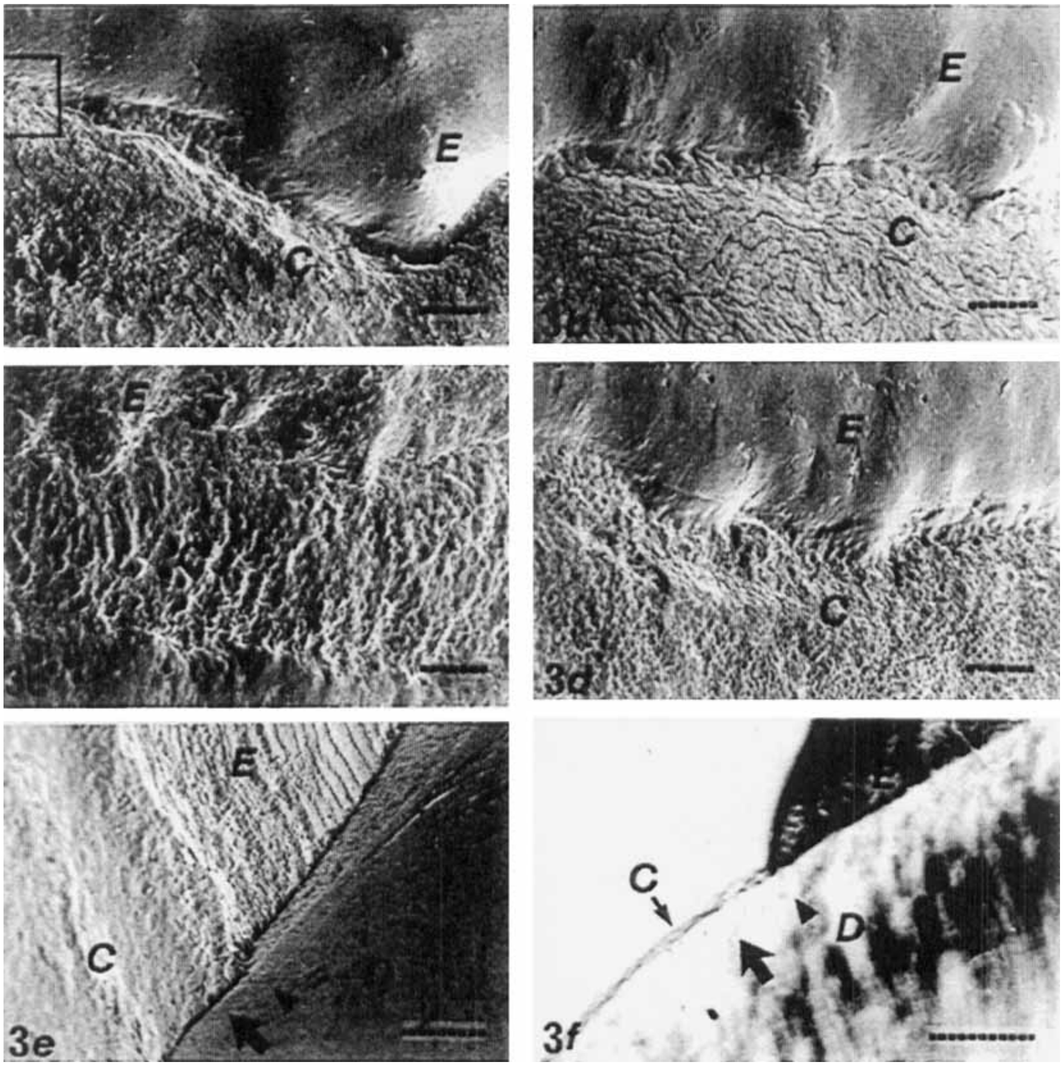

Fig. 3. Atypical relationships (gap junctions) of cementum to enamel (10% of surfaces). E = enamel; C = cementum; D = dentin. Figs. 3a, 3b. Scanning electron micrographs (SEM) of replicas in which the amelocemental junction is marked by a trough in the cementum. The cementum on the side of the trough slopes up to meet the enamel edge-to-edge. Inset in 3a indicates the area of the original tooth from which the section shown in 3e and 3f was taken. (Bar = 25 µm.) Fig. 3c. SEM micrograph of replica in which the gap appearance is due to the presence of a broad band of cementum, running parallel to the enamel boundary. (Bar = 25 µm.) Fig. 3d. SEM micrograph of replica in which there is a trough in the cementum and patches of cementum extending over the enamel. (Bar = 25 µm.) Fig. 3e. Low-voltage SEM of ground section of original tooth, through a region where SEM examination of the sputtered replica (3a, inset) suggested that there might be a gap between enamel and cementum (indicated by arrow and arrowhead). (Bar = 50 µm.) Fig. 3f. Same section as in 3e, ground and polished to < 40 µm, and photographed in polarized light. The cementum tapers off (large arrow) to an extremely thin, uneven layer as it approaches the enamel (arrowhead). The dome-shaped formations on the surface of the cementum represent the insertions of Sharpey's fibers. (Bar = 0.1 mm.)

one surface was involved in five of the teeth: gap relationships were noted on all four surfaces of one tooth, on three surfaces of one tooth, and on both approximal or the buccal and lingual surfaces of a further three teeth.

The radicular cementum featured a homogeneous, mosaic pattern of acellular extrinsic fiber cementum in which the arrangement of the fiber bundles was discernible and the insertion of Sharpey's fibers was clearly distinguishable.

The 100-μm sections of the original tooth specimens were examined in the SEM at low voltage without prior vacuum coating with a conductive layer of metal. Fig. 1e shows a section through a region in which the cementum overlaps the enamel. When this section was subsequently ground to a thickness of 30–40 μm and photographed in polarized light, the overlapping arrangement was clearly discernible (Fig. 1f).

A sectioned specimen in which the enamel and cementum meet edge to edge is shown in Fig. 2e at low magnification in SEM, and in Fig. 2f the same section has been ground to 30–40 μm and photographed in polarized light.

Figs. 3e and 3f show the appearance of a gap relationship between enamel and cementum. The cementum layer tapers off to a very thin, uneven layer as it approaches the enamel.

Discussion

The validity of data about the amelocemental junction in textbooks of oral histology has been queried recently in light microscopic and SEM studies (4–6). The results of the present light microscopic and SEM replica study of a larger, carefully selected material offer further evidence of the need for review of traditional data on the relationship between enamel and cementum.

The teeth selected for this study were young, intact premolars. Before extraction the amelocemental junction and adjacent cervical region were clinically intact and had not been exposed to the oral environment. Despite careful handling of the material and inspection in the light microscope before impression-taking, frankly damaged areas, readily identified in SEM as scratches or sharply demarcated lines of fracture, were observed on 30 (15%) of the original 200 replicas. The vulnerability of the amelocemental region to damage during extraction and subsequent use of rotating instruments to prepare specimens and the associated problems of collecting an adequate sample of teeth fulfilling the inclusion criteria for such a study have also been encountered in previous studies (5, 6).

Depressions in the radicular cementum observed in some replicas were unlikely to be artefacts and probably represent resorptive (9) or hypoplastic (10) responses to some local disturbance. As all the teeth in the study had been removed on orthodontic indications, local developmental disturbances due to crowding should not be discounted.

In an early SEM study of the cervical region (11) based on an unspecified number of erupted and unerupted permanent and deciduous teeth, the SEM micrographs show many of the morphologic features observed in the present study. The morphology of the cervical region which predominated in the teeth in the present study may therefore not be limited to premolars and may prove applicable as a descriptor in studies of other groups of teeth.

The results do not support earlier observations (5) that the morphology of the amelocemental junction varies frequently over a very short distance. In the present material the cervical regions had been protected by soft tissue until extraction; the extreme variations described earlier in teeth extracted from adults may have been a result of exposure of the cervical regions to the oral environment.

Although selection criteria, specimen preparation, and method of documentation in the present study differed from those of previous studies, the finding that an edge-to-edge relationship of enamel and cementum predominated (76%) is in general accordance with the detailed SEM observations of an edge-to-edge prevalence of around 70% on premolar teeth (6). Atypical relationships, which at low magnification could be interpreted as gaps between enamel and cementum, were observed on fewer than

10% of the surfaces in the present material; observation at higher magnification in SEM, however, showed these ditches to be covered by cementum, and in no case was exposed dentin discernible. On the ground sections, the layer of root cementum in these cases was very thin in comparison with the edge-to-edge or overlapping relationships and appeared to taper off as it approached the enamel.

The methods applied in the present study circumvented many of the errors inherent in preparation of dental hard tissues for SEM (7). The replica technique eliminated the need for dehydration and vacuum-coating of the original teeth. The 100-µm sections of the teeth were air-dried for only 1 h before direct examination in SEM at low voltage and were immediately rehydrated, to avoid friability during subsequent grinding and polishing to less than 40-µm thickness.

In previous studies in which gaps with exposed dentin have been demonstrated, even in young, recently erupted teeth, shrinkage and cracking due to dehydration (9) and the thickness of sections examined by light microscopy may have caused misinterpretation of the relationship of these atypical amelocemental regions.

The 'gap' relationship between enamel and cementum with exposed dentin at the amelocemental junction may not be developmental but a result of methodologic shortcomings in earlier studies. Correlation of the appearance of the junction on very thin sections in the light microscope with the SEM appearance of the uncoated, original specimens after sectioning eliminated the problems described in two earlier studies (3, 5), in which thick sections were examined in the light microscope.

The buccal surfaces of premolar teeth are prevalent sites of gingival recession and cervical abrasion/erosion lesions. The results of the present study provide base-line information for in vivo documentation of this region, using a modified replica technique (12).

Premolar teeth extracted on orthodontic indications are readily available and widely used in research. The results of the present study suggest that the micromorphology of the amelocemental region of these teeth and the potential source of errors of interpretation due to specimen preparation techniques should be taken into account when in vitro findings are extrapolated to clinical conditions.

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References

1. Bhaskar, editor. Orbans oral histology and embryology. St. Louis: C.V. Mosby, 1990.
2. Hopewell-Smith A. Concerning human cementum. J Dent Res 1920;2:59–76.
3. Muller C, van Wyk C. The amelocemental junction. J Dent Assoc S Afr 1984;39:799–803.
4. Mayhall C, Rose J. The relationship of enamel to cementum at the cemento-enamel junction. Ala J Med Sci 1984;21:366–73.
5. Schroeder H, Scherle W. Cemento-enamel junction—revisited. J Periodont Res 1989;23:53–9.
6. Grossman E, Hargreaves J. Variable cemento-enamel junction in one person. J Prosthet Dent 1991;65:93–7.
7. Bevenius J, Hultenby K. In vitro and in vivo replication for scanning electron microscopy of the cervical region. Scan Microsc 1991;5:731–40.
8. Boorsbom P, Wolfs B, Leydsman H, Doorn N, Ten Bosch J, Liem K. A machine for sawing 80-micrometer slices of carious enamel. Stain Technol 1987;62:119–25.
9. Jones S. The root surface: an illustrated review of some scanning electron microscope studies. Scan Microsc 1987;1:2003–18.
10. Lindskog S, Blomlöf L. Cementum hypoplasia in teeth affected by juvenile periodontitis. J Clin Periodontol 1983;10:443–51.
11. Akai M, Nakata T, Yamamoto K, et al. SEM of cementoenamel junction. J Osaka Univ Dent Sch 1978;18:83–94.
12. Bevenius J, L'Estrange P, Karlsson S, Carlsson GE. Idiopathic cervical lesions: in vivo investigations by oral microendoscopy and scanning microscopy. A pilot study. J Oral Rehab 1993;20:1–9.