The micromorphology in vivo of the buccocervical region of premolar teeth in young adults

A replica study by scanning electron microscopy

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The buccal surfaces of premolar teeth are common sites of gingival recession, generally attributed to overzealous oral hygiene. Scanning electron microscopy (SEM) of replicas made from dental impressions was applied to document the micromorphology of the buccocervical region of all premolar teeth in 27 dentally healthy, young adults. The SEM observations were correlated with clinical examination. Of the 216 sites, one-third, predominantly the maxillary first premolars, had gingival recession, but fewer than 50% were clinically discernible. The exposed roots were devoid of cementum, and the dentinal surface was smear-like or dotted with tubular apertures from which droplets of fluid extruded. SEM of replicas of gingiva, recorded as clinically healthy, frequently showed signs of inflammation: fluid exudate and distortion of gingival contour by swelling. The cervical enamel of healthy and affected sites showed characteristic periodic fissure-like cracks, probably enamel tufts. The high frequency of subclinical gingival recession, exposed cervical dentin, and gingival inflammation in dentally healthy young adults, in the absence of abrasion of hard or soft tissues, indicates the need to review conventional concepts of initiation of buccal recession and root exposure.
□ *Amelocemental junction; dentinal sensitivity; enamel tufts; gingival recession; SEM replication*

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Recent epidemiologic studies in Sweden (1, 2) and Finland (3) have reported a high prevalence of buccal gingival recession in young adults who are otherwise free from periodontal disease. The condition could be considered a localized process, separate from marginal periodontitis, with different causative or predisposing factors, primarily overzealous oral hygiene (4). The role of toothbrush abrasion is based mainly on empiric and circumstantial evidence (5). Associated trauma to the soft tissues has not been extensively investigated (6, 7).

In the absence of clinically obvious plaque and calculus, it has also been assumed that plaque-mediated gingival inflammation does not play a major role in the initiation of buccal gingival recession. However, animal studies (8) have indicated that the inflammatory response in gingival recession may be so localized that it is not detectable clinically.

An important clinical landmark in recording gingival recession is the amelocemental junction. The morphologic characteristics of this region have been documented in studies of extracted teeth (9–11). Little is known about morphologic changes in the amelocemental region once it is exposed to the oral environment by gingival recession.

A recent in vivo study of clinically manifest buccal cervical lesions, in which replicas of cervical surfaces of the teeth were documented by scanning electron microscopy (SEM), suggested that data obtained from clinical observation alone may markedly underestimate the true prevalence of root exposure (12).

For the patient, gingival recession is not only aesthetically unacceptable but is also frequently associated with distressing dentinal sensitivity, the causes of which have

only partly been resolved (13). Until quite recently, clinical management of these cervical lesions was also technically difficult. Advances in adhesive and aesthetic dentistry now allow non-invasive restoration, with high patient acceptance. Clinical studies have recorded no immediate untoward post-restorative sequelae or unfavorable soft-tissue responses to these procedures (14). Restoration of lost tooth contour and relief of symptoms are, however, not synonymous with elimination of the cause of the pathologic process.

In the present study, one of a series in which non-invasive methods of investigation are applied to document the amelocemental region of the teeth (11, 15), the status of the buccocervical region of premolars in healthy young adults, recorded by clinical examination, was correlated with the micromorphology, documented by SEM of replicas. The specific aims were to document not only the occurrence of clinical and subclinical root exposure at these sites but also to describe micromorphologic surface characteristics of the dental hard tissues and the adjacent marginal gingiva at both healthy and affected sites.

Materials and methods

The subjects were 13 women and 14 men aged 19–25 years (mean age, 19.5 years). The inclusion criteria required them to be non-smokers, in good general health, and not currently undergoing restorative or orthodontic treatment. Four of the women and 11 of the men were recent matriculants from suburban high schools in Stockholm. The other nine women and one man were recent arrivals from Eastern Europe, guest students at the Karolinska Institutet. The other two male subjects were 1st-year dental students at Karolinska Institutet, recruited within a few weeks of admission to the course, and lifetime residents of Stockholm. The subjects were informed in writing about the purpose of the study and the procedures involved. The study was approved by the ethical committee of the Karolinska Institutet.

On the day of the experiment the subjects were instructed to carry out their usual oral hygiene procedures and to attend an appointment about 90 min after breakfast.

The study included the buccal surfaces of all eight premolars, a total of 216 sites. All clinical and laboratory procedures were carried out by one author (J. Bevenius). The subjects were interviewed with regard to their awareness of 'toothbrush abrasion', or sensitive tooth necks. Gingival inflammation, sores, and bleeding associated with brushing were also noted.

The teeth were sprayed with water and air and isolated with cotton rolls. Clinically, root exposure was registered if the amelocemental junction was clearly discernible and the distance to the gingival margin was at least 1 mm (modified from Refs. 2 and 16), measured with a graduated periodontal probe (Fig. 1). The amelocemental junction had to be clearly discernible or detectable as a tactile change when a sickle probe was drawn from the gingival margin towards the occlusal surface. The buccocervical surfaces were classified clinically as either sound or having exposed roots.

The presence of vertical gingival clefts was noted by visual inspection. Gingival inflammation was recorded when the cervical gingiva bled after application of gentle pressure with the side of the periodontal probe.

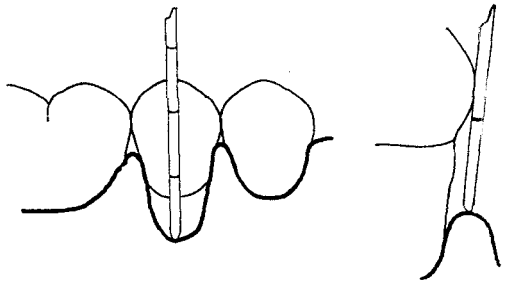


Fig. 1. Measurement of clinical gingival recession. Position of periodontal probe on the buccocervical surface of mandibular second premolar. The probe is graded at 2-mm intervals. Two major sources of error in this assessment method are the difficulty in 'reading' a clinical measurement as small as 1 mm without magnification, particularly as the buccal prominence of the tooth crown tends to obscure the area, and the uncertainty of locating the amelocemental junction visually.

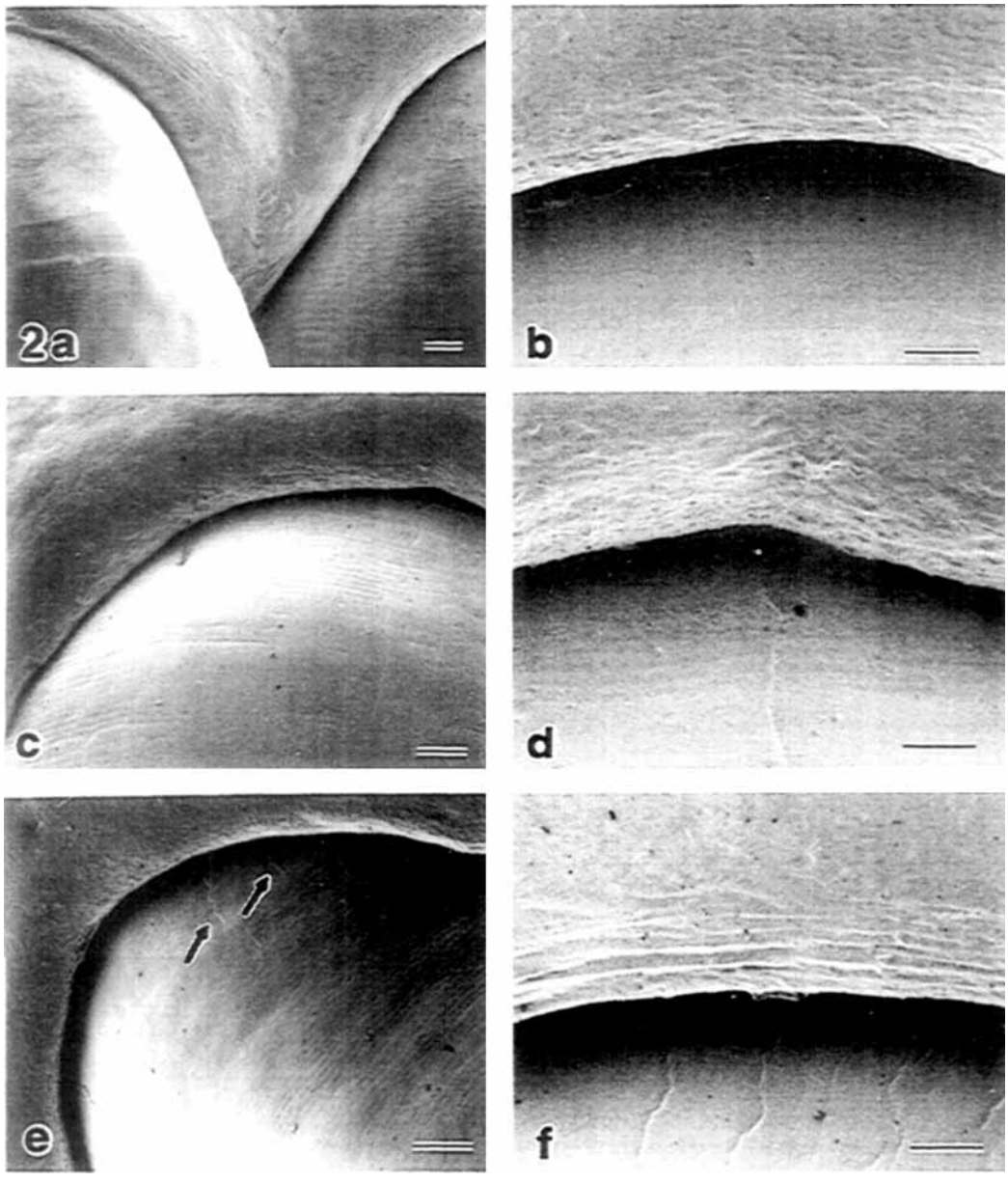


Fig. 2. Scanning electron microscopy photographs of epoxy replicas of normal buccocervical regions of premolar teeth of young adults. 2a,c,e. Low-magnification micrographs showing healthy buccocervical regions of premolar teeth. The gingiva has a regular rolled border and is closely adapted to the tooth contours. The perichymata of the enamel have been faithfully reproduced by the replica. The arrows in 2c indicate the periodic fine enamel cracks, probably enamel tufts, running from the gingival margin in an occlusal direction. Bar = 0.2 mm. 2b,d,f. Micrographs at higher magnification of the gingival margin and buccocervical enamel at healthy sites. Epithelial desquamation occurs in a regular, wave-like pattern, parallel to the gingival margin. 2f illustrates the enamel tufts, occurring with a periodicity of around 100 μm. Bar = 100 μm.

In all subjects the impression for replication for SEM was preceded by a scavenger impression, which was processed for a parallel microbiologic study of early streptococcal attachment in the buccocervical region in the same subjects. The microbiologic study required standardized procedures with regard to degree of cleanliness. The buccal surfaces of all the premolars were carefully cleaned with a slurry of pumice and water, applied in a rubber cup rotated at slow speed. The lip of the cup was gently pressed into the gingival sulcus. The surfaces were then thoroughly rinsed with water, followed by spraying with air and water. The subjects then fasted for 2 h before impression-taking, to enable the formation of 2-h salivary pellicle.

After the subjects had rinsed vigorously with water, the teeth were isolated with a parotid shield, gently rinsed with a stream of water, and dried with air. The scavenger impression was taken in polyvinylsiloxane material (Panasil Contact fast set). After 4 min the set impression was gently flexed off the teeth. The impression for SEM replication was taken immediately afterwards. The replica was prepared in an epoxy resin material (Epoxydie, Ivoclar) and prepared for SEM by vacuum-coating with a 10-nm layer of platinum (Polaron, England). Each specimen for SEM comprised the replicated buccocervical surfaces and gingival margins of a first and second premolar (Fig. 2a). The specimens were screened at 16–20× magnification in a Jeol 830 SEM. Details were photographed at magnifications up to 2000× (15). Duplicate replicas were retained unsputtered for reference and remeasurement.

One-third of the subjects were recalled after 3 months. After clinical reexamination, the teeth were cleaned as previously and new impressions taken. Replicas were prepared for SEM, and duplicate replicas were retained unsputtered for reference.

Results

Five of the female and 10 of the male subjects were school dropouts who had grown up in Stockholm and received regular preventive dental care through the school dental service, provided free of charge up to the age of 19 years. They were caries inactive, with restorations limited mainly to occlusal fissures. The oral hygiene standard was good but limited to daily use of brushing with a fluoride dentifrice: only two subjects used dental floss and a fluoride supplement daily. Four of the men in this group were elite ice hockey players; they were aware that they had an established habit of tooth-clenching and tended to clench their teeth to extremes during play. The other subjects comprised two dental students in the first weeks of their course, who had not altered their oral hygiene habits since enrollment, and nine guest students, eight female and one male, recent arrivals from Eastern Europe, with multiple approximal amalgam restorations, high standards of oral hygiene, and extremely infrequent sugar intake.

Clinical assessment of gingivocervical sites

With the exception of regions with Stillman's clefts in three male subjects, gingival health was good. A slight tendency to gingival bleeding was noted in three subjects; no calculus was observed and minimal plaque, mainly associated with vertical gingival clefts. The standard of cleanliness on the buccal surfaces of the premolars was very high.

The results of the clinical assessment of gingival recession and root exposure are presented in Table 1. Root exposure was recorded on at least 1 site in 9 of the 27 subjects (33%). One subject had clinical lesions on all eight sites, two on six sites, and one on four sites. Thus around 15% of the sample had clinically manifest exposed roots on the

Table 1. Subject distribution of clinical buccal gingival recession

	No. of lesions								
	0	1	2	3	4	5	6	7	8	Total
Men	6	2	3		1		2			14
Women	12								1	13
Total	18	2	3		1		2		1	27

Table 2. Subject distribution of clinical and subclinical buccal gingival recession

	No. of lesions								
	0	1	2	3	4	5	6	7	8	Total
Men	3	3	1		4				3	14
Women	6	1	3		1	1			1	13
Total	9	4	4		5	1			4	27

Table 3. Site distribution of clinical and subclinical buccal gingival recession

Tooth no.	Clinical lesions	Subclinical lesions	Total no. of lesions	Healthy sites
15	4	2	6	21
14	4	7	11	16
24	5	6	11	16
25	4	4	8	19
45	3	4	7	20
44	5	2	7	20
34	3	7	10	17
35	4	5	9	18
	32	37	69	147

buccal aspect of four or more premolars. One male subject had recently been treated for 'erosion-induced cervical sensitivity' with class-V restorations in glass ionomer cement on all four lower premolars (Fig. 7). The subject with clinical lesions on all eight premolars (Fig. 6) had occasional attacks of cervical sensitivity, and the lesions were painful to air-drying. None of the other subjects reported cervical sensitivity.

SEM observation of gingivocervical sites

Documentation of the epoxy replicas at low magnification in SEM disclosed sites with root exposure in 18 subjects (66%) (Table 2). Four subjects (15%) had lesions on all 8 buccal surfaces, and 10 subjects (37%) had 4 or more exposed sites. Thus low-magnification SEM disclosed 50% more root exposures than had been recorded clinically; in nine subjects (33%) with subclinical exposures only, three had at least four affected sites. Nine subjects (33%) had neither clinical nor subclinical lesions—that is, healthy buccocervical regions (Fig. 2). At least one affected site was recorded in 60% of the subjects.

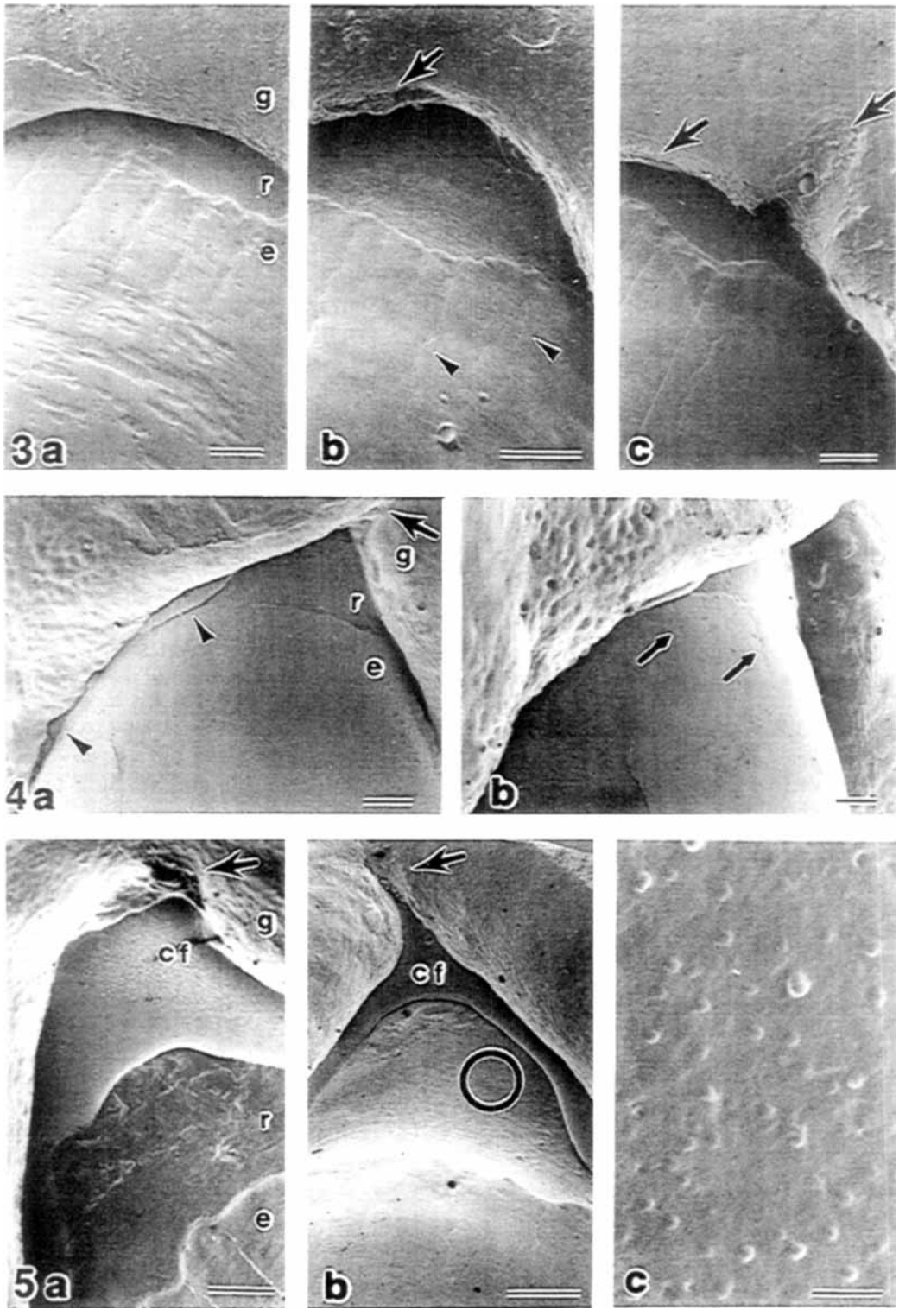
The sex distribution of all lesions, clinical and subclinical, is also presented in Table 2. Six of the 13 women (46%) and 3 of the 14 men (21%) had no lesions. In one woman (7.5%) and three men (21%) exposed roots were recorded on all eight sites. Four or more lesions were noted in three women (23%) and seven men (50%). It was of interest that in the four elite sportsmen no clinical lesions and only one subclinical lesion were recorded.

Of the total of 216 sites in the material, root exposure was recorded at clinical examination in 32 (15%). SEM disclosed a further 37 (16%) affected sites. The distribution of

Table 4. Site and sex distribution of clinical and subclinical buccal gingival recession

Tooth no.	Men				Women			
	Clin.	Subclin.	Total	Healthy	Clin.	Subclin.	Total	Healthy
15	3	2	5	9	1	0	1	12
14	3	2	5	9	1	2	3	10
24	4	4	8	6	1	2	3	10
25	3	3	6	8	1	1	2	11
45	2	3	5	9	1	1	2	11
44	4	1	5	9	1	5	6	7
34	2	2	4	10	1	5	6	7
35	3	1	4	10	1	4	5	8
	24	18	42	67	8	20	28	76

Clin. = root exposures detected by clinical examination; Subclin. = root exposures disclosed by scanning electron microscopy of replicas.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these 69 clinical and subclinical lesions, by site, is presented in Table 3. Clinical lesions were fairly evenly distributed among all the premolars. However, the distribution of all lesions shows that the sites predominantly affected were the maxillary first premolars.

Table 4 shows the distribution of the 69 lesions by sex and tooth site. More than twice as many men as women had lesions on the maxillary first premolars: 57% and 23%, respectively.

Micromorphologic observations

Some representative photomicrographs from the scanning microscopy screening are reproduced in Figs. 2–9. The healthy sites were characterized by close adaptation of the gingiva to the tooth surface (Fig. 2a, c). The gingival margin covered the cervical enamel, forming a regular, rolled border, with an even, wave-like pattern of desquamation of epithelial cells (Fig. 2b, d). On the enamel surface the perichymata were faithfully reproduced by replication (Fig. 2a–e).

At sites with subclinical root exposures (Fig. 3) the retracted gingiva showed characteristically irregular patterns of increased desquamative activity. The exposed root surface was devoid of cementum, and the cervical enamel at the amelocemental junction followed a fairly even, undulating course, accentuated by fine, fissure-like cracks with periodicity similar to those in the enamel of healthy sites. The micromorphology of sites with initial gingival clefts is shown in Fig. 4. Clinically, the distorted contour of the marginal gingivae almost completely obscured the root exposure. Crevicular fluid extruding from the sulcus was reproduced in the replica.

Sites with clinically detectable root exposures and Stillman's cleft formation had a distinctive micromorphology, characterized by copious seepage of crevicular fluid, which partly obscured the root surface (Fig. 5a, b). At higher magnification in SEM the root surface consisted of dentin, with droplets of fluid extruding from the apertures of the dentinal tubules (Fig. 5e).

A frequent feature of the cervical enamel was the occurrence of fine, fissure-like faults at quite regular intervals of around 100 μm, following a longitudinal undulating course from the gingival margin on healthy sites and from the amelocemental junction on sites with exposed roots, over the buccal prominence of the crown of the tooth, for distances of around 150–500 μm (Fig. 2e, f).

The enamel margins at the sites of all root exposures, clinical and subclinical, terminated precipitously at the amelocemental junction (Fig. 9a, b). The root surface was devoid of cementum and was either covered with a smear layer (Fig. 8c) or consisted of a fairly even dentinal surface on which the orifices of the dentinal tubules were discernible at higher magnifications (Fig. 8c, d).

With the exception of the sites with vertical gingival clefts, there were remarkably few soft-tissue lesions in the material.

In our previous in vitro replica study of the micromorphology of the amelocemental junction of premolar teeth extracted on orthodontic grounds (11), the enamel in many of the specimens terminated with a characteristically scalloped pattern. Retrospective examination of the 50 replicas of the

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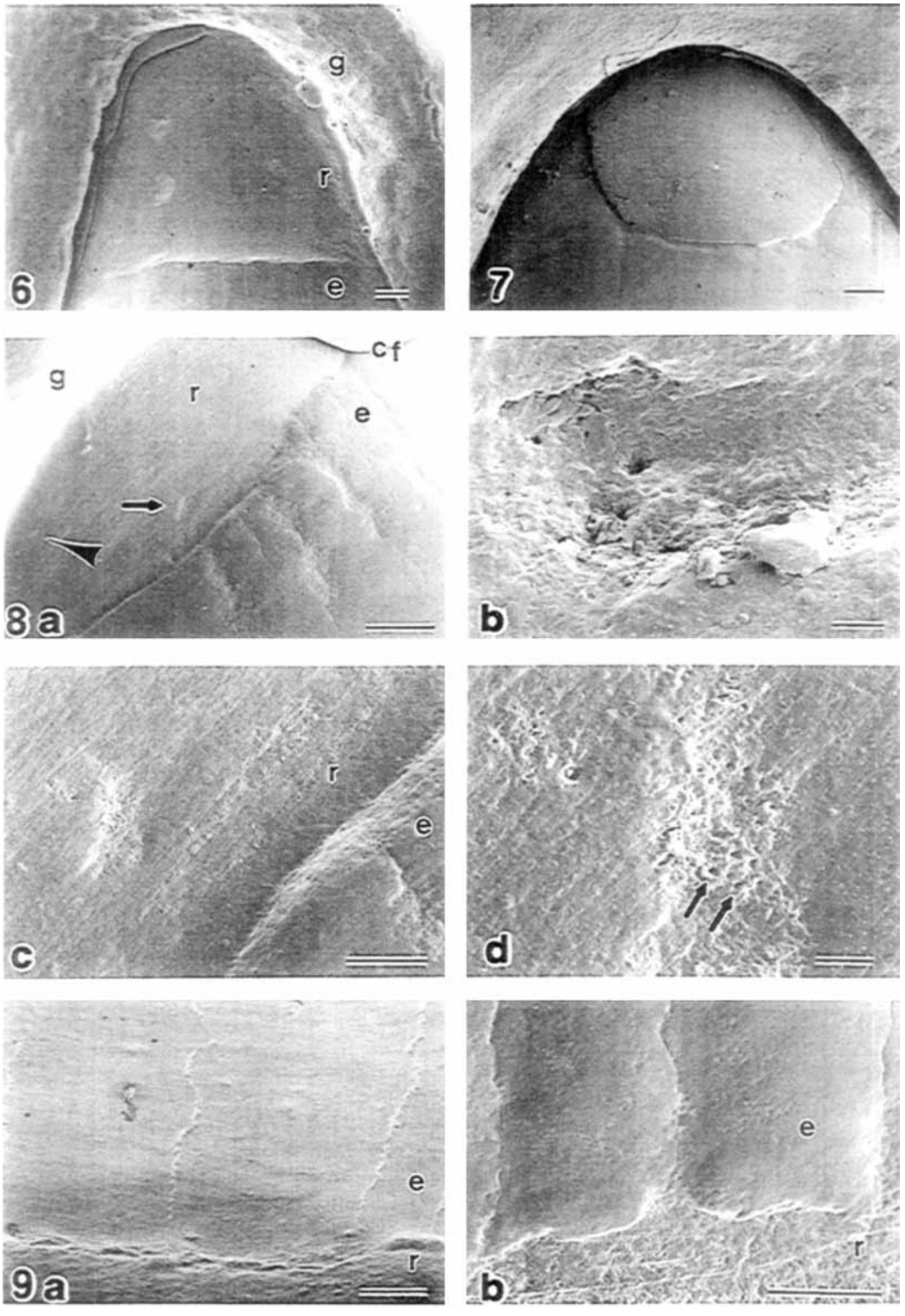
Plate I. Scanning electron microscopy photographs of epoxy replicas of buccocervical sites with gingival recession and root exposure.

Fig. 3a-c. Representative micrographs of three sites with subclinical exposures. The gingival margins showed an irregular pattern of epithelial desquamation, indicated by the arrows in b and c. Compare with Fig. 2b,d,f. The surface of the exposed dentin was furrowed and somewhat smeary (3b). The arrowheads in 3b indicate the enamel tufts originating at the amelocemental junction. Bar = 0.2 mm. g = gingiva; r = root surface; e = enamel.

Fig. 4a,b. Two views of a site with a vertical gingival cleft, arrowed in 4a. Clinically, the swollen gingivae almost completely obscured the exposed root surface. The arrowheads in 4a indicate crevicular fluid in the gingival sulcus. In 4b, the arrows indicate enamel tufts. Bar = 0.2 mm. g = gingiva; r = root surface; e = enamel.

Fig. 5a,b. Photographs of replicas of two sites with clinically diagnosed vertical gingival clefts (arrows). Crevicular fluid partly covers the exposed root surfaces. The encircled area in 5b is shown in detail in 5c. Bar = 0.1 mm. g = gingiva; cf = crevicular fluid; r = root surface; e = enamel. 5c. The dentinal surface on the exposed root, with droplets of fluid extruding from the apertures of the dentinal tubules. Bar = 2 μm.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buccal specimens in the in vitro material disclosed periodic, fine, fissure-like faults in the cervical enamel accentuating the scalloped appearance (Fig. 10a, b). A section through such a region on the original tooth specimen was examined at low-voltage SEM without a conductive coating: a fault could be traced coursing from the amelocemental junction through the enamel and terminating at the dentinoenamel junction (Fig. 10c).

Discussion

The subjects in the present study were young adults, at an age at which oral hygiene and dietary habits are considered to be well established (3). Given the limited size of the material, the findings should be interpreted with caution, but it is clear that the frequency of subclinical buccal gingival recession is high. The observation of Stillman's clefts in three of the male subjects was unexpected and warrants further investigation.

The results do not support the concept that gingival recession and buccocervical lesions in healthy young adults are initiated by overzealous oral hygiene: the lesions were fairly evenly distributed on right and left sides, no wedge-shaped lesions were observed, and there were no soft-tissue lesions resembling those induced by experimental toothbrush trauma in earlier SEM replica studies (6, 7).

The finding that fewer than 50% of the cervical lesions were detected clinically confirms our earlier pilot study, in which subjects with wedge-shaped lesions also had other sites with subclinical, mainly shallow, saucer-shaped lesions (12).

The high standard of gingival health and very low frequency of gingival recession in the elite sportsmen was unexpected (17) and warrants further investigation.

The present study also highlights the difficulty in establishing suitable clinical criteria for describing changes in the dentogingival region (18). The amelocemental junction, although an important clinical and radiographic landmark (19, 20), is not always readily recognizable clinically, even with the additional diagnostic aid of tactile change in the contour of the tooth surface. This potential source of error in both clinical and epidemiologic studies is seldom addressed (21, 22). In this context, two other assumptions should be queried—namely that when the cervical region is exposed, any change in hard tissue is limited to the root surface and that the enamel at the amelocemental junction is constant over time and therefore an appropriate landmark for measurements of loss of attachment. To our knowledge, no longitudinal studies have addressed these questions.

In the present study the clinical condition of the gingiva was assessed as healthy on the basis of conventional clinical criteria: normal color, firm texture, close adaptation to the contour of the tooth, and absence of bleeding tendency (23). Low-magnification SEM of

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Plate II.

Fig. 6. Low-magnification scanning electron microscopy (SEM) micrograph of epoxy resin replica of a clinically diagnosed lesion. The gingival contour is irregular, and desquamation is patchy. Some seepage of crevicular fluid is seen on the left side. Note the two discrete, crater-like depressions to the left, on the root surface. Bar = 0.2 mm.

Fig. 7. Low-magnification SEM micrograph of epoxy replica of a site restored with glass ionomer cement to reestablish cervical tooth contour and relieve hypersensitivity. Bar = 0.2 mm.

Fig. 8. Low-magnification SEM micrograph of subclinical gingival recession. The exposed root surface was smear-like, with occasional rough, irregular patches (arrow) and more clearly defined, crater-like depressions (arrowhead). Bar = 0.2 mm. g = gingiva; r = root surface; e = enamel; cf = crevicular fluid. 8b. Detail of crater-like depression indicated by the arrowhead in 8a. Compared with the surrounding exposed root dentin, the normal structure appears to have collapsed. Bar = 10 μm. 8c. Detail of the area in 8a, indicated by the arrow, showing the smeared surface of the exposed dentin. The 'rough' patch of patent dentinal tubules to the left is shown in detail in 8d. Bar = 50 μm. r = root surface; e = enamel. 8d. Detail of the rough patch in 8c, showing exposed dentinal tubules. The tubular apertures (arrowed) are funnel-shaped but, in contrast to the surrounding, smear-like dentin, do not contain droplets of fluid. Bar = 10 μm.

Fig. 9a–b. Details of the morphology of the amelocemental junction in two subclinical lesions, showing the rounded termination of the enamel, not abrupt as in a fracture, and the undulating path of the enamel tufts. Bar = 50 μm.

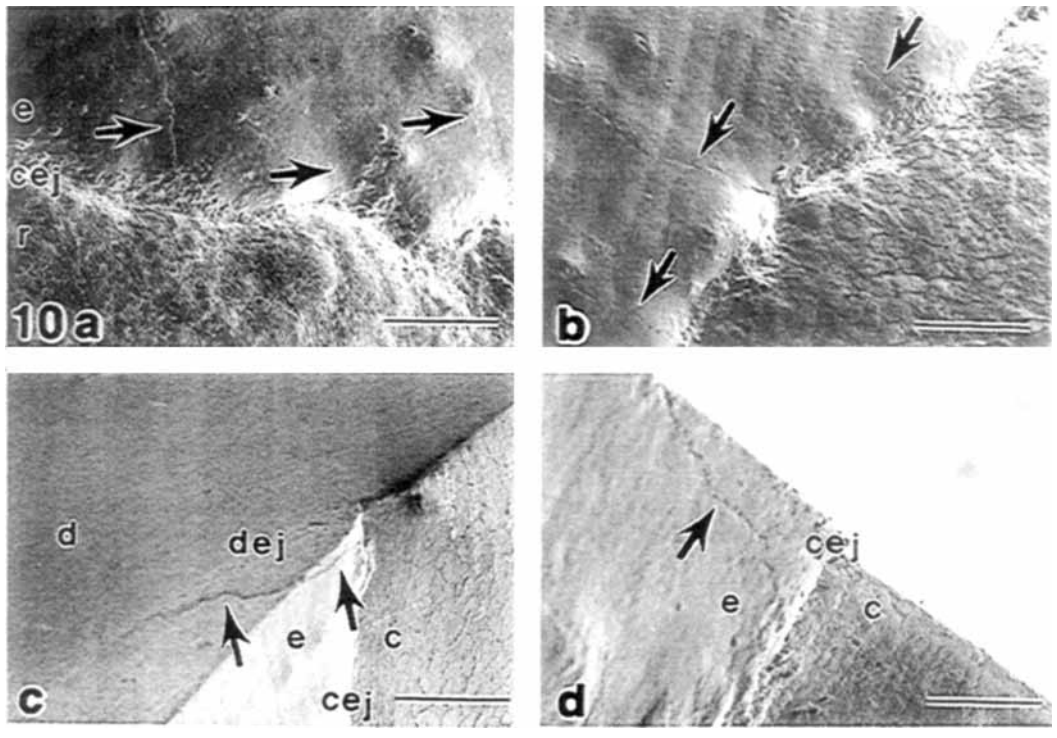

Plate III. Scanning electron microscopy (SEM) documentation of the cervical region of extracted, intact premolar teeth.

Fig. 10a–b. SEM micrographs of epoxy resin replicas of buccal aspects of extracted premolar teeth, showing enamel tufts (arrows) similar to those on the in vivo replicas in Figs. 2–9. The root surface of these healthy young teeth was covered by acellular cementum, extending some distance onto the enamel. Bar = 50 μm. e = enamel; cej = amelocemental junction; r = root surface. 10c. SEM micrograph of an extracted premolar tooth, sectioned near an enamel crack. The specimen was photographed at low voltage, without a conductive metal coating. The enamel tuft (arrowed) runs from the dentinoenamel junction to the amelocemental junction. d = dentin; dej = dentinoenamel junction; e = enamel; cej = amelocemental junction; c = cementum. Bar = 50 μm. 10d. View from the buccal aspect of the same specimen as in 10c, showing the course of the enamel tuft (arrow). e = enamel; cej = amelocemental junction; c = cementum. Bar = 50 μm.

replicas of many such healthy sites showed evidence of gingival inflammation, supporting an earlier report that very localized, clinically undetectable inflammation may be involved in the initiation of gingival recession (8). Subclinical inflammation has also been observed in histologic studies of clinically healthy tissue (24–26).

The present study has shown that the 'smear-like' appearance in SEM of replicated exposed cervical dentin (27) may be due to extrusion of droplets of dentinal fluid onto the surface. This outward flow of tubular fluid may modify the permeability of exposed cervical dentin (28) and resist the inward diffusion of molecules from the surface (29, 30). The modifying influence of such biologic variables is seldom addressed in in situ and in vitro models of root caries and dentinal sensitivity.

Periodic, fissure-like faults in cervical enamel have been described in morphologic studies of extracted teeth (31, 32), indicating them to be developmental faults, enamel lamellae, or enamel tufts (33). When demonstrated in transverse sections of teeth, these structures do not extend the full distance from the dentinoenamel junction to the enamel surface in the bulk of the enamel but might do so in the cervical region, where

the thickness of the enamel tapers off. In the present study these structures ranged from around 100 to 500 μm in length and occurred at fairly regular intervals of around 100 μm, accentuating the scalloped outline of the cervical enamel. The following characteristics indicate that these faults are developmental in origin: the length corresponds to that of penetration of enamel tufts into enamel; the occurrence at regular intervals corresponds to the periodicity of the tufts; termination at the edge of the enamel is rounded, not abrupt, as would be expected in a fracture; and the undulating path reflects the pattern of the bundles of enamel prisms with which the tufts are associated. If confirmed in further studies as enamel tufts, their characteristic morphology at the amelocemental junction in cases of root exposure may provide a valuable reference for monitoring the possible destruction of the cervical enamel at the amelocemental junction over time.

In conclusion, this study has shown the following:

When diagnosed by clinical examination only, the occurrence of buccal gingival recession and root exposure on premolar teeth in young adults is underestimated by 50%.

Even the earliest detectable exposures are devoid of cementum, and on the dentinal surfaces droplets of fluid may be seen extruding from the tubular apertures.

The margin of the cervical enamel has a regular, scalloped appearance, characterized by very fine fissures or faults. It is proposed that these faults, which have also been documented on extracted healthy premolar teeth, are not fracture lines but may be developmental faults, possibly enamel tufts.

At sites with clinically healthy gingiva, low-magnification SEM of the replicas frequently disclosed subclinical gingival inflammation.

Neither the site distribution nor the nature of the lesions supports the conventional concept that overzealous oral hygiene initiates buccal gingival recession. It is suggested that abrasion and erosion may be secondary factors, exacerbating loss of root hard tissue in an established but clinically indiscernible lesion.

The study highlights the need for critical review of current concepts of buccal gingival recession and root exposure on the basis of clinical criteria alone and for the development of strategies to reconcile the findings of in vitro and in situ studies with macroscopic observations. The replica technique, using modern hydrophilic impression materials and low-voltage SEM, offers a simple, non-invasive method for studying the surface micromorphology of the hard and soft tissues.

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