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Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study

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SUMMARY This investigation was undertaken to assess bacterial plaque accumulation adjacent to orthodontic brackets. Experiments were carried out on 11 subjects who were scheduled for orthodontic treatment including extraction of two or four premolars. Metal brackets were bonded to the premolars to be extracted using macro-filled bonding composite. A conventional elastomeric ring was placed around one bracket and a steel ligature wire around the bracket on the contralateral tooth. The subjects were told to continue their normal oral hygiene regimen. Teeth were extracted at 1, 2, or 3 weeks after bracket bonding.

Scanning electron microscopic (SEM) examination of brackets, excess composite, and buccal enamel revealed that mature plaque was present on excess composite at 2 and 3 weeks after bonding, whereas plaque on the gingival enamel surface was still at an early stage of development. The results demonstrate that excess composite around the bracket base is the critical site for plaque accumulation due to its rough surface and the presence of a distinct gap at the composite–enamel interface. The method of ligation does not appear to influence the bacterial morphotypes on both composite and enamel surfaces.

Introduction

Fixed appliances induce continual accumulation and retention of bacterial plaque, which constitute a risk of white spot lesion development during orthodontic treatment (Ingervall, 1962; Gorelick *et al*., 1982; Mizrahi, 1982, 1983; Årtun and Brobakken, 1986; Årtun and Thylstrup, 1986; O'Reilly and Featherstone, 1987; Ögaard *et al*., 1988; Ögaard, 1989; Mitchell, 1992). Bonding of brackets, using acid-etching and composite resin, has been a major advancement in orthodontic technique after the introduction of the edge-wise principle. However, design and surface characteristics of both orthodontic attachments and composite may influence plaque retention (Weitman and Eames, 1975; Zachrisson and Brobakken, 1978; Gwinnett and Ceen, 1979; Svanberg *et al*., 1984). Roughness of the composite surface predisposes to rapid attachment and growth of oral micro-organisms (Weitmann and Eames, 1975; Gwinnett and Ceen, 1979). The method of ligation of the archwire is an additional factor of importance. The labial enamel of teeth ligated with an elastomeric ring may exhibit a significantly higher number of micro-organisms in the plaque than incisors ligated with steel wire (Forsberg *et al*., 1991).

Clinical observation has indicated that a common site of demineralization is at the junction between the bonding resin and the enamel, just peripheral and commonly gingival to the bracket base (Gwinnett and Ceen, 1979). Moreover, visible enamel demineralization during fixed appliance treatment, resulting in grey and white decalcifications on the enamel at debonding, may lead to patient dissatisfaction and legal complications (Machen, 1991). Therefore, there is a great need to identify the relative role of different sites of bacterial accumulation associated with fixed appliances. The aim of this study was to assess the

accumulation of bacterial plaque at the bracket–tooth junction using scanning electron microscopy (SEM), with emphasis on the archwire ligation technique.

Materials and methods

Subjects

Nineteen patients (10–25 years) participated in this investigation. Selection criteria were that the patients required orthodontic treatment, including extraction of two or four premolars, and fixed appliances. An informed consent form was signed by the patient or the parent before the investigation started. The study was approved by the Internal Review Board at the University of Bergen. The patients were randomly assigned to either a preliminary study sample or an experimental sample.

Preliminary study sample

A total of 32 teeth to be extracted in eight patients did not have brackets bonded before extraction and these were divided into two groups: Group 1 consisted of teeth on which the morphological characteristics of naturally grown plaque without bonded brackets were assessed directly. The teeth in Group 2 were cleaned after extraction, and etched and bonded using the same procedure as in the experimental groups.

Experimental study

The experimental sample comprised 36 teeth to be extracted in 11 patients: seven patients were scheduled for extraction of four premolars, while four patients needed extraction of two upper premolars. The subjects were divided into three groups: 1-, 2-, and 3-week groups, according to the time elapsing between bonding and extraction. Although there were only 11 subjects, each group consisted of six subjects. This was because those who required extraction of four premolars had maxillary and mandibular tooth pairs extracted at two different time periods. One tooth pair was extracted at each session; one tooth with an elastomeric ring ligated bracket and the contralateral tooth with the ligature wire.

The buccal surface of all designated teeth was polished with pumice, acid etched for 30 seconds (Phosphoric Acid Gel Etchant 37.5 per cent, Kerr, Orange, CA), water rinsed for a minimum of 20 seconds, and dried thoroughly to produce a white frosted appearance of the enamel surface. Before bracket placement, the enamel surface was covered by a layer of unfilled resin (equal amounts of enamel bond resin A and B; Concise, 3M, Dental Product Division, St Paul, MN). Mesh-backed edgewise metal brackets (Unitek, 3M) were bonded on the buccal surfaces using paste/paste composite (Concise, 3M). The brackets were adjusted into position and excess composite was removed with an explorer prior to hardening. An elastomeric ring (Alastic, Unitek) and a stainless steel ligature wire (0.010-inch) were placed alternately on contralateral brackets.

All patients were told to maintain normal dietary and oral hygiene habits. No professional prophylactic care was given and none of the patients used any mouth rinse.

Surgical procedure

Tooth pairs were extracted 1, 2, or 3 weeks after bracket bonding. The teeth were luxated with a small straight elevator and removed with premolar forceps, which were engaged subgingivally so as to avoid dislodging the bracket and associated plaque accumulations.

Specimen preparation

Immediately after extraction, the premolars were rinsed in water to remove blood and debris. Plaque attached to the buccal surfaces was disclosed (Red-Cote®, John O. Butler Co., Chicago, USA) and the teeth photographed for documentation. The teeth were immersed in fixative containing 4 per cent formaldehyde and 1 per cent glutaraldehyde in phosphate buffer (McDowell and Trump, 1976) for 24 hours, followed by 0.1 M phosphate buffer for 12 hours. The root and lingual part of the crown were dissected using a high-speed bur under copious irrigation. The specimens were then dehydrated

in graded alcohols, desiccated by critical point drying, mounted on aluminium stubs, and sputter coated with gold prior to SEM examination (Philips SEM 515, Eindhoven, The Netherlands) operated at an accelerating voltage of 15 kV.

Method of plaque assessment

Plaque composition was assessed using SEM. Based on morphological characteristics, the bacteria were categorized as cocci, rods, and filaments. In addition, corn-cob formations, i.e. co-aggregation of filament and coccoid cells (Jones, 1972; Listgarten *et al*., 1973), were noted. Assessment of plaque morphology included a mid-buccal area on the excess composite resin gingival to the orthodontic bracket and on the adjacent cervical enamel surface.

Results

Preliminary study

Group 1: non-bonded extracted premolars. Colour photographs revealed disclosing stain on the buccal enamel surface. A pink colour was generally present as a narrow continuous zone covering the cervical third of the clinical crown from the mesial to distal portion of the buccal surface.

SEM at low magnification (less than ×100) showed a fine layer of plaque corresponding to the stained enamel surface. Higher magnification revealed clumps of cocci and rods aggregated in areas with surface irregularities and an extracellular amorphous matrix (Figure 1). Filamentous bacteria were occasionally noted.

Group 2: bonded extracted premolars. After prophylaxis, stained deposits were not macroscopically seen on the buccal tooth surface. At low magnification the tooth surfaces appeared clean. Excess bonding composite was present around the bracket base (Figure 2a). At higher magnification, a very rough surface texture of the bonding adhesive was evident. An abundance of large and irregularly-shaped filler particles projected from the resin matrix (Figure 2b). A gap at the composite–enamel junction was present along the edge of the bonding composite in all specimens (Figure 2a).

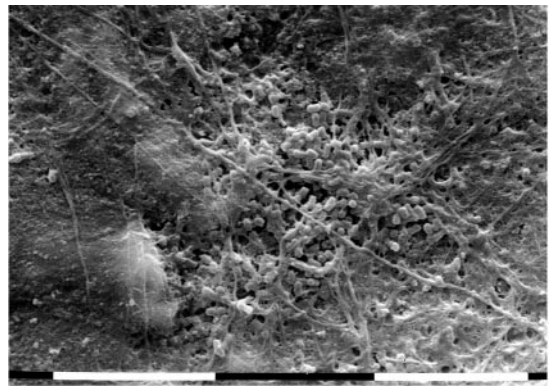

Figure 1 Characteristic cervical dental plaque on the buccal surface of a non-bonded control tooth. Clumps of cocci and rods, as well as extracellular amorphous material are present, particularly associated with enamel surface irregularities. Original magnification ×3000 (bar = 10 μm).

Experimental study

1-week group. Colour photographs revealed disclosing stain on the buccal tooth surface adjacent to the bracket base. Staining occurred generally in the cervical region, and extended mesial and distal, and in some specimens occlusal to the brackets. Stained areas coincided with the areas covered by plaque as seen by SEM at low magnification.

At higher magnification, a discrete layer of cocci and a few short rods was seen on the enamel surfaces in the cervical region and in areas lateral to the brackets (Figure 3a). Some coccoid cells appeared to form chains characteristic of *streptococci*. The surfaces of excess composite, brackets, and ligatures exhibited a more coherent layer of bacteria embedded in extracellular matrix. Cocci were prevalent; however, some rods were also present (Figure 3b). The largest deposits were found underneath bracket wings and ligatures and, notably, on composite surfaces.

A narrow gap, less than 10 μm in width and harbouring numerous bacteria, was present at the excess composite–enamel interface, extending all around the bracket base in all specimens.

2-week group. Macroscopic observation and SEM at low magnification yielded similar findings to those in the 1-week specimens. At

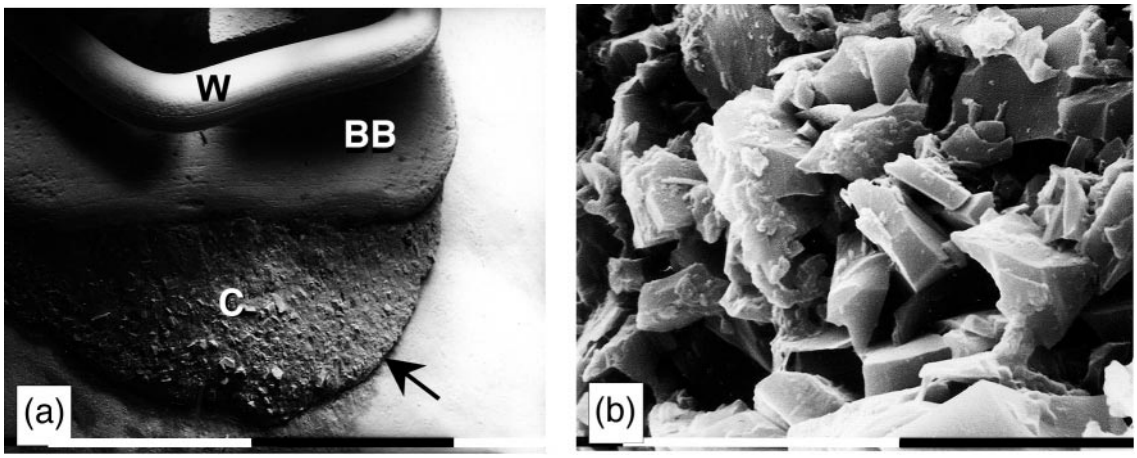

Figure 2 Surface texture of composite immediately after bonding. (a) Excess composite (C) cervical to the bracket base (BB). Note that the composite does not blend smoothly with the enamel surface, but forms a distinct gap along the periphery (arrow). W, wire ligature. Original magnification ×32 (bar = 1 mm). (b) Coarse filler particles projecting above the resin surface are clearly seen at higher magnification. Original magnification ×4200 (bar = 10 μm).

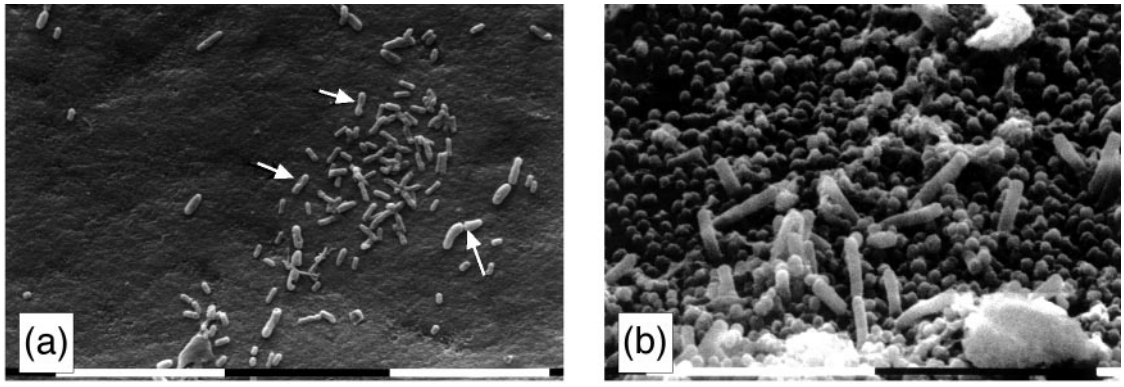

Figure 3 Plaque development on experimental tooth 1 week after bonding. (a) The enamel surface cervical to the bracket exhibits scattered cocci, some of which are forming short chains (arrows). Original magnification ×2500. (b) Excess composite cervical to the bracket base is covered by a layer of cocci and rods. Original magnification ×3600 (bars = 10 μm).

higher magnification, microcolonies of cocci and short rods and a barely detectable inter-microbial matrix were observed on the enamel surface (Figure 4a). Bacterial colonization on composite surfaces showed some degree of individual variation. Most specimens presented features of young plaque consisting of cocci and rods embedded in an inter-microbial matrix. Increased presence of organisms characteristic of mature plaque was, however, noticeable (Figure 4b). Cocci attached to filaments giving an appearance of corn-cobs were found in one subject both in the elastomeric ring and the wire ligature specimen (Figure 5). Specimen pairs from each subject generally showed comparable bacterial morphotypes on brackets and ligatures. A gap with lodged bacteria along the periphery of the composite was noticed in all specimens.

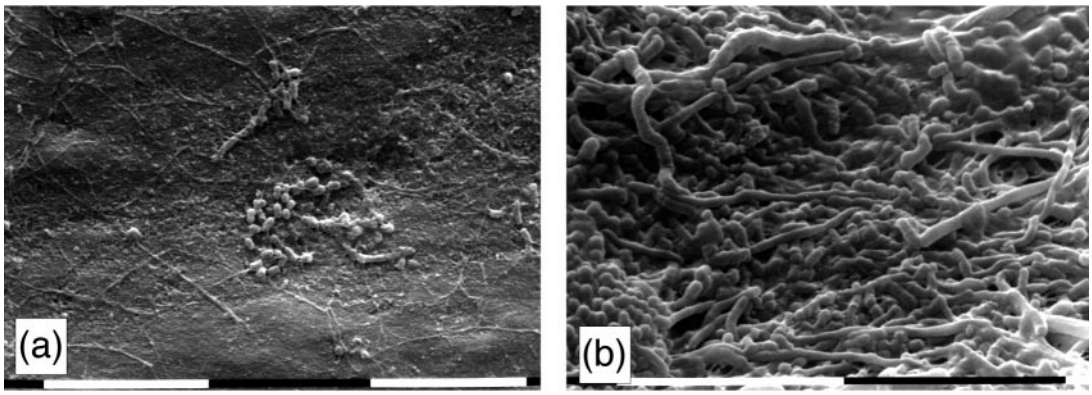

Figure 4 Plaque development 2 weeks after bonding. (a) Cervical enamel exhibits aggregates of cocci predominantly located in surface irregularities, as well as scattered cells attached to a granular pellicle. Original magnification ×2600. (b) Excess composite cervical to the bracket base exhibits a diversity of bacterial morphotypes including cocci, rods, and filaments. Original magnification ×4400 (bars = 10 μm).

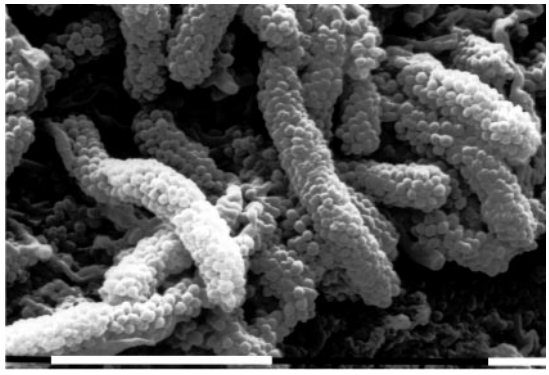

Figure 5 Corn-cobs, consisting of cocci attached to a central filament, found in one subject 2 weeks after bonding. Original magnification ×3900 (bar = 10 μm).

3-week group. Colour photographs showed abundant plaque on buccal tooth surfaces, mostly gingival and lateral to the brackets, as well as on the appliance and on excess composite (Figure 6a,b).

Low-power SEM revealed a fine layer of plaque deposition on the enamel in the cervical region, and also in areas mesial and distal to the bracket. In contrast, excess composite, brackets, and ligatures had accumulated a thick layer of bacterial plaque. Brackets ligated with elastomeric rings appeared to retain more plaque than those ligated with stainless steel wire (Figure 7a,b).

Higher magnification revealed that plaque on the cervical enamel consisted of microcolonies of cocci and short rods embedded in a prominent inter-microbial matrix in both groups of ligation, whereas filaments were infrequently present (Figure 8a). Plaque on the composite surface had a more mature composition; filaments were predominant, together with some cocci and rods (Figure 8b,c). Bacterial colonization had also occurred on bracket and ligature surfaces. Gaps at the composite–enamel junction, which harboured abundant bacteria, were conspicuous (Figure 7a, 9a,b). Some variation in the extent of bacterial colonization was found among the subjects. Spirochetes were not noted in this study.

Discussion

A SEM technique was chosen for assessing bacterial colonization in this investigation as it represents a rapid and convenient means of screening microbial samples for major morphotypes (Slots and Rams, 1992; Samaranayake, 1996). Moreover, SEM provides a large depth of focus that allows a wide area of the specimen surface to be examined in focus, and it offers a three-dimensional view of a superficial layer of bacterial colonization. A limitation in the use of

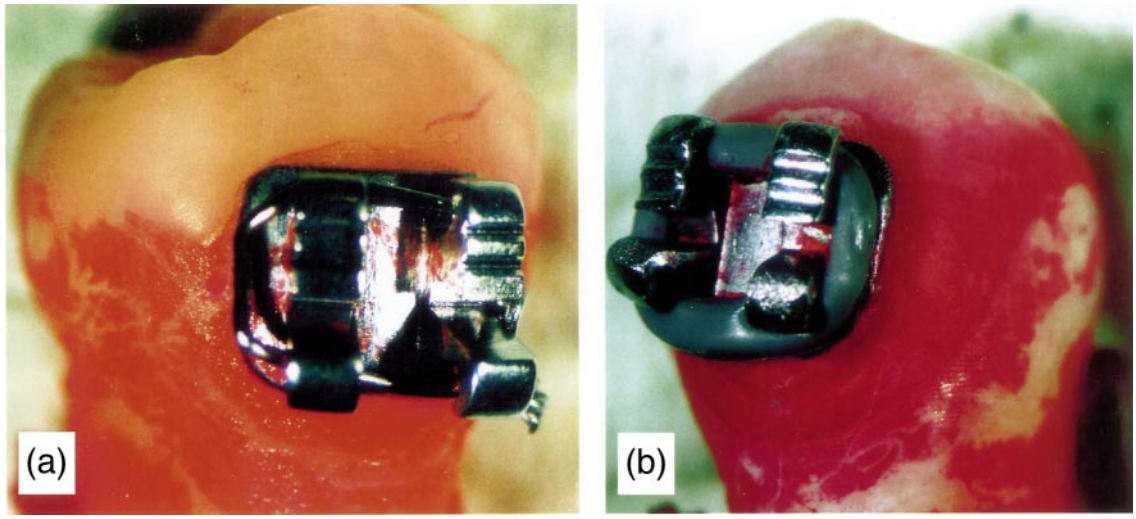

Figure 6 Colour photographs illustrating disclosed plaque 3 weeks after bonding. Stained areas are seen on excess composite and on the enamel surface cervically, as well as mesial and distal to the bracket. The bracket in (a) carries a wire ligature and in (b) an elastomeric ring.

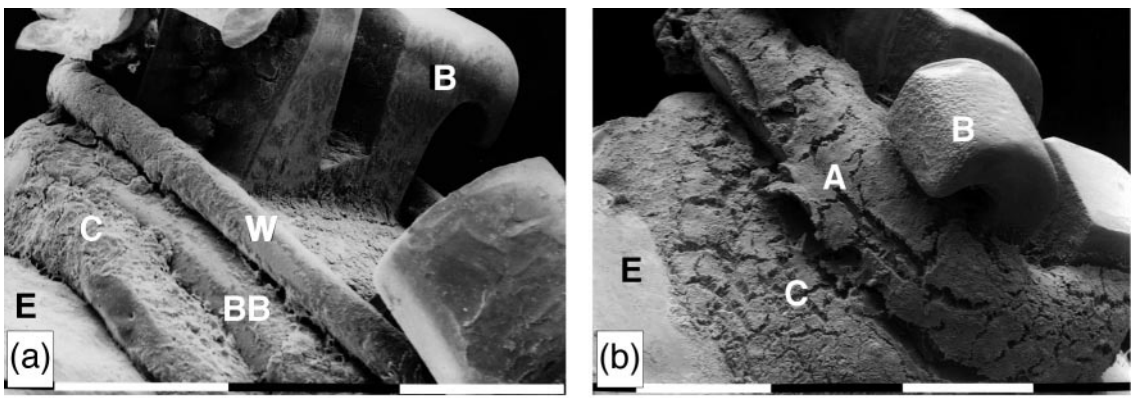

Figure 7 Low-power SEMs illustrating plaque accumulation at 3 weeks on bonded teeth. (a) Bonded tooth ligated with stainless steel wire (W) reveals abundant plaque deposition on excess composite, under bracket wings, and on and under the ligature wire, whereas sparse plaque is present on the enamel cervical to the composite. Note the gap demarcating the periphery of the composite. Original magnification ×28. (b) Bonded tooth ligated with elastomeric ring (A) displays plaque accumulation both on ring and composite, extending to the cervical enamel surface. The surface texture of the elastomeric ring is more irregular than that of the wire. In this specimen, the enamel–composite junction cannot be characterized due to the heavy plaque deposit. B, bracket wing; BB, bracket base; C, composite; E, enamel. Original magnification ×21 (bars = 1 mm).

SEM, however, is the inability to identify species; therefore, micro-organisms are classified on a morphological basis (Carrassi *et al*., 1989). The results revealed an early stage of supragingival plaque accumulation consisting of cocci on all experimental surfaces by 1 week. With time, plaque maturation included rods and filaments, as well as an increasing amount of inter-microbial matrix.

A preliminary study was carried out in order to establish the SEM appearance of a cleaned enamel surface, bonding resin, metal, and

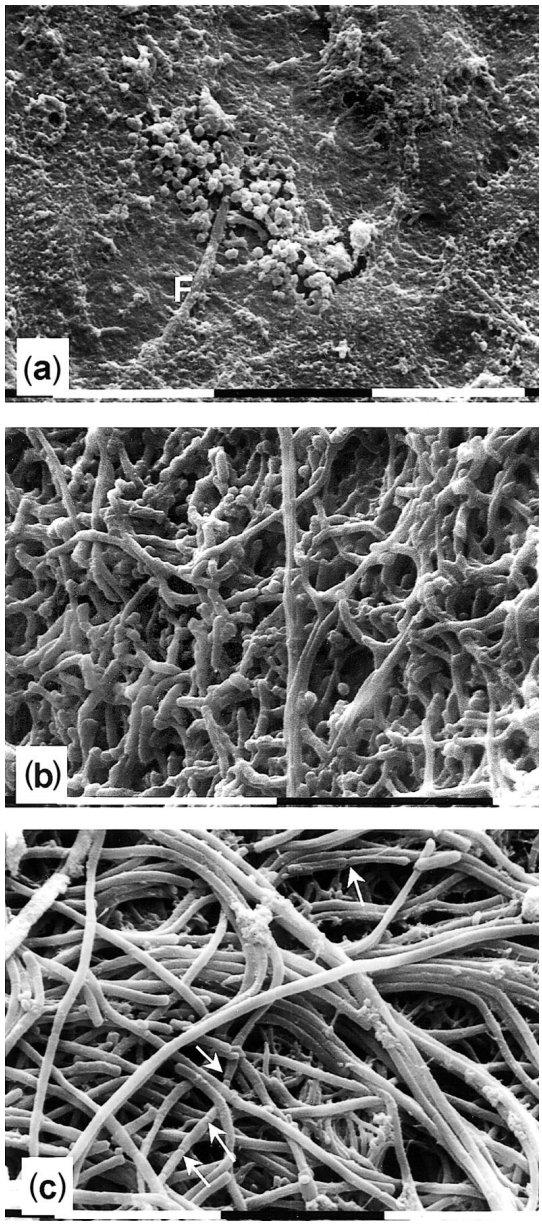

Figure 8 Plaque development 3 weeks after bonding. (a) Buccal enamel cervical to the bracket is covered predominantly by coccoid plaque exhibiting an abundant inter-microbial matrix. A filamentous organism (F) is also present. Original magnification ×2500. (b) The surface of excess composite exhibits mature plaque containing a variety of bacterial morphotypes including numerous filaments. Original magnification ×3500. (c) Mature plaque on excess composite consists predominantly of filaments and long chains of *streptococci* (arrows indicate cell segmentation). Original magnification ×2600 (bars = 10 μm).

elastomeric surfaces of the appliance, and of the habitually unclean cervical enamel of the subjects. Both monolayers and clumps of cocci and rods with a few filaments were present, mostly on the cervical third of buccal enamel surfaces. Specimens that were bonded after extraction revealed that excess composite has a very rough surface compared with pumiced enamel. This strengthens the view that excess composite is an important predisposing factor for plaque accumulation.

Filled diacrylate resin of the bis-GMA type was used as the bonding agent because it provides significantly more tensile strength than glass ionomer cements (Evans and Oliver, 1991; Hallgren *et al*., 1994). Adjustment of brackets into position and removal of excess composite around the bracket base were done to imitate an actual clinical situation. Two methods of ligation, elastomeric ring and ligature wire, were chosen, since they are the two commonly used techniques for tying archwires (Postlethwaite, 1992). However, the archwire was not ligated into the bracket because the experimental design included only one bonded tooth in each quadrant. Had the archwire and accessory attachments been present, more plaque would presumably have been retained (Mitchell, 1992). Plaque accumulation within 1–3 weeks after bonding occurred primarily gingival and lateral to the bracket base, which corresponds with areas where the majority of white spot lesions occur (Stratemann and Shannon, 1974; Gorelick *et al*., 1982; Mizrahi, 1983; Årtun and Brobakken, 1986).

Interestingly, variable amounts of composite were present on the enamel surface around the bracket base in all specimens, although an effort had been made to remove excess composite during bonding. As the bonding composite has a colour similar to the enamel surface, it is difficult to detect residual composite clinically, especially gingival to the bracket base.

Despite some inter-individual variation, the same distribution pattern of bacterial morphotypes was found within each subject with both methods of ligation. Thus, neither elastomeric ring nor wire ligature seems to affect the distribution of bacterial morphotypes on both composite and enamel surfaces.

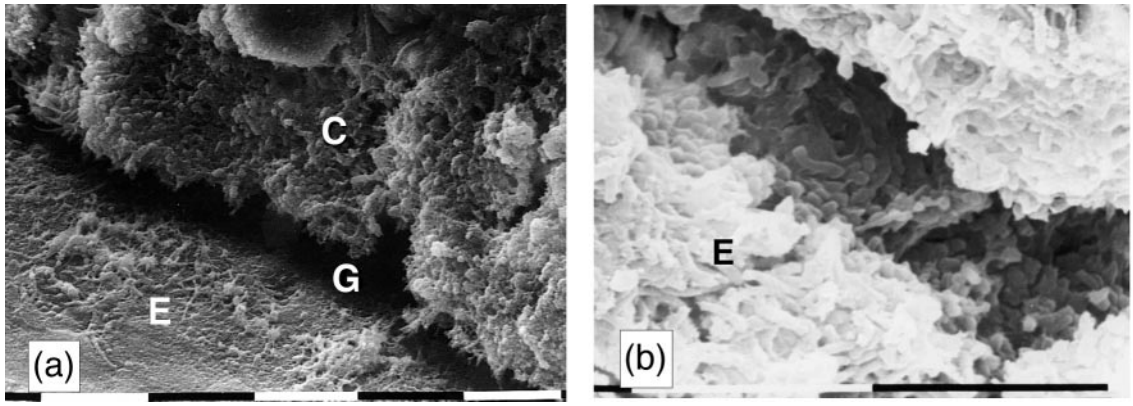

Figure 9 (a) Gap (G) along the edge of the bonding composite (C). Original magnification ×1700. (b) High-power SEM demonstrates that bacteria are present on the enamel surface (E), as well as in the gap, supporting the evidence that this separation has developed *in vivo*. Original magnification ×3800 (bars = 10 μm).

The present study differed in design from other studies of supragingival plaque formation (Frank and Houver, 1970; Theilade and Theilade, 1970; Eastcott and Stallard, 1973; Lie, 1977, 1979; Nyvad and Fejerskov, 1987; Quirynen and van Steenberghe, 1989; Zee *et al*., 1997). Earlier investigations have generally discontinued oral hygiene during experimental periods whereas, in the present study, the patients were told to maintain their normal oral hygiene regimen in order to imitate more closely the real situation of plaque development during fixed appliance treatment. The results indicate that unless patients receive specific instructions on appropriate home care, abundant plaque will form on bonded teeth within 1 week.

This SEM study revealed several interesting aspects with regard to accumulation of dental plaque on bonded teeth. Within 1 week after placement of the bracket and during the maintenance of ordinary oral hygiene, the surface of excess composite gingival to the bracket base was almost completely covered by a thick layer of bacteria, while the enamel surface gingival to the composite revealed a monolayer of bacteria. The same difference in distribution was seen at 2 and 3 weeks. This finding confirms that excess bonding composite around the bracket base is an obvious predisposing factor for plaque development due to its rough surface, and supplements earlier studies which have reported that the composite resin surface is an important factor in the accumulation of plaque (Weitman and Eames, 1975; Gwinnett and Ceen, 1979).

Significantly, a gap approximately 10 μm in width was found at the composite–enamel junction around the bracket base in all specimens. These gaps were consistent in size and shape. Bacterial accumulation was constantly detected within these gaps, indicating that they were not an artefact of specimen preparation. More likely, the gap is created by the setting shrinkage, which is an inherent property of composite during polymerization and has been reported in both restorative (Boyde and Knight, 1969; Asmussen, 1975; Jacobsen, 1975) and orthodontic bonding composite (Lee *et al*., 1986). Moreover, this marginal gap may be a consequence of the difference in the coefficient of thermal expansion between composite and tooth (Asmussen, 1985). The width of the gap corresponded more or less with that found in other studies (Brännström *et al*., 1984; Lee *et al*., 1986). The gap width may have increased secondary to the drying process (Boyde and Knight, 1969). It appears likely that the gap is another predisposing factor for bacterial accumulation, and may contribute to the frequent development of white lesions at the

bracket–tooth interface. This study highlights the importance of removing excess composite around the bracket base during bonding. Further improvement of composite bonding materials and of application technique is needed to reduce the tendency for gap formation and consequent development of white spot lesions.

Conclusions

This study shows that excess bonding composite around the bracket base is the critical site of plaque accumulation associated with fixed appliances due to its rough surface texture and the setting shrinkage gap along its periphery. A complex community of bacterial plaque may be present on the excess composite within 2–3 weeks after bonding, whereas the adjacent enamel surface still reveals early stages of plaque maturation.

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References

Årtun J, Brobakken B O 1986 Prevalence of carious white spots after orthodontic treatment with multi-bonded appliances. European Journal of Orthodontics 8: 229–234

Årtun J, Thylstrup A 1986 Clinical and scanning electron microscopic study of surface changes of incipient caries lesions after debonding. Scandinavian Journal of Dental Research 94: 193–201

Asmussen E 1975 Composite restorative resins. Composition versus wall-to-wall polymerization contraction. Acta Odontologica Scandinavica 33: 337–344

Asmussen E 1985 Clinical relevance of physical, chemical, and bonding properties of composite resins. Operative Dentistry 10: 61–73

Boyde A, Knight P J 1969 The use of scanning electron microscopy in clinical dental research. British Dental Journal 7: 313–322

Brännström M, Torstenson B, Nordenvall K J 1984 The initial gap around large composite restorations *in vitro*: the effect of etching enamel walls. Journal of Dental Research 63: 681–684

Carrassi A, Santarelli G, Abati S 1989 Early plaque colonization on human cementum. Journal of Clinical Periodontology 16: 265–267

Eastcott A D, Stallard R E 1973 Sequential changes in developing human dental plaque as visualized by scanning electron microscopy. Journal of Periodontology 44: 218–224

Evans R, Oliver R 1991 Orthodontic bonding using glass ionomer cement: an *in vitro* study. European Journal of Orthodontics 13: 493–500

Forsberg C M, Brattström V, Malmberg E, Nord C E 1991 Ligature wires and elastomeric rings: two methods of ligation, and their association with microbial colonization of *Streptococcus mutans* and lactobacilli. European Journal of Orthodontics 13: 416–420

Frank R M, Houver G 1970 An ultrastructural study of human supragingival dental plaque formation. In: McHugh W D (ed.) Dental plaque. Livingstone, Edinburgh, pp. 85–108

Gorelick L, Geiger A M, Gwinnett A J 1982 Incidence of white spot formation after bonding and banding. American Journal of Orthodontics 81: 93–98

Gwinnett A J, Ceen R F 1979 Plaque distribution on bonded brackets: a scanning microscope study. American Journal of Orthodontics 75: 667–677

Hallgren A, Oliveby A, Twetman S 1994 L(+)-lactic acid production in plaque from orthodontic appliances retained with glass ionomer cement. British Journal of Orthodontics 21: 23–26

Ingervall B 1962 The influence of orthodontic appliances on caries frequency. Odontologisk Revy 13: 175–190

Jacobsen P H 1975 Clinical aspects of composite restorative materials. British Dental Journal 139: 276–280

Jones S J 1972 A special relationship between spherical and filamentous microorganisms in mature human dental plaque. Archives of Oral Biology 17: 613–616

Lee S M H, Freer T J, Basford K E 1986 Microleak age at the etched enamel-resin interface with bonded orthodontic brackets. Australian Orthodontic Journal 9: 270–275

Lie T 1977 Early dental plaque morphogenesis. Journal of Periodontal Research 12: 73–89

Lie T 1979 Morphologic studies on dental plaque formation. Acta Odontologica Scandinavica 37: 73–85

Listgarten M A, Mayo H, Amsterdam M 1973 Ultrastructure of the attachment device between coccal and filamentous microorganisms in 'corn cob' formations of dental plaque. Archives of Oral Biology 18: 651–656

Machen D E 1991 Legal aspects of orthodontic practice: risk management concepts. American Journal of Orthodontics and Dentofacial Orthopedics 100: 93–94

McDowell E M, Trump M D 1976 Histologic fixatives suitable for light and electron microscopy. Archives of Pathology and Laboratory Medicine 100: 405–414

Mitchell L 1992 Decalcification during orthodontic treatment with fixed appliances—an overview. British Journal of Orthodontics 19: 199–205

Mizrahi E 1982 Enamel demineralization following orthodontic treatment. American Journal of Orthodontics 82: 62–67

Mizrahi E 1983 Surface distribution of enamel opacities following orthodontic treatment. American Journal of Orthodontics 84: 323–331

Nyvad B, Fejerskov O 1987 Scanning electron microscopy of early microbial colonization of human enamel and root surfaces *in vivo*. Scandinavian Journal of Dental Research 95: 287–296

Ögaard B 1989 Prevalence of white spot lesions in 19-year-olds: a study on untreated and orthodontically treated persons 5 years after treatment. American Journal of Orthodontics and Dentofacial Orthopedics 96: 423–427

Ögaard B, Rölla G, Arends J 1988 Orthodontic appliances and enamel demineralization: Part 1 Lesion development. American Journal of Orthodontics and Dentofacial Orthopedics 94: 68–73

O'Reilly M M, Featherstone J D B 1987 Demineralization and remineralization around orthodontic appliances: an *in vivo* study. American Journal of Orthodontics and Dentofacial Orthopedics 92: 33–40

Postlethwaite K M 1992 Advances in fixed appliance design and use: 2. Auxiliaries, adhesives, appliance care and debonding. Dental Update 19: 331–335

Quirynen M, van Steenberghe D 1989 Is early plaque growth rate constant with time? Journal of Clinical Periodontology 16: 278–283

Samaranayake L P 1996 (ed.) Essential microbiology for dentistry. Churchill Livingstone, New York, pp. 45–58

Slots J, Rams T E 1992 Methods for the study of oral microorganism. In: Slots J, Taubman M A (eds) Contemporary oral microbiology and immunity. Mosby, St. Louis, pp. 275–282

Strateman M W, Shannon I L 1974 Control of decalcification in orthodontic patients by daily self-administered application of a water-free 0.4 per cent stannous fluoride gel. American Journal of Orthodontics 66: 273–279

Svanberg M, Ljunglöf S, Thilander B 1984 *Streptococcus mutans* and *Streptococcus sanguis* in plaque from orthodontic bands and brackets. European Journal of Orthodontics 6: 132–136

Theilade E, Theilade J 1970 Bacteriological and ultrastructural studies of developing dental plaque. In: McHugh W D (ed.) Dental plaque. Livingstone, Edinburgh, pp. 27–40

Weitman R T, Eames W B 1975 Plaque accumulation on composite surfaces after various finishing procedures. Journal of the American Dental Association 91: 101–106

Zachrisson B U, Brobakken B O 1978 Clinical comparison of direct versus indirect bonding with different bracket types and adhesives. American Journal of Orthodontics 74: 62–78

Zee K Y, Samaranayake L P, Attström R 1997 Scanning electron microscopy of microbial colonization of 'rapid' and 'slow' dental-plaque formers *in vivo*. Archives of Oral Biology 42: 735–742