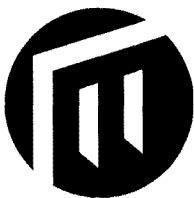




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Supragingival Plaque Formation in “Rapid” and “Slow” Plaque Formers

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無不知則無知

王陽明

*A man has real knowledge if he realizes there are things he doesn't know.
A man is ignorant when he believes there is nothing he doesn't know.*

Contents

Preface	7
Background	9
Introduction	9
Dental plaque formation on tooth surfaces	9
<i>Phase I – Deposition and adsorption</i>	9
<i>Phase II & III – Adhesion and attachment</i>	10
<i>Phase IV – Colonization</i>	10
Factors affecting microbial adherence on tooth surfaces	10
<i>Acquired pellicle</i>	10
<i>Bacterial polymers</i>	11
<i>Saliva</i>	11
<i>Gingival fluid</i>	12
<i>Diet</i>	12
<i>Physical factors</i>	12
Rate of plaque formation	13
Effect of surface active agents on the rate of plaque formation	15
Additional questions	16
Aims	16
Material and Methods	17
Selection of subjects	17
Study I	17
<i>Sampling and microbiological procedures</i>	17
Study II	18
<i>Preparation of enamel blocks</i>	18
<i>Experimental and sampling procedures</i>	18
<i>Specimen preparation</i>	19
Study III	19
<i>Replica preparation</i>	19
Study IV	20
<i>Saliva collection</i>	20
<i>Determination of salivary IgA</i>	20

Study V	21
<i>Study design</i>	21
<i>Plaque assessment</i>	21
<i>Gingivitis assessment</i>	21
Statistical analyses	22
Ethics	22
Results	22
Selection of “rapid” and ‘slow” plaque formers	22
Supragingival plaque flora of “rapid” and “slow” plaque formers	23
Morphological features of “rapid” and “slow” plaque formers	24
Extension of Plaque	24
Salivary immunoglobulin A levels	28
Effect of delmopinol hydrochloride mouthrinses	28
Discussion	28
Selection of subjects	28
Microbial flora of supragingival plaque	29
Morphological Features	30
Salivary immunoglobulin A levels in “rapid” and “slow” plaque formers ...	32
Effect of a surface active agent on plaque formation	33
Conclusions	34
References	35
Summary	41
Acknowledgments	42
Appendix	43
Study I	45
Study II	55
Study III	65
Study IV	77
Study V	85

Preface

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I. **Zee K-Y**, Samaranayake LP & Attström R. (1996) Predominant cultivable supragingival plaque in Chinese “rapid” and “slow” plaque formers. *Journal of Clinical Periodontology* **23**, 1025-1031.
- II. **Zee K-Y**, Samaranayake LP & Attström R. (1997) Scanning electron microscopy of microbial colonization of “rapid” and “slow” plaque formers *in vivo*. *Archives of Oral Biology* **42**, 735-742.
- III. **Zee K-Y**, Samaranayake LP & Attström R. (2000) An *in vivo* replica study of microbial colonization in “rapid” and “slow” dental plaque formers. *Acta Pathologica, Microbiologica et Immunologica Scandinavica* **108**, 113-121.
- IV. **Zee K-Y**, Samaranayake LP & Attström R. (2000) Salivary immunoglobulin A levels in “rapid” and “slow” plaque formers. *Oral Diseases* (submitted).
- V. **Zee K-Y**, Rundegren J & Attström R. (1997) Effect of delmopinol hydrochloride mouthrinse on plaque formation and gingivitis in “rapid” and “slow” plaque formers. *Journal of Clinical Periodontology* **24**, 486-491.

Background

Introduction

Dental plaque may be defined as bacterial colonization on the teeth or other solid oral structures (Dawes et al. 1963, Egelberg 1970, Kelstrup & Theilade 1974, Listgarten 1994). There is increasing evidence to demonstrate that the amount and composition of dental plaque is directly related to periodontal health and caries (Lindhe et al. 1980, Carlsson 1989, Haffajee & Socransky 1994). Plaque in the dento-gingival region is of primary concern to the therapists dealing with periodontal disease, and may arbitrarily be classified as supragingival plaque deposited on the clinical crowns of teeth, and subgingival plaque located in the gingival sulcus or the periodontal pocket (Theilade 1989). Supragingival plaque consists of generally densely packed bacteria embedded in a matrix containing mainly bacterial extracellular polymers and macromolecules derived from saliva or gingival fluid (Gibbons & van Houte 1975, van Houte 1983). However, the terms sub- and supragingival are relative and the position of a given bacterial deposit may shift over time. Supragingival plaque may turn into subgingival by gingival swelling and subgingival into supragingival by gingival recession.

Dental plaque formation on tooth surfaces

The mechanism behind initial plaque formation has been explained previously by mainly two hypotheses. One of these suggested that bacterial surfaces possess specific bacterial adhesins which identify and interact with components of the pellicle on oral surfaces (for reviews: see Gibbons 1980 & 1984). The other hypothesis considered bacterial adherence as a physiochemical interaction between bacteria and the pellicle surface (Glantz 1969, Busscher & Weerkamp 1987, van Dijk et al. 1987, Christersson 1991).

According to Marsh and Martin (1984), the development of a microbial film can be arbitrarily divided into four stages:

1. a reversible phase, defined as the deposition or adsorption of an organism to a surface,

which is followed by,

2. an irreversible phase in which polymer bridging between the organism and the surface plays an important role in anchoring the bacterial cell, and
3. a repeat of phases 1 and 2, but in which the adherence of depositing bacteria is to the outer layers of already attached organisms and, finally,
4. multiplication of the attached microorganisms, which eventually will lead to confluent growth and film formation.

The phases are briefly described below:

Phase I – Deposition and adsorption

As a bacterium approaches a surface, the initial interaction between the negatively charged tooth surface and the bacterial surface possessing the same charge may be affected by non-specific interactions. These interactions may include van der Waals attractive forces (operate over long, >50 nm, separation distances) and electrostatic forces (operate at 10-20 nm distances).

The result of these interactions can be explained partly by the DLVO theory (Derjaguin & Landau 1941, Verwey & Overbeek 1948), which was proposed originally to explain colloid stability. A diagram illustrating the DLVO theory is shown in Fig. 1. The total interaction energy, V_T , between a par-

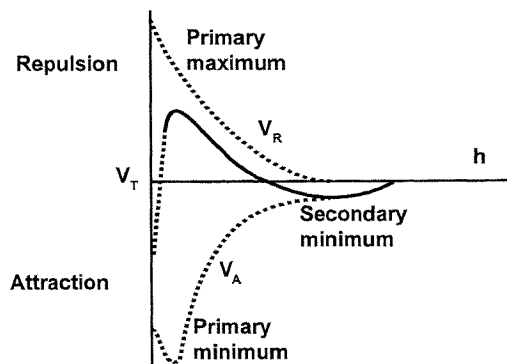


Fig. 1. Diagram illustrating the DLVO theory. The total interactive energy, V_T , between a particle and a surface is shown with respect to the separation distance, h . The total interaction curve is obtained by the summation of an attraction curve, V_A , and a repulsion curve, V_R . (Adapted from Marsh & Martin 1984)

ticle and a surface is shown with respect to the separation distance, h . The total interaction curve is obtained by the summation of an attraction curve V_A and a repulsion curve V_R . As a result, the bacteria will separate from the tooth surface at specific distances (for reviews: see Shaw 1980, Rutter 1980, Quirynen & Bollen 1995) and can be held in a weak DLVO secondary minimum for a short period of time.

Phase II & III – Adhesion and attachment

When bacteria are being held reversibly to a surface within 1 nm or less, the outer layers of bacteria are able to establish a firmer anchorage by specific interactions. This anchorage is achieved by specific extracellular proteinaceous components of the bacteria (adhesins) and complementary receptors on the surface (e.g., pellicle mucins), and is species-specific (Gibbons & van Houte 1971, van Houte 1983, Gibbons 1980 & 1984). If the bacteria in addition possess filamentous appendages with adhesins, the organisms will be capable of bridging even wider spaces up to 10 nm (Fig. 2). The adhesins are often lectins which bind to saccharide receptors, but some adhesins are thought to bind proteinaceous receptors (Ellen 1985, Gibbons 1989).

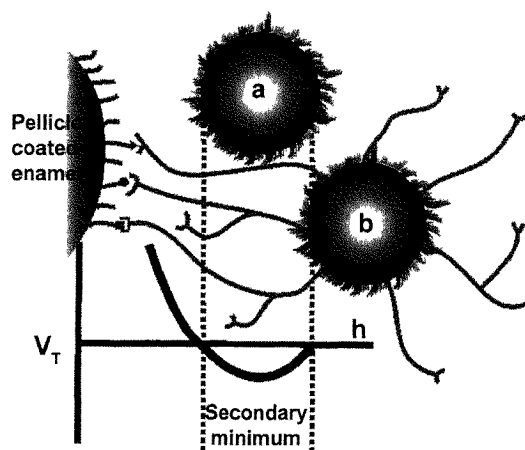


Fig. 2. Adhesion of bacteria to pellicle-coated enamel may involve (a) secondary minimum capture and (b) polymer bridging in which, the critical DLVO distances are spanned. (Modified from Marsh & Martin 1984)

Phase IV – Colonization

Once the bacteria are firmly attached onto the surfaces, a further growth of the plaque mass occurs primarily by multiplication of already adherent bacteria (Brex et al. 1983). The bacteria may also arrange themselves into special configurations such as corn-cob formation in which, for example, streptococci adhere to filaments of *Bacterionema matruchotii* (Mouton et al. 1980) or *Actinomyces* species (Cisar 1982), or “bristle-brush” formation composed of filamentous bacteria to which the gram-negative rods adhere (Listgarten 1976).

In conclusion, the deposition, growth, removal and reattachment of bacteria is a dynamic process and a microbial film such as dental plaque undergoes constant reorganization. This suggests that both hypotheses, that is either specific adhesion or physiochemical interaction, might not be necessarily contradictory but instead, complementary.

Dental plaque is considered to be a natural biofilm, which forms an important class of potentially pathogenic relationships between microbe and man (Liljemark et al. 1997). Biofilms are difficult to remove once they are formed, and are frequently intractable to antimicrobial therapies (Gilbert et al. 1997). Therefore, it is important to understand the detailed biology of this biofilm in order to prevent and treat caries and periodontal diseases.

Factors affecting microbial adherence on tooth surfaces

The adherence stages described above are influenced not only by the interaction between surface structures of bacteria and available colonizable surfaces, but also by the activity of saliva as a suspending fluid (Carlsson 1989). Hence, properties and composition of the acquired pellicle (both on the tooth and bacterial surface), the bacterial surface and saliva can affect plaque formation.

Acquired pellicle

Within seconds after a tooth surface is cleaned, salivary glycoproteins are adsorbed forming the acquired pellicle. It has been shown that the acquired pellicle can alter the adhesiveness of all surfaces exposed to the oral environment (Baier &

Glantz 1978). Sönju and Glantz (1975) reported that the composition of the early pellicle is dependent on the nature of the solid on which it forms. However, the newly formed pellicle gives different materials placed in the oral cavity of an individual the same clinical adhesiveness in spite of differences between the basic surface chemical properties of these solids (Jendresen & Glantz 1981). In an *in vivo* study on the clinical adhesiveness of the tooth surface, Jendresen and Glantz (1980) found that the adhesiveness of pellicles covering normal tooth enamel was essentially the same over a wide human population with moderate plaque formation tendency. They also speculated that “non-plaque formers” might have pellicles with a critical surface tension of wetting that is lower than that for the average person.

Bacterial polymers

Most bacterial surfaces are coated by highly hydrated matrices called glycocalyxes (Costerton et al. 1981). In some bacteria the glycocalyx consists of a regular array of rod-like glycoprotein appendages, but more often it is composed of a matrix of polysaccharide fibers. Many bacteria bear long nonflagellar appendages at their surfaces, which extend beyond the surface of the glycocalyx. They are usually called pili or fimbriae. Bacteria pili or fimbriae have been shown to process molecules called adhesins (Mergenhagen et al. 1987). The carbohydrate groups of the glycoproteins of the pellicle may serve as receptors for such bacterial adhesins (Sharon 1987). These appendages are important to enable microorganisms to firmly attach onto the surfaces.

Beside the glycocalyxes, bacteria have in addition the capacity to form extracellular homo-polysaccharides from sucrose (Guggenheim 1970, Walker & Jacques 1987), which include highly branched glucans, which can be soluble (dextran) or insoluble (mutan), and fructan. These insoluble glucans are considered to play an important role as a matrix in the microbial aggregations on the teeth. Fructan can be used as a source of carbohydrate by some members of the oral microbiota and enhance their growth (McGhee & Michalek 1981).

Saliva

Since the aforementioned adherence phenomena in the oral cavity occur within a milieu bathed in saliva, the impact of the latter as a suspending fluid is worthy of consideration. Saliva is a complex mixture of many different polymers and its composition is known to vary between individuals and with time. The major salivary factors which may affect plaque formation are discussed below.

Salivary bacteria-agglutinating factors

Ericson and Magnusson (1976) suggested that bacteria-agglutinating glycoproteins in a fluid medium reduce the attachment of microorganisms to tooth enamel surfaces. Such substances can promote the formation of aggregates and hence act as a host protection mechanism. Large conglomerates of organisms would be more susceptible to removal by shear forces from salivary and crevicular fluid flow, and thus be removed from the mouth by swallowing. However, such agglutinins when adsorbed to a tooth surface may potentiate the attachment of microorganisms (Hay et al. 1971, Ericson et al. 1976).

Salivary antibodies

Heremans (1968) reported that salivary secretory IgA forms complexes with other proteins in mucus, and it may thus act as an “antiseptic paint” on different surfaces. Williams and Gibbons (1972) suggested that secretory IgA forms non-adhering aggregates with oral bacteria. However, antibodies are also found in the pellicle and this could enhance deposition in a similar manner to aggregate-inducing substances. Previous work has indicated that salivary IgA exerts a direct inhibitory effect on the adherence of microorganisms to host mucosal epithelial cells (Abraham & Beachey 1988) and to saliva coated hydroxyapatite (Reinholdt & Kilian 1987) by blocking of adherence determinants, reduction of hydrophobicity of bacteria and agglutination of bacteria (Kilian & Bratthall 1986). Such properties, however, have been confirmed only by *in vitro* experiments (Williams & Gibbons 1972, Kilian et al. 1981, Reinholdt & Kilian 1987).

Salivary antimicrobial factors

Lactoferrin is an iron-binding protein present in saliva, which reduces the amount of iron, and is essential for bacterial growth, in the microbial environment (Kochan 1973). Lactoferrin has been reported to possess a bactericidal effect (Arnold et al. 1977) and to potentiate the hydroxyl-radical formation from polymorphonuclear leukocytes (Ambruso & Johnston Jr 1981).

Lactoperoxidase is a salivary enzyme that catalyses the oxidation of thiocyanate by hydrogen peroxide during the formation of hypothiocyanite ions. These ions interfere with bacterial metabolism and thus inhibit bacterial growth (Hoogendoorn et al. 1977). It was also suggested that salivary peroxidase and thiocyanate protect bacteria from hydrogen peroxide, which is more toxic for the cells than hypothiocyanite ions (Adamson & Carlsson 1982).

Lysozyme is another antimicrobial enzyme in saliva that can break the glycosidic bonds between N-acetylmuramic acid and N-acetylglucosamine in bacterial cell walls (Salton 1961, Brandtzaeg & Mann 1964). Twetman et al. (1986) suggested that lysozyme can interfere with the sugar-transporting mechanisms of the bacterial cell membrane and may thus be of importance in limiting glycolysis in oral bacteria. However, such bacteriostatic or bactericidal effects were shown *in vitro* and it is not clear what role this enzyme has in the microbial ecology of the oral cavity (Iacono et al. 1982).

Al-Hashimi & Levine (1989) used Western transfer analyses and radiolabeling techniques to identify salivary components involved in pellicle formation. They reported that salivary α -amylase, neutral cystains, high-molecular-weight salivary mucin (MG1) and secretory IgA were the major components participating in the formation of the 2-hour *in vivo* pellicle.

Gingival fluid

Apart from saliva, gingival crevicular fluid has some impact on supragingival plaque development.

Saxton (1973) found a correlation between gingival health and the rate of deposition of microorganisms on tooth surfaces. This finding was taken as an indication that the gingival exudate plays an important role as a nutrient source for plaque bacteria and for their colonization of tooth surfaces. A number of previous investigations also found that the rate of plaque formation increased with the degree of gingivitis (Goldman et al. 1974, Hillam & Hull 1977, Rönström et al. 1975, Goh et al. 1986, Quirynen et al. 1991 and Ramberg et al. 1995).

Diet

Another factor that has been implicated in plaque formation is the diet of an individual. It has been shown that dental plaque may form on human teeth in the absence of oral food intake (Howitt et al. 1928). However, diet may also modify both the amount of plaque formed and its composition. Vigorous chewing has been shown to have a beneficial plaque-reducing activity in dogs (Egelberg 1965). In man, a similarly marked effect has not been observed (Lindhe & Wicén 1969, Wade 1971). Anatomical differences between dog and human teeth appear to be the reason for this difference. Nutrients from the diet, especially a high intake of carbohydrates, were shown to promote plaque formation (Carlsson & Egelberg 1965a). Sucrose was found to be particularly significant in promoting plaque formation compared to glucose or a soft diet devoid of sucrose and cereals (Carlsson & Egelberg 1965b). Results from a number of studies on the effect of carbohydrates on plaque formation are contradictory, possibly due to the diversity of possible metabolic pathways of dietary sugar in plaque (Sheinin & Mäkinen 1971, Folke et al. 1972, Brex et al. 1981, Rateitschak-Pluss & Guggenheim 1982, Lim et al. 1986).

Physical factors

Microorganisms which have poor adhering properties can still remain on the tooth surface due to physical forces such as retention. These organisms are usually found associated with the anatomy of the tooth in cracks and fissures, and other receptive tooth surfaces as evident clinically after staining with a disclosing solution. Such factors initiating and favouring plaque growth are the curvature

of the crown of the tooth (Simonsson et al. 1987a), malposition of a tooth (Behlfelt et al. 1981), the retentive area in the dento-gingival region (Gibbons & van Houte 1975), the roughness and irregularities of enamel surfaces (Mierau 1984, Nyvad & Fejerskov 1987a, Quirynen et al. 1989), and the rough cementum surface (Nyvad & Kilian 1987), all of which provide a sanctuary for these less adherent bacteria.

In conclusion, it is evident from the discussion above that bacterial adherence on a tooth surface is a complex process which involves the interaction between bacteria surface, tooth surface and the bathing medium, saliva. Any factor present in these systems may affect microbial adherence. The current knowledge is still insufficient to understand the complex process of bacterial adherence. Hence, further research is warranted to provide more detailed and clinically relevant information, not only for the purpose of preventing dental diseases, but also for other disciplines where biofilm formation involving a fluid medium and a surface has crucial implications.

Rate of plaque formation

In one of the pioneering studies of "Experimental gingivitis in man", the authors observed that subjects who required a longer period of time to exhibit gingivitis accumulated plaque at a slower rate than those who developed gingivitis more rapidly (Theilade et al. 1966). Since then, quite a number of workers have reported similar observations related to differences in the rate of supragingival plaque formation, and yet only a few have investigated factors regulating such differences.

Ritz (1970) reported the role of aerobic *Neisseriae* in the initial formation of dental plaque. The extent of plaque development on the incisor teeth of 11 subjects was stained and photographed, along with bacterial sampling performed throughout a 5-day experimental period. The author arbitrarily divided the 11 subjects into 3 classes representing different rates of plaque formation; "rapid" plaque formers with an average of 67% of the tooth surfaces covered after 5 days (4 subjects), "intermediate" plaque formers with an average of 20% of the tooth surfaces covered (6 subjects) and "slow"

plaque formers with less than 3% plaque coverage (1 subject). The results suggested that there was a relative constancy in the rate of plaque formation as well as the level of *Neisseria* in plaque i.e. the more rapid the rate of plaque formation, the higher the level of *Neisseria* in plaque.

Listgarten et al. (1975) studied the development of dental plaque on epoxy resin crowns in man using both light and electron microscopy. Although they used the terms "heavy plaque former" and "slow plaque former", strict criteria or definition of these were not stated. Nevertheless, the authors observed in some detail the differences in the microbial samples between these two types of subjects throughout the experimental gingivitis period i.e. day 1, day 3, day 7, day 21 and 2 months. The quantity of plaque varied from practically no bacterial deposits to a well-organized bacterial mat up to 80 mm thick among different subjects on day 1. In the day 3 samples, the subject who had a well-structured bacterial plaque after day 1 showed a highly organized bacterial plaque with well-defined columnar microcolonies consisting primarily of rather large cocci. "Corn cob" formations could also be observed. Those who had little, if any, plaque deposits at the 1-day interval also failed to demonstrate substantial plaque deposits after 3 days. Similar observations were seen throughout the experimental period. At the 2-month interval, the person who had been a poor plaque former from the beginning of the experiment and whose plaque at all intervals tended to be predominantly coccoid in nature, was also the only person without spirochetes in the samples. As we can deduce from the results of this early study, there seems to be quite distinct differences in the microbial composition between the "rapid" and "slow" plaque formers.

Magnusson et al. (1976) studied the relationship between the rate of plaque formation on newly cleaned tooth surfaces and the relative concentration of the specific agglutinin for *S. mutans* serotype c, in resting whole saliva. They evaluated in five dentists the rate of plaque formation on the buccal surfaces of the upper right canines, premolars and first molars in a period 16-20 hours. Rapid plaque formers were those with visible colonies at the gingival margin after the first 16 hours. Medium plaque formers were those who developed

no visible colony within the first 16 hours but visible colonies in the left side between 16-20 hours. Slow plaque formers were those in whom, no visible colonies were found after 20 hours. Magnusson and co-workers also found that saliva collected from persons with a low rate of plaque formation had a high relative concentration of the “aggregation inducing substance” and *vice versa*. The rate at which resting whole saliva induced aggregation of a tested strain of *S. mutans* correlated well with the appearance of visible colonies *in vivo* on the cleaned tooth surfaces of the test subjects. Rapidly induced aggregation of *S. mutans* was related to slow appearance of visible colonies and *vice versa*. Results of this study tend to suggest that subjects with a high level of salivary aggregation factors are slow plaque formers.

Although the above studies reported the existence of variation in the rates of plaque formation among different individuals, little detailed information was available to understand this phenomenon. Simonsson et al. (1987a,b) were the first to investigate the rate of dental plaque formation in a more systematic manner, and attempted to give some insight through clinical studies. These authors studied in detail the clinical, biochemical, biophysical and microbiological features of plaque formation in “heavy” and “light” plaque formers.

The rate of plaque formation was determined using the plaque index (PI, Silness & Løe 1964), recorded on the buccal surfaces of premolars and first molars after 3 days of plaque accumulation. They selected, from a group of 133 subjects, a total of 12 individuals with the highest and another 12 with the lowest mean PI. In one comprehensive study (Simonsson et al. 1987a), a number of factors possibly related to plaque formation was investigated and included: i) periodontal status, ii) restorations and caries, iii) dietary habits, iv) salivary secretion rate and buffer effect, v) *S. mutans* and *Lactobacillus* counts in saliva, vi) salivary content of IgA, lactoferrin, lactoperoxidase and lysozyme, vii) saliva-induced aggregation of certain oral streptococci, viii) gel electrophoresis of saliva, ix) amino acid composition of saliva and the acquired pellicle, and x) retention depth of the dentogingival area.

On comparison of the results between the two groups of plaque formers, statistically significantly more rapid parotid saliva-induced aggregation of a strain of *S. sanguis*, lower content of glutamic acid in the acquired pellicle and smaller retention depth of the dentogingival area for maxillary premolars were found in “light” than the “heavy” plaque formers. As large variations were found for most of these factors, the authors concluded that several co-factors are involved in plaque formation and hence, none of the studied variables alone could explain the significant differences in the amount of plaque formed after 3 days between the “heavy” and “light” plaque formers.

In another study, Simonsson et al. (1987b) investigated biophysical and microbiological features of the “heavy” and “light” plaque formers. The adhesiveness of pellicle covered teeth was determined through contact angle measurements with various test liquids *in vivo*. Samples for bacteriologic investigations of 2- and 6-hour-old plaque were collected from buccal surfaces of the first and second premolars in the maxillary left or right quadrants. No statistically significant difference was observed between the two groups either for the contact angles and the calculated work of adhesion of the test liquids or the colony forming units (CFU) from 2- and 6-hour samples. Multiple linear regression analysis was performed on the results including the factors studied in the study mentioned above. The analysis revealed that 4 of the studied variables were crucial for about 90% of the variation observed in the number of CFU, for both the 2- and 6-h samples. Namely,

1. the calculated work of adhesion for two polar test liquids (glycerol and thiodiglycol) and one non-polar test liquid (propylene carbonate),
2. the rate of unstimulated salivary secretion,
3. the “retention depth” of the maxillary premolars, where the bacterial samples were taken, and
4. the aggregation of *S. mitior* TH 11 with whole, unstimulated saliva, as measured by planimetry.

When studying the early plaque growth and plaque topography in 15 young adults with healthy periodontium during a 4-day period of oral hygiene

abstinence, Quirynen and van Steenberghe (1989) found clear differences in the rate of plaque growth between tooth types, and the growth pattern seemed to be closely related to irregularities on the tooth surfaces. They also reported that the rate of supragingival plaque growth was not linear with time. For example, during a 12-h period which included over night rest, the plaque growth rate decreased 50% in some individuals when compared with the day growth.

In conclusion, although it is generally accepted that there are great differences in the rate of plaque formation among different individuals, not much is known about the qualitative differences in the supragingival plaque flora between subjects with extreme plaque formation rates. It is also not clear whether such differences occur only in the early stages of plaque formation.

Effect of surface active agents on the rate of plaque formation

Delmopinol hydrochloride (HCL) is a surface active agent with an antimicrobial effect which is 5 to 125 times lower than that of chlorhexidine digluconate against both oral and non-oral bacterial species (Simonsson et al. 1991). This low degree of antimicrobial activity has also been con-

firmed in an *in vivo* study of cell vitality of dental plaque after 4 days of mouthrinsing with delmopinol HCL (Rundegren et al. 1992). Moreover, Collaert et al. (1993) could not find a significant change in the salivary microbiological counts after rinsing with 10ml 0.2% delmopinol HCL for 2 weeks in a cohort of 45 individuals. Nevertheless, studies have shown its effectiveness against plaque formation *in vitro* and *in vivo* as well as against gingivitis *in vivo*, presumably explained by the high interfacial tension reduction property of delmopinol (Simonsson et al. 1991, Collaert et al. 1992, Moran et al. 1992).

In a delmopinol dose response study of 64 subjects (Collaert et al. 1992), the values for plaque extension over surface obtained after 2 weeks of rinsing with the placebo solution were found to vary between 16% to 90%. The response to delmopinol treatment in subjects with high plaque values obtained after placebo rinsing showed relatively less plaque extension after delmopinol rinsing than subjects who had low placebo plaque values. These comparisons were made visually using line graphs and were statistically evaluated. It seems therefore, that a surface active agent may have a differential efficacy in subjects with different plaque formation rates.

Additional questions

The above discussion raises the following questions:

1. Are there differences in the microbiological and morphological aspects of early plaque formation between “rapid” and “slow” plaque formers?
2. Will the difference of plaque formation between “rapid” and “slow” plaque formers occur only in the early stage or throughout a specific period of plaque formation?
3. Will the salivary immunoglobulin A concentration be a factor in affecting plaque formation rate *in vivo*?
4. Does a surface active agent has variable efficacy in subjects with extreme plaque formation rates?

Aims

In an attempt to answer the above questions, a series of studies were conducted with the following aims:

1. To compare the composition of microbial colonization of “rapid” and “slow” plaque formers under “experimental gingivitis” conditions.
2. To compare morphological structure of the early and subsequent plaque formation of “rapid” and “slow” plaque formers using scanning electron microscopy.
3. To compare the salivary immunoglobulin A concentration in “rapid” and “slow” plaque formers.
4. To compare the impact of a delmopinol hydrochloride mouthrinse in “rapid” and ‘slow” plaque formers.

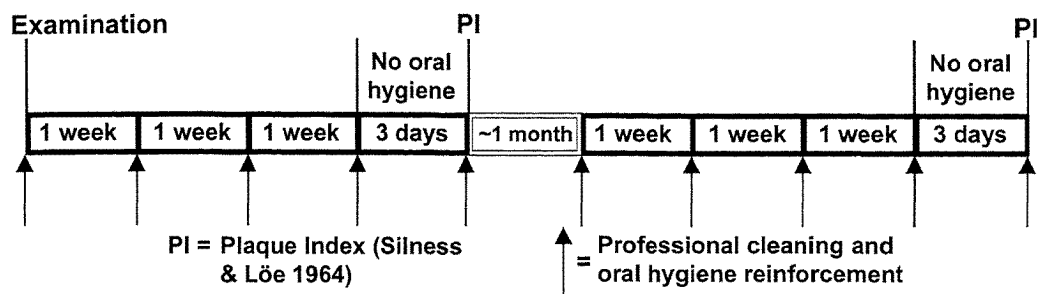


Fig. 3 Study design to determine the screening period for selecting “rapid” and “slow” plaque formers (Studies I - V)

Material and Methods

Selection of subjects

Volunteers were screened to assess their plaque formation rate after 3 days of plaque accumulation using the plaque index (PI, Silness & Løe 1964). Fig. 3 shows the study design for the selection period. Prior to this period, the subjects were examined to exclude those with extensive interproximal or buccal restorations, untreated caries or periodontal disease, treatment with antibiotics and smokers. Suitable subjects entered a 3-week oral hygiene period, comprising visits once a week for professional cleaning and oral hygiene instructions. At the start of the plaque accumulation periods, gingival health of each volunteer was confirmed by absence of gingival cervical fluid (GCF) using standardized paper strips according to Løe and Holm-Pedersen (1965). PI on the buccal surfaces of selected teeth were recorded. This procedure was repeated after 1 month to confirm the results from the first period, after which mean 3-day plaque scores were calculated for each individual.

There were differences in the procedures employed for the selection of subjects between Studies I - IV and Study V as follow:

Number of volunteers

For Studies I-IV, a total of 49 subjects volunteered for the study. They were dental students, dental surgery assistants and dental hygienists from the Prince Philip Dental Hospital, Hong Kong. All of them were born in Hong Kong and were ethnic Chinese. For Study V, a total of 71 dental students, postgraduate students and personnel at the Center

for Oral Health Sciences in Malmö, Sweden joined the selection procedures.

Number of teeth used to score PI

For Studies I-IV, all buccal surfaces of canines, premolars and 1st molars were used and the number of recorded surfaces for each individual was thus 16. For Study V, buccal surfaces of all premolars and 1st molars were used amounting to 12 recorded surfaces.

Number of “rapid” and “slow” plaque formers selected

For Studies I-IV, 5 “rapid” plaque formers with highest mean PI scores and 6 “slow” plaque formers with lowest mean PI scores were selected. 12 subjects with highest mean plaque scores and 11 with lowest mean plaque scores were selected in Study V.

Study I

Sampling and microbiological procedures

Before the start of the 14-day experimental gingivitis period, the selected 11 subjects once again went through a 3-week professional tooth cleaning regimen as described above to ensure gingival health (Fig. 4). At Day 0, GCF was taken to confirm the gingival health of all the subjects and a thorough professional tooth cleaning was done to make sure that no plaque was present on the sampling surfaces. The 14 days of no oral hygiene period started thereafter.

A single plaque sample was collected on day 1, day 3, day 7 and day 14 from the buccal surface of

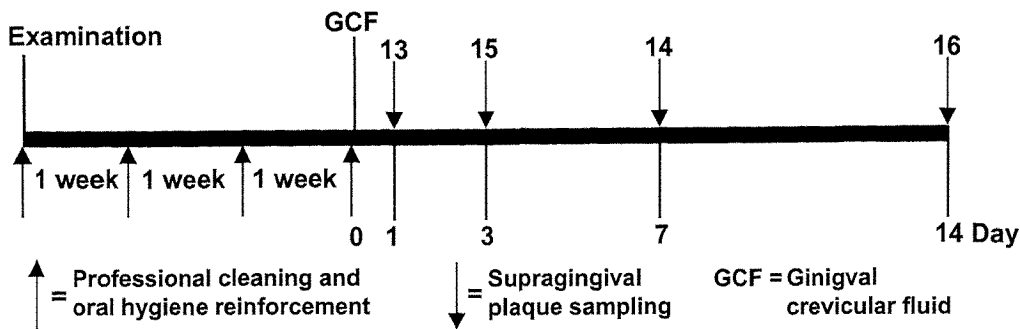


Fig. 4. Clinical schedule employed for supragingival plaque sampling.

upper right canine, 2nd premolar, 1st premolar and 1st molar, respectively. A sterile curette, passing along the gingival margin, was used to remove as much supragingival plaque as possible. The sample was then dispensed into small screw-capped bottles filled with sterile tryptic soy broth (TSB; Difco Laboratories, Detroit, U.S.A.) and delivered to the laboratory for processing within 30 min. In the laboratory, the samples were vortexed for 30s, serially diluted and spiral plated onto Columbia blood agar base (Difco Laboratories) supplemented with 5% defibrinated horse blood, 5 mg/l hemin and 500µg/l menadione (CBABS). The plates were incubated anaerobically at 37°C for 3-4 days in an anaerobic chamber supplied with a gas mixture of 80% N₂, 10 % H₂ and 10% CO₂. A region with ~40 well separated colonies were marked and 20-30 different colonies were subcultured on CBABS to obtain pure isolates. The pure isolates were identified according to their colony and cell morphology, Gram stain reaction, oxygen requirement and biochemical tests (using the RapID STR, ANA II and NH system, Innovative Diagnostic Systems Inc., Atlanta, G.A.). Gram-positive and gram-negative anaerobic bacteria still unidentified by these means were subjected to gas liquid chromatography for identification.

Study II

Preparation of enamel blocks

Human enamel blocks fixed on the buccal surfaces of the teeth of each volunteer were used to investigate plaque formation. Each enamel piece measuring approximately 2x2x1 mm was sectioned from the intact buccal or lingual surfaces of an extracted tooth stored in thymol. After sectioning, the enamel

pieces were cleaned, sterilized and stored in distilled water before use.

Experimental and sampling procedures

The morphological investigations were conducted over a 14-day period. At first the selected 11 subjects went through a repetition of once a week prophylaxis and oral hygiene instructions for 3 consecutive weeks to ensure gingival health (Fig. 5). Dietary sheets were issued to all 11 subjects for recording every meal during the 14-day period. On Day 0, gingival health of the subjects was confirmed by the absence of gingival crevicular fluid (GCF) flow according to Løe and Holm-Pedersen (1965) and a thorough professional cleaning was done to remove observable plaque deposits on tooth surfaces.

Two enamel pieces were then bonded with composite resins (APH, Dentsply International Inc., Milford, USA.) onto each buccal surface of upper left canine, premolars and the first molar, giving a total of eight *in situ* enamel pieces per subject. All the enamel blocks were carefully bonded slightly coronal to the gingival margin in order to prevent plaque that accumulated at the margins extending onto the enamel blocks. When all the enamel pieces were attached, the subjects started a 14-day experimental gingivitis period during which they refrained from all oral hygiene measures.

Thus, after allowing for undisturbed microbial colonization on the enamel pieces, they were retrieved sequentially from the left first molar, first premolar, canine and second premolar at 3-hour, 6-hour, 1-day, 3-day, 7-day and 14-day intervals, respectively; the remaining pieces were removed on day 14 (see Fig. 5). Prior to detachment, each enamel piece was rinsed gently with distilled water. Finally,

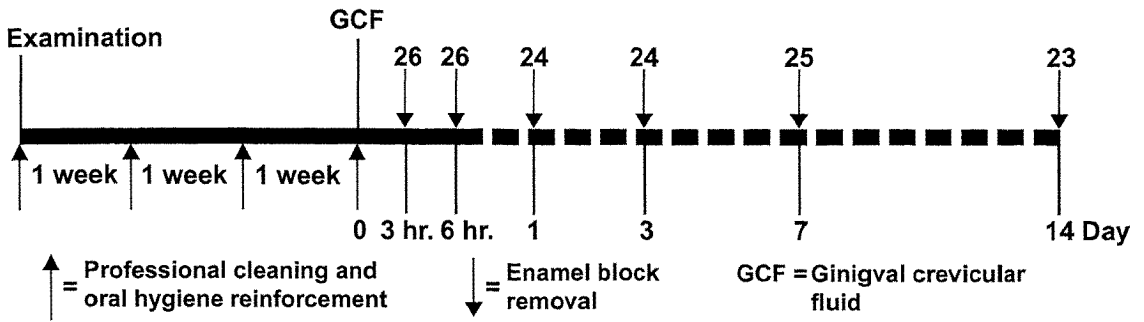


Fig. 5. Clinical schedule employed for removal of enamel blocks.

the residual composite resins were removed and a thorough prophylaxis performed on each volunteer.

Specimen preparation

Immediately after detachment, the enamel pieces were placed for a minimum of 24 hours in a plastic chamber saturated with a fixative of 25% glutaraldehyde and 10% paraformaldehyde in 0.2M cacodylate buffer (adjusted to pH 7.4) (Karnovsky 1965). The enamel pieces were then rinsed 4 times in 0.1M cacodylate buffer (pH 7.4) and immersed in 1% osmic acid in 0.1M cacodylate buffer for 30 minutes, followed by 4 rinses in cacodylate buffer. Subsequently, the pieces were dehydrated by serial transfer in ascending concentrations of acetone (50-100%), critical point dried in CPD 020 (Balzers Union, Liechtenstein, Germany), mounted on copper stubs and sputter coated with gold to a thickness of approximately 15nm (Fine Coat Ion Sputter JFC-1100, JEOL Ltd., Tokyo, Japan). All the samples were examined with a scanning electron microscope (JXA-840, JEOL Ltd.) at 5-10 kV. Micrographs were taken at 100X and 1000X magnifications for all enamel pieces and at higher magnifications whenever appropriate.

Study III

Replica preparation

Before the start of the 14-day experimental gingivitis period, the selected 11 subjects went through once a week prophylaxis and oral hygiene instruction for 3 weeks to ensure gingival health. At day 0, GCF was taken to confirm the gingival health of all the subjects and a thorough professional cleaning was done to remove observable plaque deposits on tooth surfaces. During the 14-day experimental gingivitis period, undisturbed plaque accumulation was facilitated by refraining from all oral hygiene measures, although the subjects were advised to maintain their routine dietary habits.

The buccal surface of the upper right central incisor of each subject was replicated using the method of Lambrechts et al. (1982). A light body silicone elastomeric impression material (President, Coltène® AG, Altstätten, Switzerland) was used. Impressions were taken on day 0, 1, 3, 7 and 14 after the tooth surface was thoroughly rinsed with distilled water and gently dried by air (Fig. 6).

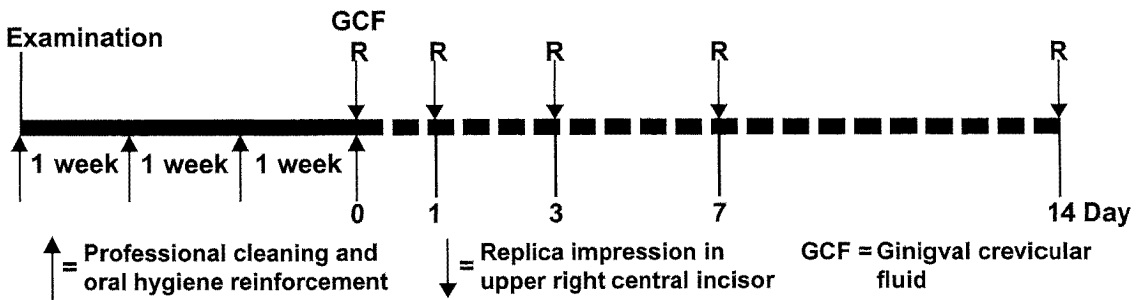


Fig. 6. Clinical schedule employed for obtaining replica impressions.

The polymerized impression was then removed from the tooth surface and rinsed sequentially in running tap water and distilled water. After air drying, epoxy resin (Taab 812, Taab Laboratory Equipment, England) was poured onto the impression to produce a positive replica within 3 hours. The resin replica was polymerized for 18 hours at 60°C in an oven and subsequently sputter coated with gold for 8 minutes in an ion sputter coater (JFC-1100, JEOL Ltd., Tokyo, Japan). All the replicas were examined with a scanning electron microscope (JXA-840, JEOL Ltd.) at 5-10 kV. Micrographs were taken at 500X to 4000X magnifications at 8 sites in each replica as shown in Fig. 7 so that similar areas could be compared within and between different subjects. Micrographs from other areas were also taken whenever appropriate.

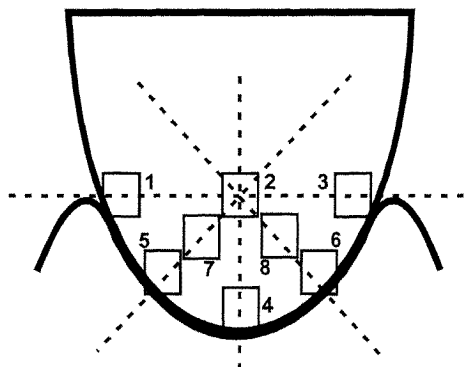


Fig. 7. A schematic diagram of the eight regions of the tooth surface examined using the replica technique and scanning electron microscopy.

The extent of the plaque colonization on each tooth surface was quantified using the replicas with the help of an image analyzer (IBAS2000, Lecia, Germany) and expressed as the percentage of the tooth surface covered by at least a monolayer of plaque. Image analysis was performed by a single examiner who was unaware of the specimen groups.

Study IV

Saliva collection

The subjects were asked to expectorate unstimulated saliva into a ice-chilled beaker at least one hour after a meal. After collection, the saliva was immediately delivered to the laboratory for

centrifugation at 10 000g for 15 min., and the supernatants were stored in Eppendorf tubes at 70°C. Before analysis, the samples were thawed at room temperature.

Determination of salivary IgA

Polystyrene microtiter plates were coated with goat anti-human IgA (Sigma BioSciences, St. Louis, USA) by adding 200µl of antiserum diluted 1:10 000 in phosphate buffered saline (PBS; pH 7.3) per well. The plates were then wrapped in plastic sheets and kept at room temperature overnight. Before use, the plates were washed 3 times in water containing 0.15M NaCl, 0.5% (v/v) Tween 20 and 0.02% (w/v) sodium azide in an automatic ELISA washing machine (Tri-Continent Scientific Inc., Grass Valley, USA).

Reference standard curves for IgA were established by testing serial dilutions of purified human colostrum IgA (Sigma BioSciences). The IgA was diluted with PBS containing 5mg/ml bovine serum albumin (PBS/BSA) plus 0.02% sodium azide, and 200µl was applied per well. The plates were incubated at 37°C overnight.

Test samples of whole saliva were diluted 1:10, and 200µl was applied per well. After overnight incubation as described above, the plates were washed and 200µl of goat anti-human IgA alkaline phosphatase conjugate (Sigma BioSciences) diluted 1:28 000 in PBS/BSA was applied per well. The plates were incubated at 37°C for 2 hours, washed 5 times and incubated with 1.0 mg/ml *p*-nitrophenyl phosphate (*p*NPP, Sigma BioSciences) in 10% diethanolamine buffer, pH 9.8 with 0.5 mM MgCl₂ in the dark for 30 minutes at room temperature. The colour change was then read in a spectrophotometer (SpectraMax 340, Molecular Devices Corporation, California, USA) at 405nm. Each plate included duplicate negative controls (coated wells reacted with PBS/BSA), duplicate references (coated wells reacted with standard IgA), duplicated standard IgA in uncoated wells and triplicate test samples. Results of the concentration of salivary IgA were calculated from the reference curve and expressed as µg/ml. Repeated analyses of the same samples were performed after 1 week in order to test the reproducibility.

Study V

Study design

The study was double-blind with parallel design between the “rapid” and “slow” plaque formers and cross-over design between two active treatment periods and a placebo period. Each test period was 5 days as illustrated in Fig. 8. Once a week professional cleaning was provided for 3 consecutive weeks before the start of the test periods. During the test periods the subjects were asked to discontinue their normal oral hygiene procedures. Un-supervised rinsing with 10ml of the mouthwash was performed for 60 seconds in the morning and in the evening. The subjects were instructed not to eat within 30 minutes after rinsing. The placebo solution was an aqueous solution containing a herb flavor (eucamint 0.02%), sodium saccharine 0.01% and ethanol 1.5%. The two active solutions were similar to the placebo except both with delmopinol (either 0.1% or 0.2%) and sodium hydroxide (at pH=5.7) added. The test solutions were supplied in identical 200ml glass bottles with only subject codes marked. Remaining test solutions in the bottles were measured to monitor the compliance of the subjects. Neither the examiner nor the subjects knew whether the test solutions were active or placebo. The subjects were assigned to treatment groups according to a computer-generated randomized list.

Plaque assessment

Supragingival plaque was disclosed with erythrosine at the end of each rinsing period (Fig. 8). PI was scored for buccal and lingual surfaces of all teeth, except 3rd molars. Standardized color photographs for planimetric analyses of plaque extension were taken from the canines and premolars (Carlsson et al. 1965b). They were enlarged 6.5 times and projected onto white paper. The extension of the stained plaque on the canines and premolars were measured with computerized digitization and expressed as relative area units.

Gingivitis assessment

Gingival crevicular fluid (GCF) was collected with a standardized filter paper strip at the start and the end of the each rinsing period according to Löe and Holm-Pedersen (1965). All the buccal surfaces of upper canines and premolars were sampled. After collection, the filter paper strips were mounted on glass slides and stained with ninhydrine. The stained length on the paper strips was then measured with computer digitization. Bleeding on probing (BOP) was recorded after gentle probing with a WHO-probe to the bottom of the gingival crevice at 6 sites around each tooth viz: mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual (Fig. 8). All clinical assessments were made by the same examiner (ZKY).

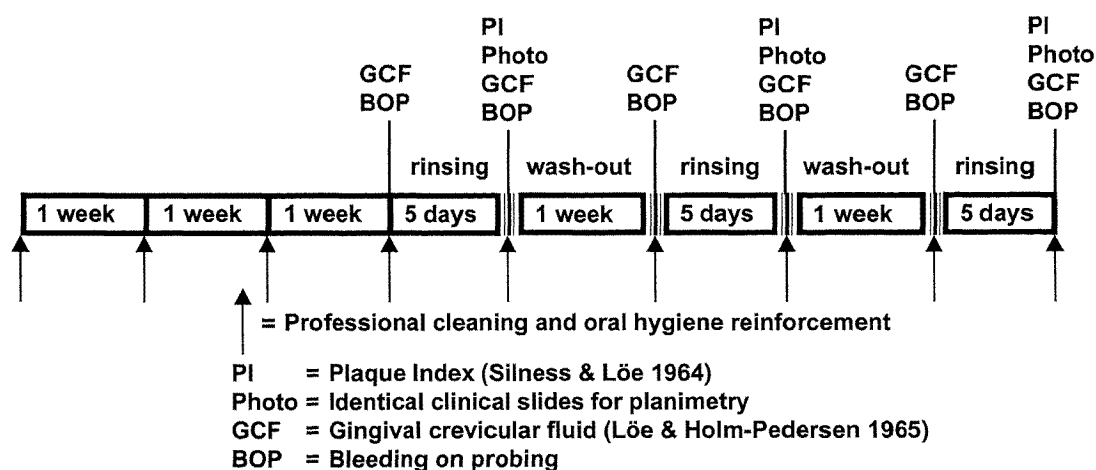


Fig. 8. Experimental design of the delmopinol study.

Statistical analyses

Selection of Subjects

The plaque forming capacities of the “rapid” and “slow” plaque formers in the first and second 3-day plaque accumulation periods were compared using the Wilcoxon Signed Ranks Test.

Study I

Comparison of the proportion of the different groups of bacteria between the “rapid” and “slow” plaque formers were performed by MANOVA test with “group” and “day” as the crossed factors and “subject” as the nested factor using the SAS software package (SAS Institute, Cary NC, U.S.A). Groups were regarded as significantly different from each other if $p < 0.05$.

Study III

The extension of the plaque as measured on the replica surfaces from the “rapid” and “slow” plaque formers was compared using Student’s t -test.

Study IV

Differences between the results of the repeated measurements were tested using paired t -test while differences in the mean concentration of the salivary IgA between the two groups of plaque formers were compared using Student’s t -test.

Study V

All statistical analyses were performed with non-parametric tests. Wilcoxon rank sum test was used to test the difference between rapid and slow plaque formers using the percentage of placebo response. No adjustment for multiple comparisons of the calculated p -values was performed.

Ethics

Permissions for Studies I - IV were sought and granted by the Ethics Committee of the Faculty of Dentistry, The University of Hong Kong. Study V was approved by the local Ethics Committee at the University of Lund and by the Swedish National Board of Health and Welfare. Prior to each study, every subject was given full verbal and written in-

formation regarding objectives, the procedures, and possible risks involved. Informed consent was obtained from all the subjects.

Results

Selection of “rapid” and ‘slow” plaque formers

Studies I-IV

The mean PI of the 49 subjects after 3 days of no oral hygiene ranged from 0.84 to 2.63. For the 5 “rapid” plaque formers, the mean PI was 2.5 (range: 2.41 - 2.63). The mean PI of the 5 “slow” plaque formers was 1.02 (range: 0.84 - 1.16). Differences in the mean PI from the first and second 3-day plaque accumulation periods of the 11 subjects were not statistically significant ($p = 0.3552$). Fig. 9 shows the percentages of tooth surfaces with different PI scores in both groups during the two plaque accumulation periods. The mean PI of the unselected 38 subjects was 1.74 (range: 1.19 - 2.31).

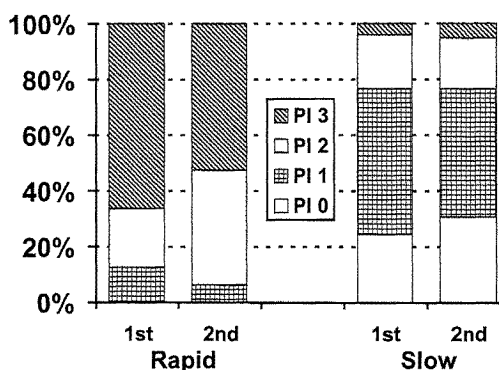


Fig. 9. Percentage of tooth surfaces and the corresponding plaque indices for “rapid” (80 surfaces) and “slow” (94 surfaces) plaque formers, in the first and second 3-day plaque accumulation periods (Studies I - IV).

Study V

The mean PI score obtained from the two 3-day plaque accumulation periods of the 71 subjects ranged from 0.08 to 2.88. The mean PI score of the 11 “slow” plaque formers was 0.67 (range: 0.08-0.96) while that of the 12 “rapid” plaque formers was 2.09 (range: 1.75-2.88).

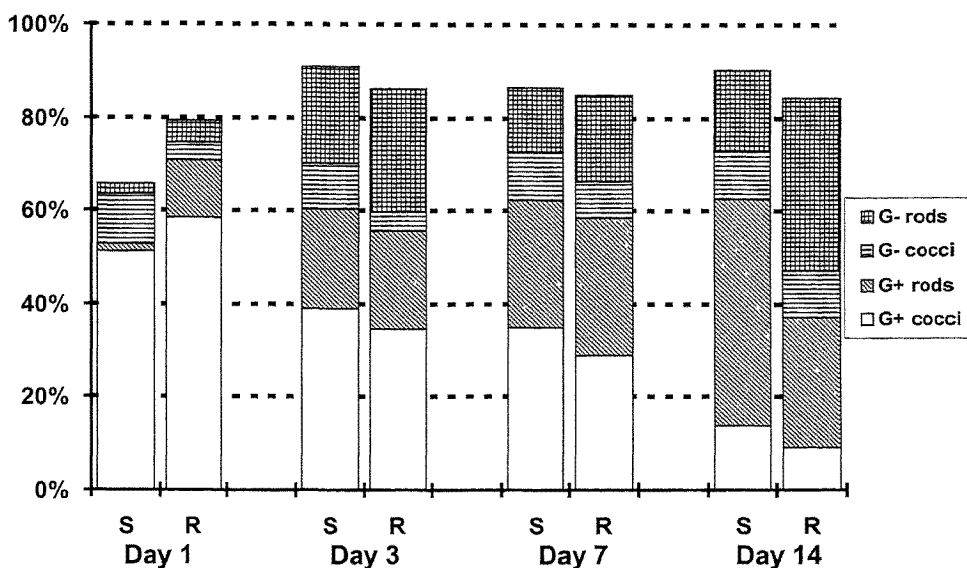


Fig. 10. The mean proportions of predominant cultivable bacteria in supragingival plaque samples of "slow" (S) and "rapid" (R) plaque formers during the 14-day period of no oral hygiene.

Supragingival plaque flora of "rapid" and "slow" plaque formers

A total of 44 plaque samples were obtained for microbiological analysis from the 11 subjects and the number of samples from the "rapid" and "slow" plaque formers were 20 and 24 respectively. After purification, 1 095 isolates were cultured from the 44 plaque samples for identification. However, only 83.5% were speciated and the rest were lost on sub-culture and unable to be speciated.

Results showed similar trends in plaque development in the two groups (Fig. 10). Gram-positive bacteria were the predominant cultivable species from day-1 to day-7 ("rapid": 71↔58%; "slow": 53↔62%) and gram-negative species increased in percentage with the plaque age ("rapid": 8↔47%; "slow": 13↔28%). "Slow" plaque formers showed a lower proportion of gram-positive bacteria than "rapid" plaque formers on day-1 but overtook the "rapid" plaque formers from day-3 to day-14. The proportion of gram-negative bacteria was higher in the "slow" plaque formers than the "rapid" plaque formers at day-1 but as the proportion of gram-negative rods increased in the "rapid" plaque formers, the overall proportion of gram-negative

bacteria (47%) became higher than that of gram-positive (37%) at day-14. The difference in the mean relative proportion of the gram-positive and gram-negative bacteria between the "rapid" and "slow" plaque formers were statistically significant ($p = 0.0162$). There was no statistical significant difference in the mean relative proportions of gram-positive and gram-negative bacteria, between the subjects within the same group ($p = 0.4055$).

Streptococcus spp. were the most predominant cultivable gram-positive species whereas *Actinomyces* spp. were the most common gram-positive rod identified in both groups (Fig. 11). Initially the proportion of the *Actinomyces* species increased in both groups, but on day-14, its proportion in the "slow" plaque formers was higher than that of the "rapid" group. *Veillonella dispar* and *Neisseria mucosa* were the only gram-negative cocci found in all the samples with the *Veillonella* spp. being the predominant type throughout the 14-day period. *Fusobacterium* spp. and *Capnocytophaga* spp. were the two most predominant cultivable gram-negative rods. There were some black-pigmented *Bacteriodes* spp. identified, but only in small numbers.

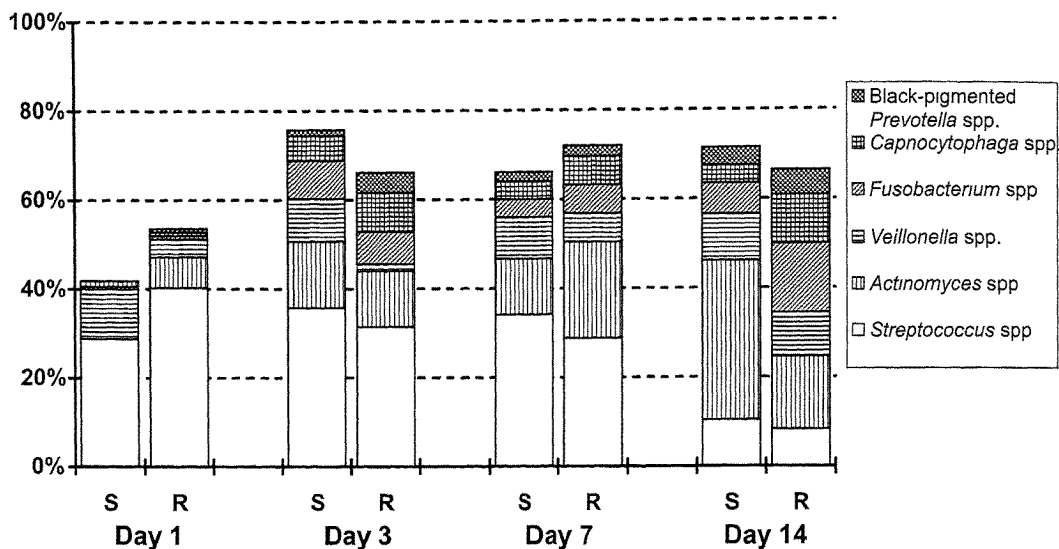


Fig. 11 Comparison of the percentage change in major bacterial species in supragingival plaque samples of "slow" (S) and "rapid" (R) plaque formers during the 14-day period of no oral hygiene.

Morphological features of "rapid" and "slow" plaque formers

Scanning electron microscopy of supragingival plaque on enamel blocks (Study II) and on replicas (Study III) showed that "rapid" plaque formers attained a relatively more complex supragingival plaque flora than the "slow" plaque formers. The day-1 enamel block specimens of the "slow" group showed a less complex supragingival plaque structure than the "rapid" group (Fig. 12a & e). From day-3 to day-14, during the maturation period of supragingival plaque, no discernible differences were noted between the two groups (Figs. 12b, c, d, f, g & h). The morphological features as observed on the day-1 and day-3 replicas showed that the supragingival plaque structure of "slow" group was less complex than the "rapid" group (Figs. 13a, b, e & f). Both the enamel blocks and replicas showed, no discernible differences between the two groups from day-7 to day-14 (Figs. 13c, d, g & h).

Rough textured, globular deposits were observed which coalesced to form a monolayer of confluent bacterial growth eventually developing into a continuous undulating surface throughout the 14-day study period in both the "slow" and "rapid" plaque formers (Figs. 14a & b). The surfaces of these globular structures were covered by bacteria (Figs. 14c & d). Under higher magnification, it was apparent that they comprised bacteria embedded in an intermicrobial matrix (Fig. 14e & f).

Extension of Plaque

The mean percentage plaque covered tooth area as observed on the replica surfaces (Study III) during the 14-day period of oral hygiene abstinence is shown in Fig. 15. The most obvious difference in the plaque coverage was observed on day-1 where the area of microbial growth in the "rapid" plaque formers was almost 24 times greater than that of the "slow" group. However, this initial difference in the plaque coverage between the two groups gradually decreased and on day-7 and day-14, when similar percentage coverage was observed in both groups.

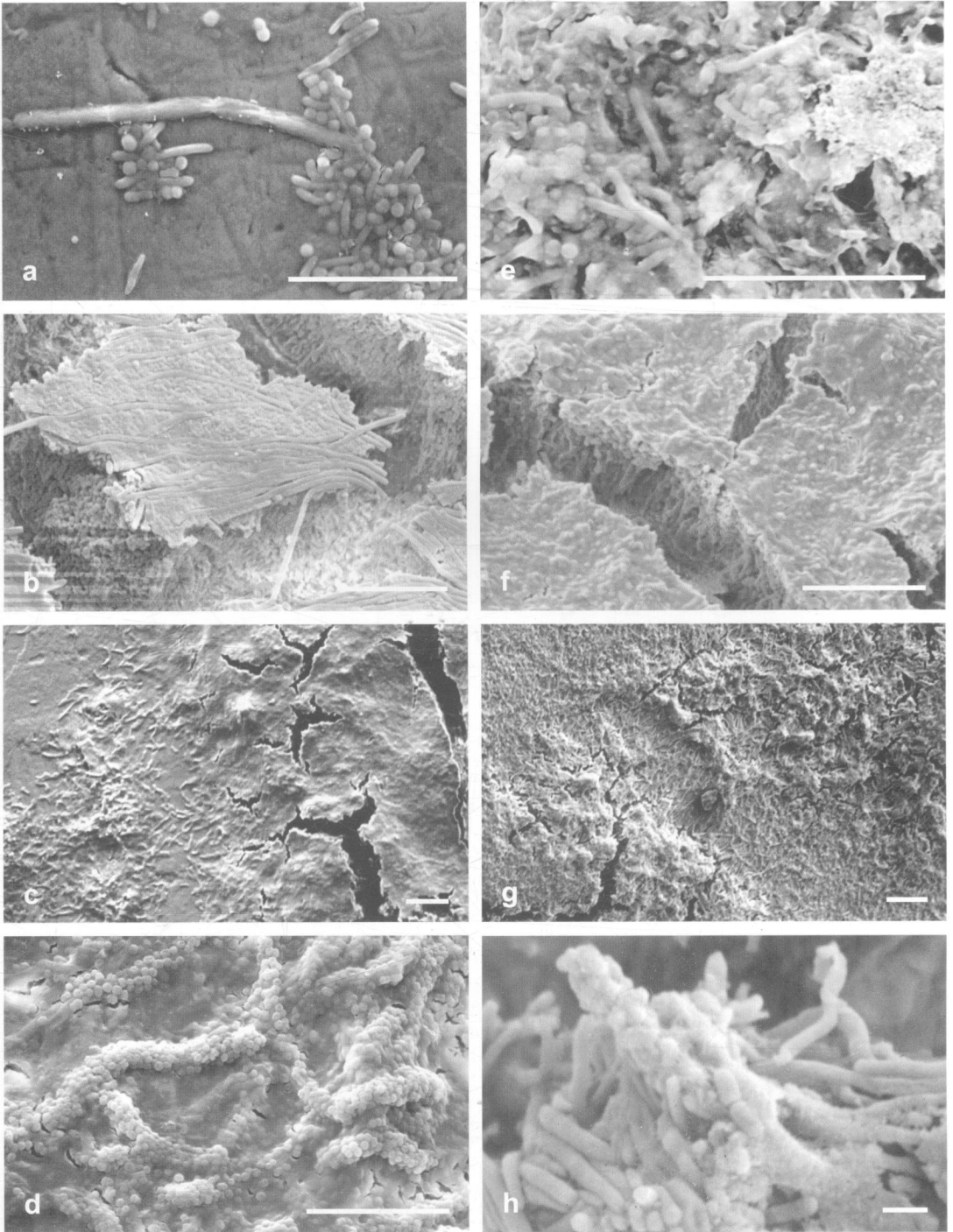


Fig. 12. Scanning electron micrographs of enamel blocks retrieved from “slow” (a-d) and “rapid” (e-h) plaque formers on day-1 to day-14 of the undisturbed plaque development period. All bars represent 10 μm except h (bar = 1 μm). (a) Day-1 block: coccoid and rod-shaped bacteria attached onto the enamel surface. (b-c) & (f-g) Day-3 to day-7 blocks: a thick layer of bacteria with a complex structure. (d) & (h) Day-14 blocks: corn-cob formation in which, coccoid forms were attached to rod-shaped bacteria, indicating a mature plaque flora.

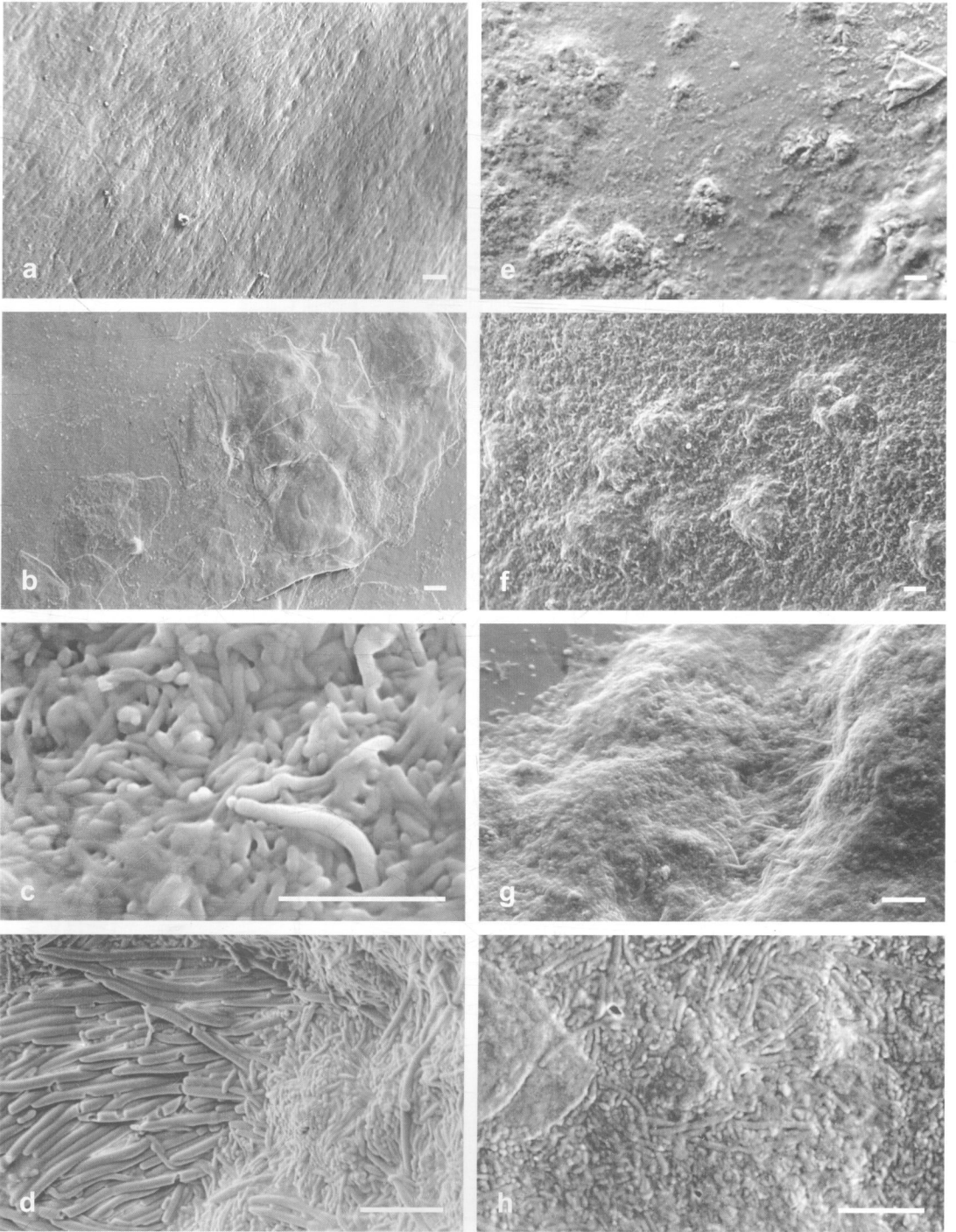


Fig. 13

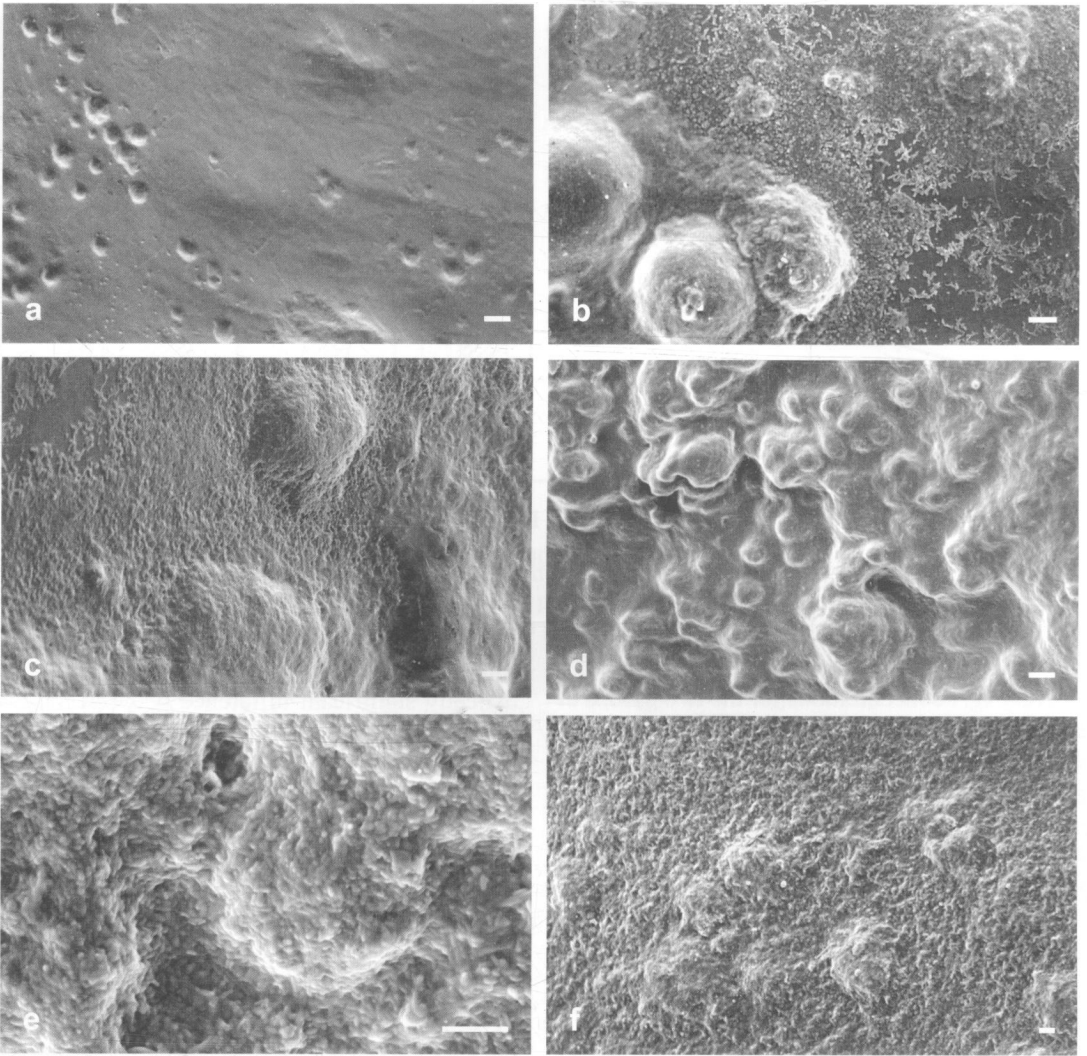


Fig. 14

Fig. 14. Scanning electron micrographs of replicas obtained from “slow” and “rapid” plaque formers on day-1 to day-14 of the undisturbed plaque development period. All bars represent 10µm. (a) Discrete globular deposits ranging in size from 1-10 µm could be seen on day-1. (b) Globular deposits spread widely and coalesced with monolayers of bacteria. (c) - (f) Bacteria could be seen on the surface of the globular deposits and embedded in an intermicrobial matrix.

Fig. 13 (left side). Scanning electron micrographs of replicas obtained from “slow” (a-d) and “rapid” (e-h) plaque formers on day-1 to day-14 of the undisturbed plaque development period. All bars represent 10 µm. (a) Day-1 replica: scratch marks with very few bacteria. (b) Day-3 replica: epithelial cells attached to the tooth surface with discrete bacteria. (e) Day-1 replica: monolayers of bacteria and globular deposits were observed on the tooth surface. (f) Day-3 replica: globular deposits coalescing with monolayers of bacteria forming an undulating surface. (c-d) & (g-h) Day-7 & Day-14 replicas: rod-shaped and filamentous bacteria running in different directions.

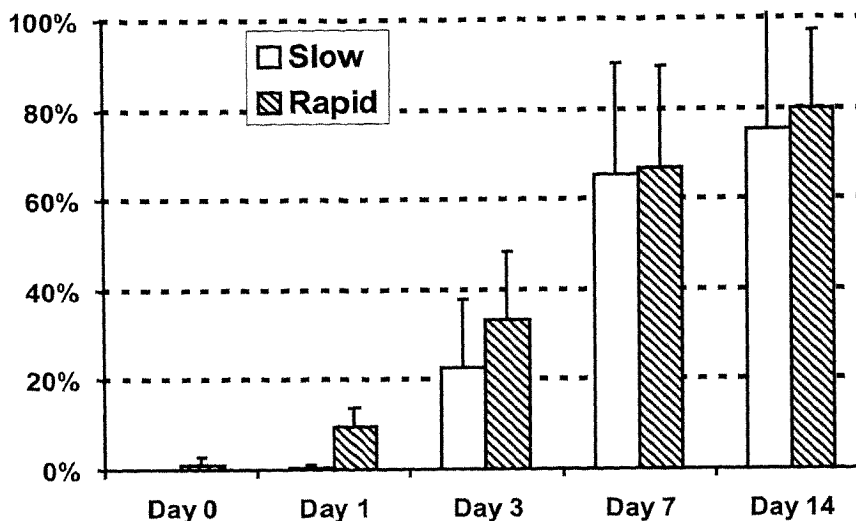


Fig. 15. Relative percentage plaque coverage detected on the surfaces of the replicas taken from the "slow" and "rapid" plaque formers during the 14-day period.

Salivary immunoglobulin A levels

Salivary IgA concentrations for the two groups of plaque formers as measured on two separate occasions are shown in Table 1. "Rapid" plaque formers showed a statistically significantly lower mean concentration of salivary IgA (9.2/9.0 µg/ml) than the "slow" plaque formers (15.6/16.8 µg/ml; $p < 0.05$). There was no significant difference between the first and the repeat analysis of the samples.

Effect of delmopinol hydrochloride mouthrinses

The PI, plaque extension, GCF and BOP results of the "rapid" and "slow" plaque formers using 0.1

%, 0.2% delmopinol, and placebo after 5 days (Study V) are shown in Fig. 16. There was no statistically significant difference between the two groups for any of these parameters.

Discussion

Selection of subjects

The method used to select "rapid" and "slow" plaque formers in the present study is based on the work of Simonsson et al. (1987a & b). The method included a 3-week oral hygiene period, comprising once a week professional cleaning and oral hygiene instruction, followed by 3 days of undisturbed plaque accumulation. The 3-day duration was chosen since Lindhe and Rylander (1975) showed that

Table 1. Mean Salivary IgA concentrations (µg/ml ± S.D.) of the "rapid" and "slow" plaque formers as measured by ELISA on two different occasions. (n.s. = not statistically significant)

	1 st Measurement	2 nd Measurement	<i>p</i> value
"Rapid" group	9.20±2.59	9.00±1.87	0.3974 (n.s.)
"Slow" group	15.60±4.04	16.80±6.30	
<i>p</i> value	0.0175		

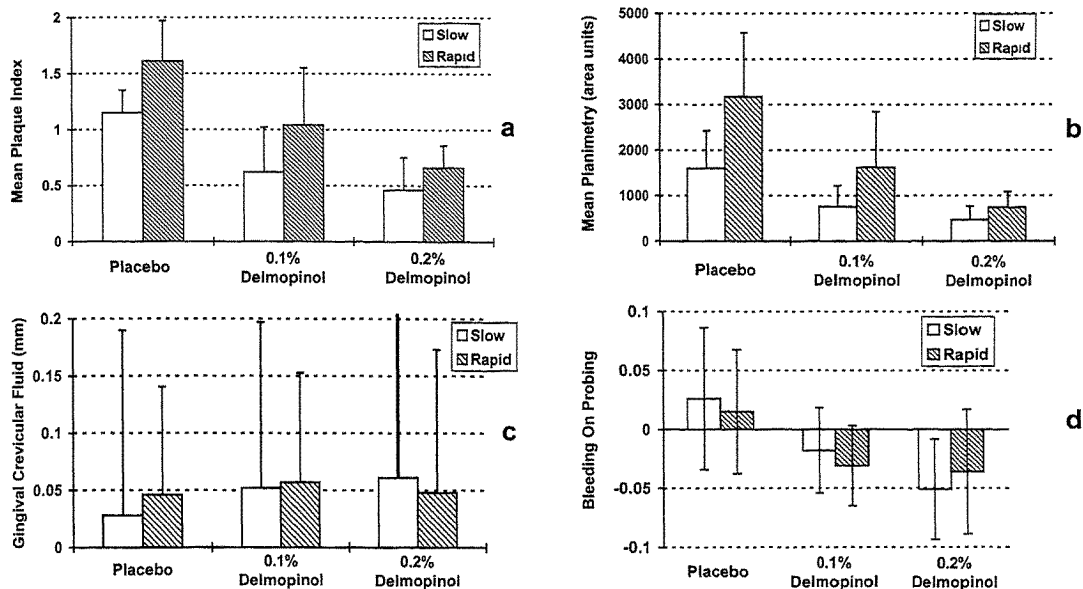


Fig. 16. Variations in the four different parameters studied in the “slow” and “rapid” plaque formers after 5 days of rinsing with placebo, 0.1% and 0.2% delmopinol HCL. a. plaque index, b. buccal plaque extension (in arbitrary area units), c. gingival crevicular fluid (in mm) d. bleeding on probing (none showed p levels < 0.5 between the “slow” and “rapid” groups).

gingival exudate can first be retrieved in noticeable amounts after four days of plaque formation. It is therefore less likely that gingival fluid would have influenced the plaque accumulation during the 3-day period.

In order to ensure that subjects with extreme rates of plaque formation were selected, plaque accumulation rates were determined on two separate occasions. Statistical analyses showed that there was no significant difference between the two periods of plaque accumulation for the selected “rapid” and “slow” plaque formers.

Diet is considered an important factor affecting plaque formation. To ensure uniformity of plaque formation rate among subjects, they were requested to adhere to their usual diet. Dietary sheets were then given to the 11 subjects of Studies I – IV to monitor their dietary intake throughout the experimental gingivitis period. Results (not presented here) showed that all participants maintained a basic Chinese diet with rice as the staple carbohydrate, supplemented by vegetables and proteins in smaller amounts and with few or no sugary snacks between meals.

The present thesis evaluated only 5 “rapid” and 6 “slow” plaque formers in Studies I - IV, which are

small numbers of subjects. One reason for this was due to the fact that only 49 subjects volunteered to participate in the screening of “rapid” and “slow” plaque formers. If more subjects had been selected from the 49 volunteers, it would inevitably incorporate some with non-extreme plaque formation rates thus making the data interpretation very complex. The other reason was the limitation in handling the samples in Study I. A total of 11 samples were cultivated for the predominant bacterial species in each sampling occasion. The subsequent subculturing and identification together with the following samples taken within the 14-day period involved a vast amount of work. Hence, with such a small number of subjects, it is important to bear in mind that individual variations may affect the results.

Microbial flora of supragingival plaque

Study I is the first attempt at investigating the supragingival plaque formation in Chinese subjects with different rates of plaque formation, using the experimental gingivitis model. Results of the study are basically in broad agreement with previous reports using similar methodology. Gram-positive bacteria were the predominant cultivable species in early plaque, and the proportion of gram-nega-

tive bacteria increased with the plaque age (Socransky et al. 1977, Syed & Loesche 1978, Moore et al. 1982, Moore et al. 1987). However, in Study I previously unreported differences were found between the two groups. Thus, the "slow" plaque formers showed a statistically significantly higher proportion of gram-positive bacteria and a lower proportion of gram-negative bacteria, when compared with the "rapid" plaque formers, from day-3 to day-14. The mean proportion of the gram-positive bacteria in the "slow" group remained at around 60% from day-3 to -14, while that of the "rapid" group showed a reduction in these organisms, from 56% to 37%. This may suggest that the "rapid" plaque formers tend to attain a complex flora earlier than the "slow" plaque formers. All of the comparisons between the two groups were based on the relative proportions of different species. Whether the absolute counts of the different species would show a similar trend remains to be determined.

It is important to note that the longitudinal supragingival plaque samples taken during the 14-day period were from different sites. Whether the differences in the surface characteristics and position of the different sites will affect the composition of the bacterial flora as obtained from this study is not known.

In one study on the role of aerobic *Neisseriae* in the initial formation of dental plaque, Ritz (1970) reported a positive correlation between the rate of plaque formation and the level of *Neisseria* in plaque i.e., the more the rapid rate of plaque formation, the higher the level of *Neisseria* in the plaque. In the present study, *Neisseria* was not a major component of the plaque flora of all subjects, and we were unable to detect any differences between the "rapid" and "slow" plaque formers. This discrepancy may be explained by our use of a non-selective medium, and/or a true difference in supragingival plaque composition in our subjects.

Morphological Features

The following plaque formation patterns were observed from the results of both groups of subjects in Studies II and III. The bacteria first colonised the enamel surface via a surface coating material

(Fig. 17a) and occasionally as individual organisms retained in pits and grooves (Fig. 17b), followed by the formation of micro-colonies (Fig. 17c). These proliferated to form larger colonies, coalesced and spread out to form a monolayer of cells (Fig. 17d). Similar observations have been reported in other electron microscopic studies (Lie 1979, Lie & Gusberti 1979, Nyvad & Fejerskov 1987a). Study II & III also showed that "rapid" plaque formers attain mature plaque morphology earlier than the "slow" plaque formers during the initial 1 – 3 days of undisturbed plaque formation (Figs. 17e & f). However, the morphological features of day-7 to day-14 plaque in both groups were remarkably similar (Figs. 17g & h). This indicates that the differences in the rate of plaque formation in these two groups arise mainly as a result of early rather than late plaque development.

The most prominent microscopic feature reported in Study II was the presence of globular deposits throughout the 14-day study period in both the "slow" and "rapid" plaque formers (Figs. 14a & b). This phenomenon has been reported by previous workers using the replica method (Saxton 1971, 1973 & 1976, Sheinin et al. 1971, Lie et al. 1978, Lambrechts et al. 1982), whereas others who used different materials and scanning electron microscopy did not observe such deposits (Berthold et al. 1971, Eastcott & Stallard 1973, Nyvad & Fejerskov 1987a, Walsh et al. 1991). Saxton (1973) suggested that the globular deposits are likely to be bacterial aggregates attached to the tooth surfaces, while Lie & Gusberti (1978) surmised them to be artifacts due to air bubbles or moisture derived from saliva or gingival exudate. According to the current observations, the surfaces of these globular structures were always covered by bacteria (Figs. 14c & d), and under higher magnification, it was apparent that they comprised bacteria embedded in an intermicrobial matrix (Fig. 14e). We are unable to agree that such globular deposits are randomly distributed focal bacterial aggregates as proposed by Saxton (1973). On the contrary, we propose that the globular deposits are first formed by bacteria which attach onto pits and grooves of the enamel in the initial plaque formation stage. These pioneer bacteria appear to proliferate and develop into micro-colonies and eventually coalesce with the monolayers of neighboring bacteria on the subja-

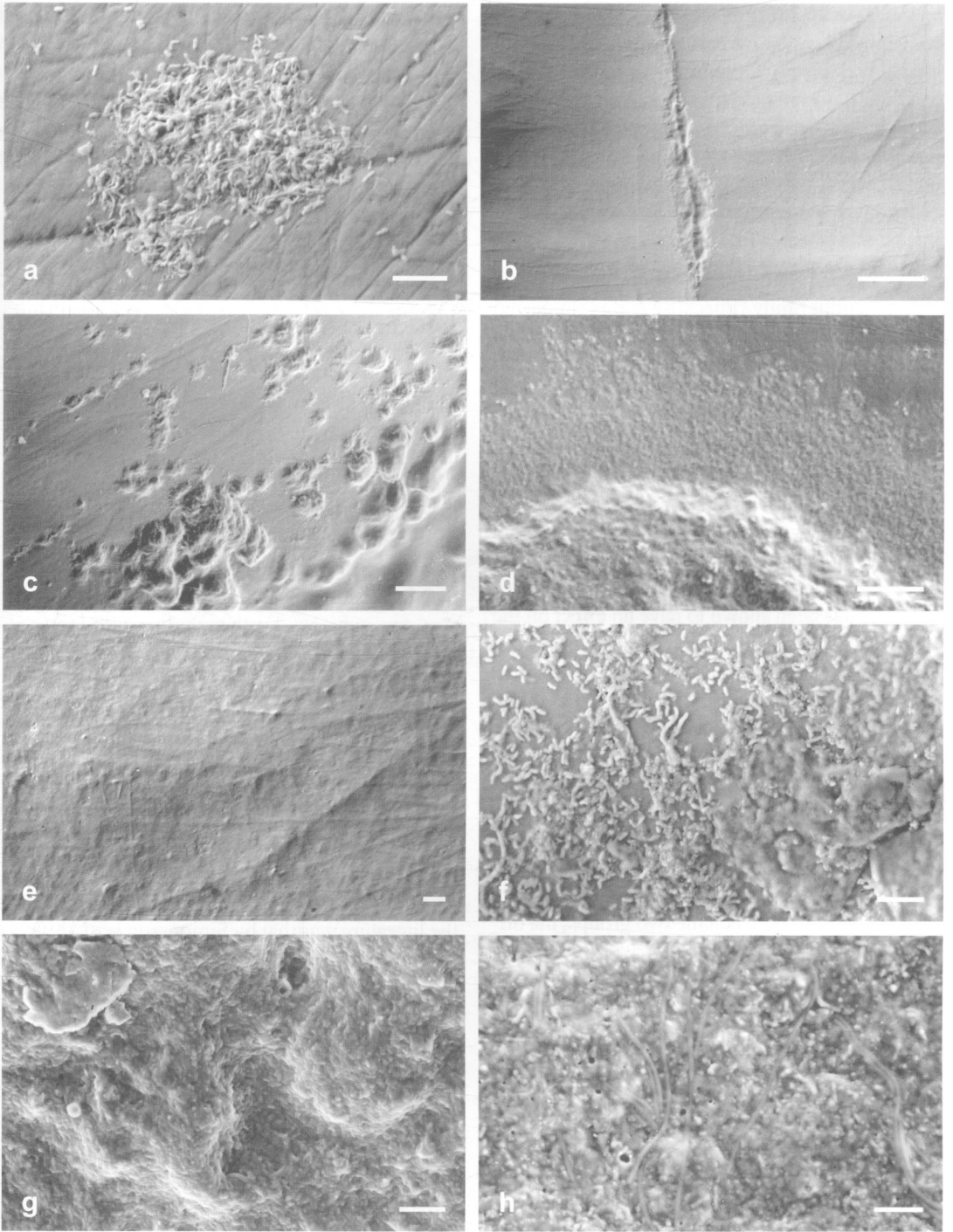


Fig. 17. Scanning electron micrographs of replicas obtained from the “slow” and “rapid” plaque formers on day-1 to day-14 of the undisturbed plaque development period. All bars represent 10 μm . Refer to text for the descriptions.

cent tooth surfaces (Fig. 14b). Similar observations were reported by Björn & Carlsson (1964) in an early study of plaque formation, using clinical photography. They observed focal bacterial aggregates with hemispherical appearance in early phase of dental plaque development within a matrix of organic material, possibly polysaccharide in nature, that were subsequently covered by salivary components.

Enamel blocks were chosen to study the plaque development in Study II because it was suggested that natural tooth surfaces should not be replaced by artificial substrates with an unknown surface morphology in studies investigating early microbial colonization as the deposition and retention of pioneer plaque bacteria is highly dependent on the microstructure of the surface (Lie 1979, Nyvad & Fejerskov 1987a & b). On the other hand, various surface irregularities are present on the enamel blocks which enhance initial bacterial attachment (Lie 1979, Nyvad & Fejerskov 1987a) and surface roughness has been shown to have an effect on the bacterial adhesion (for review: see Quirynen & Bollen 1995). For instance in an *in vitro* experiment, Swartz and Phillips (1957) reported a 10 fold increase in colony forming units after surface roughening of enamel surfaces. Therefore the observed quantitative and qualitative differences in early supragingival plaque formation between the "rapid" and "slow" plaque formers may have been influenced to some extent by these cofounders. Nonetheless, as great care was taken to randomise the enamel blocks between the two groups, the differences in plaque formation observed are likely to be authentic.

The major advantage of using replicas as compared to enamel blocks is the possibility of observing successional microbial structures on natural tooth surfaces and the sequential changes that occur during undisturbed plaque development *in vivo*. A drawback with replicas is that the impression material is rather sensitive to moisture and this may affect the setting period and the sensitivity of the technique. For this reason, Lie and Gusberti (1978) recommended repeating the procedure a number of times to improve reproduction details of the colonization pattern. The good quality scanning elec-

tron microscopy images we obtained in Study III imply that with care and correct manipulation of the materials, this technique is acceptable as a method investigating plaque development *in vivo*.

Salivary immunoglobulin A levels in "rapid" and "slow" plaque formers

Salivary IgA has been proposed to inhibit the bacterial adherence to oral structures by blocking adherence determinants, reducing hydrophobicity and agglutination of bacteria (Kilian & Bratthall 1986). Such properties have been confirmed *in vitro* (Williams & Gibbons 1972, Kilian et al. 1981, Reinholdt & Kilian 1987). Results from Study IV indicated that subjects with a slow rate of plaque formation have significantly higher salivary IgA levels than individuals with rapid plaque formation rates. Although this is the first time that salivary IgA levels are shown to correlate with extremes of undisturbed plaque formation rate, it is important to bear in mind that the results were obtained from two groups with very few subjects. Further studies with larger cohorts are necessary to confirm this finding.

On the other hand, the lower level of salivary IgA in "rapid" plaque formers may due to the fact that more salivary IgA are bound to microorganisms than the "slow" group, owing to a higher bacterial load in the oral cavity of the "rapid" group. It may be worthwhile to use saliva directly collected from the ducts in future studies to test this hypothesis.

The only comparable study that could be found in the literature was that by Simonsson *et al.* (1987a), who investigated clinical and biochemical characteristics and the rate of plaque formation in a group of "heavy" and "light" plaque formers. They were unable to show differences in salivary IgA, lactoferrin, lactoperoxidase and lysozyme levels between the "heavy" and "light" plaque formers. The differences between this study and ours might be due to the variations in the methodology used for detection of salivary IgA, and/or the ethnic differences between the investigated cohorts. All subjects in the current study were Chinese as opposed to a Scandinavian cohort used by Simonsson and co-workers.

In the same study, Simonsson and co-workers also

reported significant differences in the saliva-induced aggregation of early colonizers including *S. sanguis* and *S. mitis*. The authors suggested that their findings may support that theory that bacterial aggregates are more easily removed from the oral cavity than single cells, and it may be the case that saliva-induced aggregation of oral bacteria is an important factor in the rate of plaque formation. Salivary IgA, which is known to co-aggregate bacteria, may modulate this phenomenon.

Effect of a surface active agent on plaque formation

Results of the PI and planimetry of plaque extension reported in Study V tended to show a higher percentage reduction of plaque formation in the “rapid” plaque formers than the “slow” plaque

formers, when compared with the placebo. However, these differences were not statistically significant. This may suggest that the clinical effect of delmopinol mouthrinse is similar in subjects with different rates of plaque formation. In a previous dose response study, delmopinol treatment in subjects with high plaque values after placebo rinsing showed relatively higher reduction in plaque when measured by planimetry than subjects with low placebo plaque values (Collaert et al. 1992). However, the rinsing period for each treatment was only 5 days in contrast to 28 days in the latter study. On the other hand, their subjects were not specifically screened for the rate of plaque formation while the 12 “rapid” and 11 “slow” plaque formers in the present study represented extreme groups from a population of 71 subjects. Further studies with longer treatment periods may be necessary to confirm the present findings.

Conclusions

Based on the results from the microbiological and morphological studies of plaque formation in “rapid” and “slow” plaque formers, and the effect of a surface active agent on these two groups, the following conclusion can be drawn:

- There was a statistically significant higher proportion of gram-negative bacteria in the supragingival plaque samples of “rapid” plaque formers when compared with the “slow” group, from day-3 to day-14.
- The plaque extension in “rapid” plaque formers was significantly greater than that of the “slow” plaque formers in the early (3-hr - 1-day) supragingival plaque development.
- The morphological features in the “rapid” plaque formers showed a more complex structure than the “slow” plaque formers during the early (3-hr - 1-day) supragingival plaque formation.
- Subjects with a slow rate of plaque formation appear to have higher salivary IgA levels than individuals with rapid plaque formation rates.
- A surface active agent – delmopinol hydrochloride mouthrinse had similar efficacy in terms of plaque reduction in subjects with both “slow” and “rapid” plaque formation rates.
- When comparing the morphological studies using enamel blocks and replicas, it appears that images obtained from the replica technique are of comparable quality to the direct observation of enamel blocks using scanning electron microscopy. This may suggest that the replica technique is preferable for sequential observation of plaque formation *in vivo* as the same surface or site can be repeatedly studied throughout the investigation period.
- This series of studies showed that subjects with extreme differences in early plaque formation rates do exist. It may therefore be important for future studies, which utilise the experimental gingivitis model, to screen the subjects for their plaque formation rates before the experimental period in order to minimize error due to qualitative and quantitative variations in such plaque biology. This is particularly relevant for clinical trials on efficacy of plaque control agents as variation in plaque formation rates may significantly affect the overall results.

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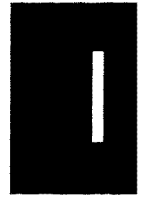
Summary

Supragingival plaque formation was studied in a group of subjects with two extreme plaque formation rates. Thus a “rapid” group and a “slow” group of plaque formers were selected from a cohort of healthy individuals for investigations related to i) microbiology and morphology of supragingival plaque, ii) salivary immunoglobulin A (IgA) concentration, and iii) response to a mouthrinse with a surface active agent.

Results showed a statistically significant difference between the “rapid” and “slow” plaque formers in both the qualitative and quantitative changes of the cultivable flora of the supragingival plaque. The morphological features of the “rapid” plaque formers were more complex than the “slow” plaque formers, especially during the early (3 hr - 1 day) plaque growth. Subjects with a slow rate of plaque formation seemed to have higher salivary IgA levels than “rapid” plaque formers. A mouthrinse containing the surface-active agent delmopinol hydrochloride showed similar efficacy in supragingival plaque reduction in subjects with extreme plaque formation rates.

This series of studies illustrates prevalence of extremes of plaque formation in population subgroups and there are differences in the microbiology and morphology of their supragingival plaque formation. It may therefore be important in future investigations utilising the experimental gingivitis model, to screen subjects for their plaque formation rate in order to minimize the undesirable spurious impact of such qualitative and quantitative variations in supragingival plaque. This appears particularly relevant for clinical trials investigating the efficacy of plaque control agents, where variation in plaque formation rates may affect the results.

Appendix



Reprinted from Journal of Clinical Periodontology, Vol. 23, Zee K-Y, Samaranayke LP, Attström R, Predominant cultivable supragingival plaque in Chinese “rapid” and “slow” plaque formers, 1025-1031, Copyright © (1996), with permission from Munksgaard International.

Predominant cultivable supragingival plaque in Chinese "rapid" and "slow" plaque formers

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Abstract. The aim of this study was to compare the predominant cultivable bacterial flora in supragingival plaque samples of Chinese "rapid" and "slow" plaque formers, using the experimental gingivitis model. 11 Chinese subjects (5 "rapid" and 6 "slow" plaque formers) were selected from 49 healthy young adults. The selection was based on the plaque index on the buccal surfaces of all the canines, premolars and 1st molars after 3-days without plaque control. The 11 subjects began a 14-day no oral hygiene period after prophylaxis. Plaque samples were collected on day 1, day 3, day 7 and day 14 from the buccal surface of upper right canine, 2nd premolar, 1st premolar and 1st molar, respectively. The samples were then dispensed into a tryptic soy broth, and cultured anaerobically to obtain pure isolates which were then identified by conventional means. Results showed similar trends in plaque development in the two groups; gram-positive bacteria were the predominant cultivable species ("rapid": 71-37%; "slow": 53-63%) and gram-negative species increased in proportion to the plaque age ("rapid": 9-47%, "slow": 13-28%). "Rapid" plaque formers showed a statistically significant higher percentage of gram-negative rods (38%) than the "slow" group (17%) in the 14-day samples. The difference in the proportions of other groups of bacteria between the "rapid" and "slow" plaque formers were also found to be statistically significant using the MANOVA test ($p=0.0162$). Most of the cultivable gram-negative rods belonged to *Fusobacterium* and *Campylobacter* species. Besides the quantitative difference observed clinically, there seems to be a qualitative difference in the cultivable flora between subjects with different rates of plaque formation.

Key words: microbiology; supragingival plaque, rate of plaque formation, experimental gingivitis

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Supragingival plaque consists of densely packed bacteria embedded in a matrix of bacterial extracellular polymers and macromolecules derived from saliva or gingival fluid (Gibbons & Van Houte 1975). Once this microbial flora colonizes the gingival crevice, an inflammatory response with migration of neutrophilic granulocytes and macrophages through the junctional epithelium will be initiated in healthy gingivae (Attström & Egelberg 1971). The classical experimental gingivitis studies of

Löe et al. (1965) have demonstrated the causal relationship between accumulation of plaque and gingival inflammation.

In another human experimental gingivitis study, Theilade et al. (1966) observed that subjects who required longer period of time to exhibit gingivitis accumulated plaque at a slower rate than those who developed gingivitis more rapidly. This might suggest that the rate of plaque formation would affect the rate of gingivitis development.

Ritz (1970) reported the role of aerobic *Neisseriae* in the initial formation of dental plaque. The author arbitrarily divided the subjects into 3 classes representing different rates of plaque formation. Namely, "rapid", "intermediate" and "slow" plaque formers with 67%, 20% and <3% of the tooth surfaces being covered with plaque after 5 days, respectively. The results suggested that there was a correlation in the rate of plaque formation and the level of *Neisseriae* in plaque i.e. the more rapid the

rate of plaque formation, the higher the level of *Neisseriae* in plaque.

Relatively recently, Simonsson et al. (1987a, b) studied the rate of plaque formation and related clinical, biochemical, biophysical and microbiological characteristics in "heavy" and "light" plaque formers. Large variations were found for most of the factors. Hence, the authors concluded that several factors might be involved in plaque formation and none of the studied variables alone could explain the large difference in the amount of plaque formed after 3 days between the "heavy" and "light" plaque formers.

It is evident from the foregoing that there are significant differences in the rate of plaque formation among different individuals, but thus far no study has reported the composition of the supragingival plaque in these individuals. The aim of the present study, therefore, was to compare the cultivable flora of the supragingival plaque in "rapid" and "slow" plaque formers using the experimental gingivitis model, in a cohort of Chinese individuals.

Material and Methods

Selection of "rapid" and "slow" plaque formers

A total of 49 subjects volunteered for the study. They were dental students, dental surgery assistants and dental hygienists from the Prince Philip Dental Hospital, Hong Kong. All the subjects were born in Hong Kong and were Chinese in ethnic origin. The subjects were screened to assess their plaque formation rate after 3 days of plaque accumulation using the Plaque Index (PI, Silness & Løe 1964). Fig. 1 shows the study design for the selection period. At the beginning of this period, the subjects were screened to exclude those with extensive interproximal or buccal restorations, untreated caries or periodontal diseases, and treatment with antibiotics. All of the subjects were not smokers. Informed consent were obtained from all the subjects. After screening, suitable subjects entered a 3-week oral hygiene period, comprising visits once a week for professional cleaning and oral hygiene instructions. At the start of the plaque accumulation periods, gingival health of the subjects was confirmed by the measurements of gingival crevicular fluid (GCF) according to Løe & Holm-Pedersen (1965). PI on the buccal surfaces of all canines,

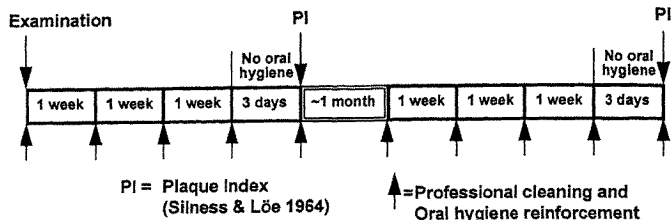


Fig. 1. Study design for the screening period for selecting "slow" and "rapid" plaque formers.

premolars and 1st molars were registered. The number of registered surfaces for each individual thus was 16. This procedure was repeated after 1 month, with all subjects, to confirm the results from the first period, after which mean plaque scores were calculated for each individual. After the recordings, 5 "rapid" plaque formers with highest mean PI scores and 6 "slow" plaque formers with lowest mean PI scores were selected for subsequent studies (Simonsson et al. 1987b).

Sampling and microbiological procedures

Before the start of the 14 days experimental gingivitis period, the selected 11 subjects went through again once a week prophylaxis and oral hygiene instruction for 3 weeks to ensure gingival health (Fig. 2). At Day 0, GCF was taken to confirm the gingival health of all the subjects and a thorough prophylaxis was done to make sure that no plaque was presence on the sampling surfaces. The 14 days of no oral hygiene period started thereafter.

A single plaque sample was collected on day 1, day 3, day 7 and day 14 from the buccal surface of upper right canine, 2nd premolar, 1st premolar and 1st molar, respectively (Fig. 2). A sterile curette, passing along the gingival margin, was used to remove as much supragingival plaque as possible. The sample was then dispensed into small screw-capped

bottles filled with sterile tryptic soy broth (TSB; Difco Laboratories, Detroit, U.S.A.) and sent to the laboratory for processing within 30 min. In the laboratory, the samples were vortexed for 30s, serially diluted and spiral plated onto Columbia blood agar base (Difco Laboratories) supplemented with 5% defibrinated horse blood, 5 mg/l hemin and 500 µg/l menadione (CBABS). The plates were incubated anaerobically at 37°C for 3–4 days in anaerobic chamber supplied with a gas mixture of 80% N₂, 10% H₂ and 10% CO₂. A region with ~40 well separated colonies were marked and 20–30 different colonies were subcultured on CBABS to obtain pure isolates. The pure isolates were identified according to their colony and cell morphology, Gram stain reaction, oxygen requirement and biochemical tests (using the Rapid STR, ANA II and NH system, Innovative Diagnostic Systems Inc., Atlanta, G.A.). For those unidentified gram-positive and negative anaerobic bacteria, gas liquid chromatography were used.

Permission was sought and granted by the Ethics Committee of the Faculty of Dentistry, University of Hong Kong, to conduct the study.

Statistics

Comparison of the percentage proportion of the different groups of bacteria between the "rapid" and "slow"

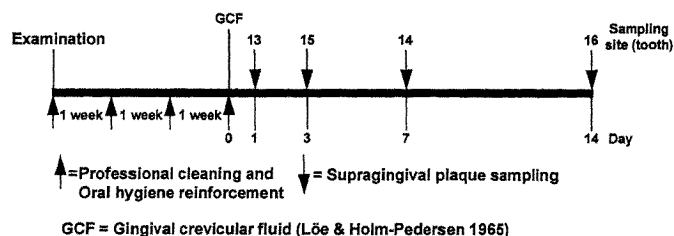


Fig. 2. Schedule of the supragingival plaque sampling during the experimental period.

plaque formers were performed by MANOVA test with "group" and "day" as the crossed factors and "subject" as the nested factor using the SAS soft-

ware package (SAS Institute, Cary NC, USA). Groups were regarded as significantly different from each other if $p < 0.05$.

Results

The mean PI of the 49 subjects after 3 days of no oral hygiene ranged from

Table 1. Mean % isolates of cultivable supragingival plaque flora of 5 "rapid" and 6 "slow" plaque formers during 14 days of no oral hygiene

Cultivable bacteria	Day 1		Day 3		Day 7		Day 14	
	slow	rapid	slow	rapid	slow	rapid	slow	rapid
Gram-positive cocci								
<i>Gemella haemolysans</i>	-	-	1.3	0.8	0.7	-	1.4	0.8
<i>Micrococcus</i> spp.	-	0.8	-	-	-	-	-	-
<i>Peptostreptococcus prevotii</i>	0.7	-	-	-	-	-	0.7	-
<i>Staphylococcus epidermidis</i>	19.6	16.3	1.3	-	-	-	1.4	-
<i>Streptococcus acidominimus</i>	3.5	2.3	-	-	0.7	0.8	2.1	0.8
<i>Streptococcus anginosus</i>	-	-	-	-	-	-	0.7	-
<i>Streptococcus bovis</i>	-	-	-	-	-	-	-	0.8
<i>Streptococcus constellatus</i>	3.5	-	10.4	3.9	8.0	5.6	1.4	0.8
<i>Streptococcus equinus</i>	-	-	-	-	-	2.4	-	-
<i>Streptococcus intermedius</i>	-	1.6	0.6	1.6	2.0	2.4	-	-
<i>Streptococcus mitis</i>	2.1	1.6	1.3	3.9	2.7	2.4	-	0.8
<i>Streptococcus morbillorum</i>	4.9	17.1	1.9	4.7	2.0	4.8	3.4	2.5
<i>Streptococcus mutan</i>	-	-	0.6	-	0.7	-	-	-
<i>Streptococcus pneumoniae</i>	1.4	0.8	1.3	0.8	-	0.8	0.7	0.8
<i>Streptococcus salivarius</i>	1.4	2.3	4.5	3.9	1.3	-	0.7	-
<i>Streptococcus sanguis I</i>	2.8	5.4	5.8	7.1	3.3	4.0	0.7	0.8
<i>Streptococcus sanguis II</i>	9.1	9.3	9.1	5.5	13.3	5.6	0.7	0.8
Unidentified Gm+ve cocci	-	1.6	0.6	1.6	-	-	0.7	-
Gram-positive rods								
<i>Actinomyces israelii</i>	-	3.9	11.0	4.7	9.3	12.8	31.7	15.6
<i>Actinomyces meyeri</i>	-	0.8	-	3.1	-	0.8	-	-
<i>Actinomyces naeslundii</i>	-	0.8	-	-	-	1.6	-	-
<i>Actinomyces odontolyticus</i>	-	0.8	3.9	4.7	3.3	5.6	4.1	0.8
<i>Actinomyces pyogenes</i>	-	0.8	-	-	-	-	-	-
<i>Actinomyces viscosus</i>	-	-	-	-	-	0.8	-	-
<i>Bacillus</i> spp.	0.7	-	1.3	3.1	4.0	-	-	1.6
<i>Bifidobacterium</i> spp.	-	-	-	-	1.3	1.6	0.7	-
<i>Clostridium difficile</i>	-	-	-	-	0.7	-	0.7	-
<i>Clostridium hastiforme</i>	-	-	3.9	-	1.3	0.8	-	-
<i>Lactobacillus jensenii</i>	-	-	-	-	1.3	-	-	-
<i>Propionibacterium acnes</i>	-	-	-	-	-	-	-	0.8
<i>Propionibacterium granulosum</i>	-	3.1	-	-	3.3	1.6	6.9	5.7
<i>Propionibacterium propionicus</i>	-	-	-	-	-	0.8	-	-
Unidentified Gm+ve rods	1.4	1.6	1.9	7.1	3.3	3.2	4.1	3.3
Gram-negative cocci								
<i>Neisseria mucosa</i>	-	-	-	2.4	0.7	1.6	-	-
<i>Veillonella dispar</i>	11.2	3.9	9.7	1.6	9.3	6.4	10.3	9.8
Gram-negative rods								
<i>Bacteroides capillosus</i>	-	-	-	0.8	1.3	-	-	-
<i>Bacteroides gracilis</i>	-	-	-	-	-	-	-	0.8
<i>Bacteroides ureolyticus</i>	-	0.8	-	-	-	-	0.7	-
<i>Capnocytophaga</i> spp.	1.4	0.8	5.8	8.7	4.0	6.4	4.1	10.7
<i>Eikenella corrodens</i>	-	-	-	-	0.7	-	-	-
<i>Fusobacterium mortiferum</i>	-	-	0.6	0.8	-	-	-	-
<i>Fusobacterium nucleatum</i>	-	-	0.6	0.8	-	-	-	-
<i>Fusobacterium</i> spp.	0.7	0.8	7.1	5.5	4.0	6.4	6.9	15.6
<i>Haemophilus parainfluenzae</i>	-	-	0.6	-	-	-	-	-
<i>Kingella denitrificans</i>	-	-	-	2.4	-	0.8	-	2.5
<i>Prevotella buccae</i>	-	-	-	-	-	-	-	0.8
<i>Prevotella corporis</i>	-	-	-	-	0.7	-	-	-
<i>Prevotella intermedia</i>	-	0.8	1.3	3.9	0.7	1.6	2.8	3.3
<i>Prevotella loescheii</i>	-	-	-	0.8	0.7	0.8	-	0.8
<i>Prevotella melaninogenica</i>	-	-	-	-	-	-	1.4	1.6
<i>Prevotella oralis</i>	-	-	-	-	-	-	0.7	-
<i>Wolinella</i> spp.	-	-	-	-	-	1.6	-	-
Unidentified AnO ₂ Gm-ve rods	-	1.6	4.5	2.4	2.0	0.8	0.7	1.6

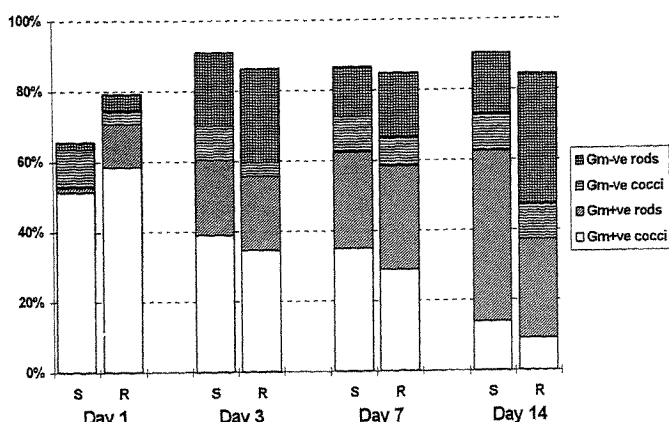


Fig. 3. Comparison of the mean relative proportions of predominant cultivable bacteria in supragingival plaque samples of "slow" (S) and "rapid" (R) plaque formers during the 14-day period of no oral hygiene

0.84 to 2.63. For the 5 "rapid" plaque formers (3 females and 2 males), the mean PI was 2.5 with a range of 2.41 to 2.63. The mean PI of the 6 "slow" plaque formers (5 males) was 1.02 with a range of 0.84 to 1.16. The mean PI of the remaining subjects not selected was 1.74 with a range of 1.19 to 2.31.

All the subjects showed a clinically healthy gingivae with minimal amount of GCF detected before the start of the no oral hygiene period. A total of 44

plaque samples were obtained for microbiological analysis from the 11 subjects and the number of samples from the "rapid" and "slow" plaque formers were 20 and 24 respectively. After purification, 1095 isolates were cultured from the 44 plaque samples for identification. However, only 83.5% were speciated and the rest were lost on sub-culture and unable to be speciated.

Results showed similar trends in plaque development in the two groups

(Fig. 3). Gram-positive bacteria were the predominant cultivable species from day 1 to day 7 ("rapid": 71→58%; "slow": 53→62%) and gram-negative species increased in percentage with the plaque age ("rapid": 8→47%, "slow": 13→28%). "Slow" plaque formers showed a lower proportion of gram-positive bacteria than "rapid" plaque formers on day 1 but overtook the "rapid" plaque formers from day 3 to day 14. The proportion of gram-negative bacteria was higher in the "slow" plaque formers than the "rapid" plaque formers at day 1 but as the proportion of gram-negative rods increased in the "rapid" plaque formers, the overall proportion of gram-negative bacteria (47%) became higher than that of gram-positive (37%) at day 14. The difference in the mean relative proportion of the gram-positive and gram-negative bacteria between the "rapid" and "slow" plaque formers were statistically significant ($p=0.0162$). There was no statistical significant difference in the mean relative proportions of gram-positive and gram-negative bacteria, between the subjects within the same group ($p=0.4055$).

Table 1 shows the mean proportions of organisms speciated in the two groups of subjects. *Streptococcus* spp. are the most predominant cultivable gram-positive species whereas *Actinomyces* spp. are the most common

Table 2 Frequency occurrence (in percentage) of bacterial species in the cultivable supragingival plaque flora of 5 "rapid" and 6 "slow" plaque formers during 14 days of no oral hygiene, only species with frequency of isolation $\geq 50\%$ of the subjects in any one of the sampling day were included (i.e. >2 of the 5 subjects in the "rapid" group and ≥ 3 of the 6 subjects in the "slow" group)

Cultivable bacteria	Day 1		Day 3		Day 7		Day 14	
	slow	rapid	slow	rapid	slow	rapid	slow	rapid
Gram-positive cocci								
<i>Staphylococcus epidermidis</i>	100	80	33	-	-	-	33	-
<i>Streptococcus acidominimus</i>	50	20	-	-	17	20	33	20
<i>Streptococcus constellatus</i>	33	-	67	80	50	60	33	20
<i>Streptococcus equinus</i>	-	-	-	-	-	60	-	-
<i>Streptococcus mitis</i>	33	40	17	60	50	40	-	-
<i>Streptococcus morbillorum</i>	67	100	33	60	50	40	17	20
<i>Streptococcus sanguis I</i>	33	80	67	60	50	40	17	20
<i>Streptococcus sanguis II</i>	83	80	100	80	83	100	17	20
Gram-positive rods								
<i>Actinomyces israelii</i>	-	40	50	80	83	100	100	80
<i>Actinomyces odontolyticus</i>	-	20	67	80	50	60	50	20
<i>Bacillus</i> spp.	17	-	33	33	67	-	-	-
<i>Propionibacterium granulosum</i>	-	40	-	-	50	20	33	40
Gram-negative cocci								
<i>Veillonella dispar</i>	67	80	83	20	100	60	83	60
Gram-negative rods								
<i>Capnocytophaga</i> spp	17	20	83	60	67	80	67	80
<i>Fusobacterium</i> spp	17	20	100	60	67	100	83	100
<i>Prevotella intermedia</i>	-	20	17	40	17	20	50	40

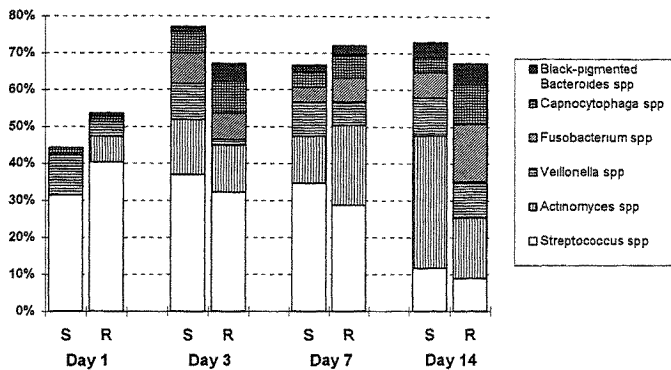


Fig. 4. Comparison of the percent change in major bacterial species in supragingival plaque samples of "slow" (S) and "rapid" (R) plaque formers during the 14-day period of no oral hygiene.

gram-positive rod identified in both groups. Initially the proportion of the *Actinomyces* species increases in both groups, but at day 14, its proportion in the "slow" plaque formers is higher than that of the "rapid" group. *Veillonella dispar* and *Neisseria mucosa* are the only gram-negative cocci found in all the samples with the *Veillonella* spp. being the predominant type throughout the 14 day period. *Fusobacterium* spp. and *Capnocytophaga* spp. are the 2 most predominant bacteria among the cultivable gram-negative rods. There is also some black-pigmented *Bacteroides* spp. identified, but only in small proportion.

Table 2 shows only those species with a frequency of occurrence $\geq 50\%$ in any one of the samples. Among the 17 gram-negative rod species isolated and identified (Table 1), only 3 i.e., *Capnocytophaga* spp., *Fusobacterium* spp. and *Prevotella intermedia* were detected the most often. For the other groups of bacteria, there are also very few species occur $\geq 50\%$ in each sample.

Fig. 4 shows the transition of the major species in both groups. The proportions of the *Streptococcus* spp. decrease both in the "rapid" and "slow" plaque formers to a similar extent but the increase in the *Actinomyces* spp. is higher in the "slow" than in the "rapid" group. The proportions of gram-negative rods, i.e., the *Fusobacterium* spp. and *Capnocytophaga* spp. increase both in the "rapid" and "slow" group but the cumulative proportions of these were higher in the "rapid" group at day 14. There is also a small percentage of culti-

vable isolates (4-6%) identified as dark-pigmented *Bacteroides* spp. in both groups, at day 14.

Discussion

Although supragingival plaque formation has been studied extensively, very limited information on this topic is available in the Chinese population, and further, the differences in the rate of plaque formation in different individuals are not clear. The present study is the first attempt at investigating the supragingival plaque formation in Chinese subjects with different rates of plaque formation, using the experimental gingivitis model. Results of the present study are broadly in agreement with previous reports using similar methodology where gram-positive bacteria were the predominant cultivable species in early plaque, and the proportion of gram-negative bacteria increases with the plaque age (Socransky et al. 1977, Syed & Loesche 1978, Moore et al. 1982, Moore et al. 1987). However, in the present study previously unreported differences were found between the two groups. Thus the "slow" plaque formers showed a statistically significant higher relative proportion of gram-positive bacteria and a lower relative proportion of gram-negative bacteria, when compared with the "rapid" plaque formers, from day 3 to day 14. The mean proportion of the gram-positive bacteria in the "slow" group remained at around 60% from day 3 to 14 while that of the "rapid" group a reduction in these organisms, from 56% to

37%, was noted. This may suggest that the "rapid" plaque formers tends to attain a complex flora earlier than the "slow" plaque formers in 14 days. All of the comparison between the two groups were based on the relative proportions of different species. Whether the absolute counts of the different species will show a similar trend remains to be determined.

In the present study, a method similar to that described by Simonsson et al. (1987a, b) was used, i.e., 3 days of no oral hygiene to identify subjects with different rate of plaque formation. Based on their results, Simonsson et al. (1987a, b) surmised that salivary flow conditions, saliva-induced aggregation of oral streptococci and clinical wettability are factors responsible for 90% of the variation in the number of bacteria initially colonizing tooth surface. Furthermore, the authors suggested that biophysical factors such as the rate of unstimulated salivary secretion rate and the "retention depth" of the tooth surfaces may be important in bacterial accumulation during the early phases of plaque formation. Whether the qualitative differences in the cultivable flora of the "rapid" and "slow" plaque formers identified in the present study could be explained by the salivary factors and other biophysical and ecological phenomena are not known as we have not investigated the quality of the saliva or salivary flow rates in our cohorts.

Cao et al. (1990) in a relatively recent study compared the plaque microflora of Chinese and Caucasians. Their results showed that there were notable differences in the composition of supragingival plaque between the Chinese and Caucasian population groups. The Chinese group had a higher mean proportions of spirochetes, motile rods, *Fusobacterium* spp. and dark-pigmented *Bacteroides* species, while the Caucasian group had higher mean proportions of cocci, total *Actinomyces* spp., *A. viscosus* and total *Streptococcus* spp. The results of 14-day samples in our study also show similar proportions of *Fusobacterium* spp. and dark-pigmented *Bacteroides* species comparable to the Chinese group in the latter study. However, such direct comparison is merely indicative due to the differences in the methodology, including microbiological procedures and identification, and the fact that their samples were of unknown age while ours were well-defined.

When Quirynen & van Steenberghe (1989) studied the early plaque growth in 15 young adults going through a 4-day period of no oral hygiene, they reported that the plaque growth rate decreased by up to 50% during the night and there were high standard deviations of the mean plaque scores among the individuals due to the presence of 'slow' and 'fast' plaque formers. Clear differences in plaque growth rate observed between types of teeth and the plaque growth pattern seemed to be closely correlated to irregularities of the tooth surfaces. In the present study, each sampling session was scheduled in the morning so as to avoid the possible diurnal differences in the plaque growth rate. Samples were taken from the same tooth type for each subject in order to obviate variation. However, there may also be surface irregularities such as crack lines or micro-grooves present on the tooth surfaces which may have affected the rate of plaque formation. Other factors such as gingival fluid (Hillam & Hull 1977, Brex et al. 1980, Quirynen et al. 1991), salivary components (Williams & Gibbons 1972, Ericson & Magnusson 1976, Arnold et al. 1977, Adamson & Carlsson 1982, Twetman et al. 1986, Rudney et al. 1993) and diet (Carlsson & Egelberg 1965, Martin 1991) may contribute to the differences found between the "rapid" and "slow" plaque formers in the present study.

The present study is one of a series of investigations that were undertaken to enquire into the differences in the microbiology of the supragingival plaque between "rapid" and "slow" plaque formers. The other complementary studies involving the morphological and structural aspects of supragingival plaque in these subjects may yield further qualitative data on this phenomenon in subjects with extreme rates of plaque formation. Although the current data are based on a total number of 11 subjects, a relatively small number to give conclusive results, we have demonstrated for the first time a statistically significant difference in the qualitative changes of the cultivable flora of the supragingival plaque samples between the "rapid" and "slow" plaque formers during a 14-day period of no oral hygiene. It would therefore seem that both qualitative and quantitative differences in the supragingival plaque exist in subjects with different rate of plaque formation. The clinical implications, if

any, of such differences need to be investigated by further studies.

Acknowledgements

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Zusammenfassung

Vorherrschender kultivierbarer, supragingivaler Plaque bei chinesischen Patienten mit der Neigung zu "schneller" und "langsamer" Plaqueanlagerung

Das Ziel der vorliegenden Studie bestand darin, die prädominierende, kultivierbare Bakterienflora im supragingivalen Plaque von "schnell" oder "langsam" plaquebildenden Chinesen, bei Anwendung des Versuchsmodells für Gingivitis zu vergleichen. Aus einer Population von 49 gesunden chinesischen Jugendlichen wurde eine Gruppe von 11 Probanden (5 "schnell" und 6 "langsam" plaquebildende) zusammengestellt. Nach 3 Tage lang eingestellter Plaquekontrolle war die Auswahl der Probanden nach den Plaqueindexwerten der bukkalen Oberflächen ihrer Eckzähne, Prämolaren und 1. Molaren getroffen worden. Nach einer Prophylaxebehandlung stellten die 11 Probanden 14 Tage lang ihre Mundhygiene ein. An den Versuchstagen 1, 3, 7 und 14 wurden an der bukkalen Oberfläche des oberen rechten Eckzahns, des 2. Prämolaren und des 1. Molaren, Plaqueabstriche in genannter Reihenfolge entnommen. Um reine, mit herkömmlichen Mitteln identifizierbare, Isolate zu erhalten, wurden die Proben in einem Transportmedium (Tryptic Soy Broth; TSB) in ein Labor transportiert und anaerob kultiviert. Bei beiden Gruppen zeigten die Ergebnisse ähnliche Entwicklungstendenzen des Plaque; die prädominierenden, kultivierbaren Spezies waren grampositive Bakterien ("schnell": 71–37%, "langsam" 53–63%) und die Zahl der gramnegativen Spezies erhöhte sich mit zunehmendem Alter des Plaque ("schnell": 9–47%, "langsam" 13–28%). In 14 Tage alten Plaqueproben beobachtete man bei den Probanden mit "schneller" Plaquebildung einen statistisch abgesicherten, höheren prozentualen Anteil gramnegativer Stäbchen (38%) als bei den Probanden der "langsam" Gruppe (17%). Zwischen Probanden mit "schneller" und "langsamer" Plaquebildung waren, nach dem MANOVA Test, die Unterschiede der Verhältniswerte auch für andere Bakteriengruppen statistisch signifikant ($p=0.0162$). Die meisten kultivierbaren gramnegativen Stäbchen gehörten zu den *Fusobacterium*- und *Capnocytophaga*-Arten. Neben dem klinisch beobachteten, quantitativen Unterschied scheint zwischen Proban-

den mit mengenmäßig unterschiedlicher Plaqueanlagerung auch ein qualitativer Unterschied der kultivierbaren Flora vorzukommen.

Résumé

Plaque dentaire sus-gingivale mise en culture chez des chinois à formation de plaque rapide ou lente

Le but de cette étude a été de comparer la flore bactérienne cultivable prédominante dans des échantillons de plaque dentaire sus-gingivale de chinois formant lentement ou rapidement la plaque dentaire en utilisant le modèle de la gingivite expérimentale. Onze chinois (5 rapides et 6 lents dans la formation de leur plaque) ont été sélectionnés de 49 adultes sains. La sélection était basée sur l'indice de plaque des surfaces vestibulaires de toutes les canines, prémolaires et premières molaires après trois semaines sans hygiène buccale. Les onze sujets sélectionnés ont débuté une période sans hygiène buccale de 14 jours après prophylaxie. Des échantillons de plaque dentaire ont été pris aux jours 1, 3, 7 et 14 de la surface vestibulaire de la canine, deuxième prémolaire, première prémolaire et première molaire supérieures droites. Ces échantillons ont été placés dans un bouillon de tryptic soy et mis en culture anaérobie afin d'obtenir des isolats purs qui ont ensuite été identifiés par les méthodes conventionnelles. Les résultats ont montré des tendances semblables dans le développement de la plaque dentaire des deux groupes. Des bactéries Gram-positif représentaient les espèces cultivables prédominantes (rapide: 71 à 37%; lent 53 à 63%) et les espèces Gram-négatif augmentaient en proportion de l'âge de la plaque (rapide 9 à 47%; lent 13 à 28%). Les rapides montraient une différence statistiquement significative d'un plus important % de bâtonnets Gram-négatif (38%) que les lents (17%) dans les échantillons du jour 14. La différence dans les proportions des autres groupes de bactéries entre formeurs de plaque lents et rapides était également statistiquement significative lorsque le test MANOVA était utilisé ($p=0.0162$). La plupart des bâtonnets Gram-négatif cultivables appartenaient aux espèces du *Fusobacterium* et des *Capnocytophaga*. Mise à part la différence quantitative observée cliniquement, il semble y avoir une différence qualitative dans la flore cultivable des sujets avec différents taux de formation de plaque dentaire.

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SCANNING ELECTRON MICROSCOPY OF MICROBIAL
COLONIZATION OF 'RAPID' AND 'SLOW' DENTAL-
PLAQUE FORMERS *IN VIVO*KWAN-YAT ZEE,¹* LAKSHMAN P. SAMARANAYAKE¹ and ROLF ATTSTRÖM²¹Faculty of Dentistry, 3/F. Prince Philip Dental Hospital, University of Hong Kong, 34 Hospital Road, Hong Kong and ²Department of Periodontology, Centre for Oral Health Sciences, Lund University

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Summary—The aim was to investigate the morphological features of supragingival plaque development in 'rapid' and 'slow' plaque formers using scanning electron microscopy (SEM). Forty-nine healthy volunteers were screened for their plaque-formation rate after 3 days of oral hygiene abstinence using the Plaque Index (PI). Five with the highest and six with the lowest mean PI were selected as 'rapid' and 'slow' plaque formers, respectively. Six enamel blocks measuring 2 × 2 × 1 mm were bonded onto the buccal surfaces of the upper left canine, premolars and first molar of each selected participant after a series of prophylaxes and oral hygiene instruction to ensure clinical gingival health. A 14-day period with no oral hygiene began thereafter. An enamel block was removed at 3 hr, 6 hr, 1 day, 3 days, 7 days and 14 days from each individual, fixed, and processed for SEM. Quantitatively, more bacteria were observed on the 3-hr to 1-day enamel blocks of the 'rapid' plaque formers. The day-1 specimens of the 'rapid' group showed a more complex supragingival plaque structure than those of the 'slow' group. From days 3 to 14, during the maturation period of supragingival plaque, there were no discernible differences between the two groups except that intermicrobial matrix was more prominent in the 'rapid' group. In general, the development of supragingival plaque followed known patterns. These observations indicate that morphological features of supragingival plaque in 'slow' and 'rapid' plaque formers differ, especially in the early developmental phases. © 1997 Elsevier Science Ltd

Key words: SEM, supragingival plaque, morphology, rate of plaque formation, experimental gingivitis.

INTRODUCTION

Dental plaque, defined as bacterial aggregations on the teeth or other solid oral structures (Egelberg, 1970; Kelstrup and Theilade, 1974; Listgarten, 1994) is recognized as a primary aetiological factor in the development of periodontal diseases, and there is increasing evidence that the amount and composition of plaque is directly related to the degree of periodontal health (Lindhe *et al.*, 1980; Haffajee and Socransky, 1994).

Most of the information on the composition of supragingival plaque has come from *in vivo* cultural and morphological studies. For this purpose, various materials have been used to mimic enamel surfaces, including Mylar strips (Theilade and Theilade, 1970), Vestopal 'W' (Berthold *et al.*, 1971), epoxy resin (Listgarten *et al.*, 1975) and plastic films (Brex *et al.*, 1981). However, a study on early microbial colonization of human enamel and root surfaces showed that the pattern of bacterial colonization was dependent on the surface structure and topographic features of the tooth (Nyvad and

Fejerskov, 1987 a,b). Therefore, artificial surfaces are a poor substitute for teeth in studies on structural aspects of oral microbial colonization.

The rate of plaque formation varies between individuals (Theilade *et al.*, 1966; Ritz, 1970; Listgarten *et al.*, 1975; Magnusson *et al.*, 1976; Rundegren and Ericson, 1981), but there have been very few attempts to investigate the variables involved in these differences. Simonsson *et al.* (1987 a,b), in a pioneering study, characterized the rate of plaque formation and the effect of clinical, biochemical, biophysical and microbiological variables on 'heavy' and 'light' plaque formers. They concluded that several factors might be involved, although none of the studied variables could alone explain the large differences in the amount of plaque formed.

There is no published information on the ultrastructural features of the supragingival plaques that appear to be forming at relatively different rates in different individuals. Our aim now was to compare the microbial colonization of supragingival plaque in a group of 'rapid' and 'slow' plaque formers, using enamel blocks, during a period in which they refrained from normal oral hygiene, i.e., under 'ex-

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Abbreviations: PI, Plaque Index, SEM, Scanning electron microscope(-y).

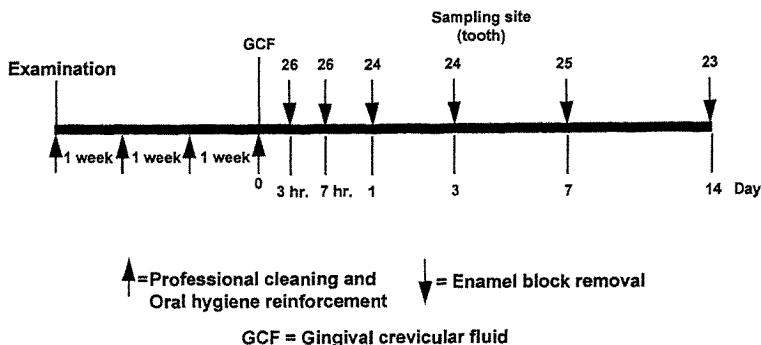


Fig. 1 Clinical schedule for the experimental period.

perimental gingivitis' conditions. SEM was used to evaluate the morphological features.

MATERIALS AND METHODS

Selection of 'rapid' and 'slow' plaque formers

Forty-nine ethnic Chinese volunteered for the study: they were dental students, dental surgery assistants and dental hygienists from the Prince Philip Dental Hospital, Hong Kong. The volunteers were screened to exclude those with extensive approximal of buccal restorations, untreated caries or periodontal diseases, and recent treatment with antibiotics; none was a smoker. After this preliminary screening, all the participants entered a 3-week oral hygiene period, comprising once-a-week professional cleaning and oral hygiene instruction. Their rate of plaque formation was then assessed using the PI (Silness and Løe, 1964) after 3 days of undisturbed accumulation. PI was measured on the buccal surfaces of all canines, premolars and first molars, yielding a total of 16 sites per individual. To ascertain the screening results, the above procedure was repeated after 1 month and mean plaque scores recalculated for each individual. The collated data from the first and second periods of plaque accumulation were utilized to select five 'rapid' plaque formers with highest and six 'slow' plaque formers with lowest mean PI scores (Simonsson *et al.*,

1987b). These 11 individuals (8 males, 3 females) were used in the final study.

Permission to conduct the study was sought and granted by the Ethics Committee of the Faculty of Dentistry, the University of Hong Kong. Informed consent was obtained from all the participants.

Preparation of enamel blocks

Blocks of human enamel fixed on the buccal surfaces of the teeth of each volunteer were used to investigate plaque formation. Each enamel piece measured approx. $2 \times 2 \times 1$ mm and was cut from intact buccal or lingual surfaces of extracted teeth stored in thymol. The enamel surfaces were all free from major cracks and defects as observed under a dissection microscope. After cutting, the pieces were cleaned, sterilized and stored in distilled water before use.

Experimental and sampling procedure

The morphological investigations were conducted over a 14-day period as in a previously described study on experimental gingivitis (Zee *et al.*, 1996). First the 11 selected participants went through a repetition of once-a-week prophylaxis and oral hygiene instruction for three consecutive weeks to ensure gingival health (Fig. 1). They were issued with sheets for recording every meal during the 14-day period. On day 0, the presence of healthy gingiva on all upper first molars, premolars and

Fig. 2. Scanning electron micrographs of enamel blocks retrieved from 'slow' (a-e) and 'rapid' (f-j) plaque formers during 3 hr to 1 day of undisturbed plaque development. All bars represent $10 \mu\text{m}$. (a) 3-hr block: granular material covering scratch marks on enamel surface (original magnification: $\times 1000$). (b) 6-hr block: bacteria in pits and grooves of enamel surface (original magnification: $\times 1000$). (c) Day-1 block: very few bacteria in enamel pits and grooves (original magnification: $\times 1000$). (d) Day-1 block: microcolonies of cocci and rod-shaped bacteria (original magnification: $\times 1000$). (e) Day-1 block: aggregated rod- and coccoid-shaped bacteria (original magnification: $\times 4000$). (f) 3-hr block: epithelial cells with some bacteria attached to the surface (original magnification: $\times 1000$). (g) 6-hr block: bacteria reside in the enamel pits and grooves (arrow) (original magnification: $\times 1000$). (h) Day-1 block: thick layer of bacteria covered by surface-coating material; cracks are due to preparation artefacts (original magnification: $\times 1000$). (i) Day-1 block: a complex flora including filamentous, rod and cocci-shaped aggregates in the matrix (original magnification: $\times 1000$). (j) Day-1 block: coccoid and rod-shaped bacteria embedded in a granular intermicrobial matrix (original magnification: $\times 5000$).

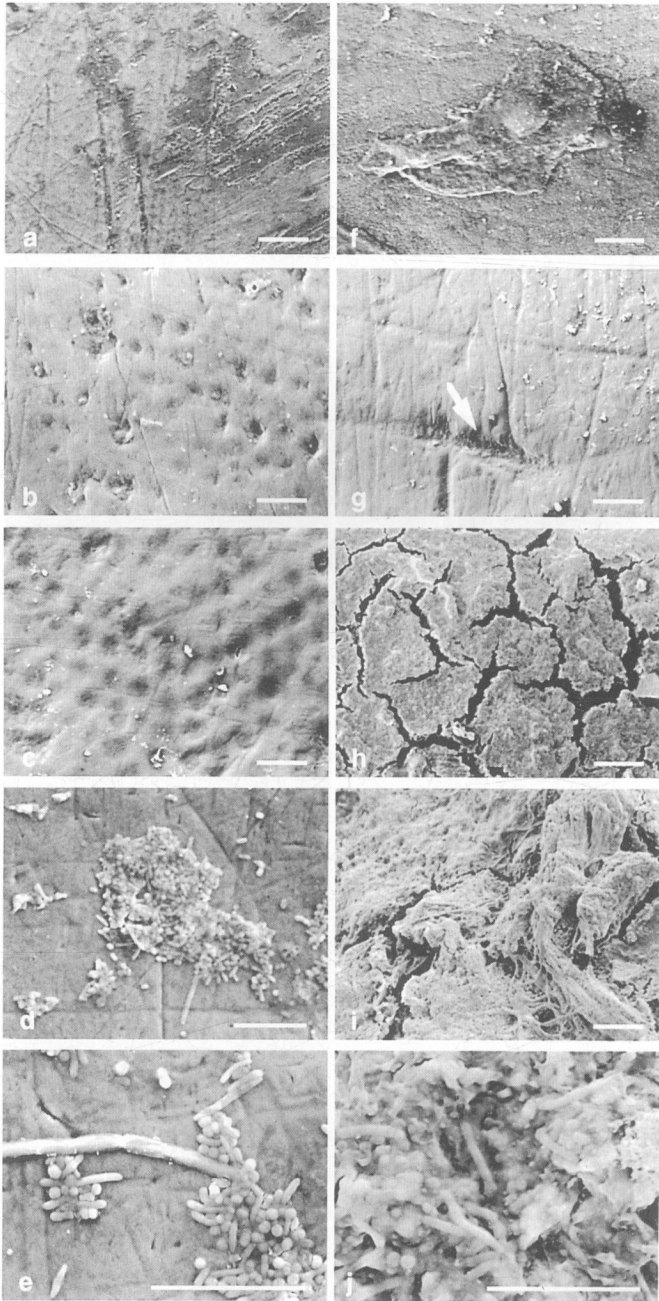


Fig. 2.

canines was confirmed by measurement of gingival crevicular fluid flow according to L oe and Holm-Pedersen (1965), and a thorough prophylaxis was done to remove visible plaque deposits on tooth surfaces.

Two enamel pieces were then bonded with composite resin (APH; Dentsply International Inc., Milford, USA) to the buccal surfaces of the upper left canine, premolars and first molar, giving a total of eight enamel pieces per volunteer. Six pieces were intended to be retrieved while the two extra pieces were put in to compensate for any premature losses. All the blocks were bonded slightly coronal to the gingival margin in order to prevent marginal plaque accumulations from extending onto the blocks. When all the enamel pieces had been attached, the volunteers started the 14-day period of experimental gingivitis during which they refrained from all oral hygiene measures; they were advised to maintain their normal dietary habits.

After allowing for undisturbed microbial colonization on the enamel pieces, the pieces were retrieved sequentially from the left first molar at 3-hr and 6-hr, the first premolar at 1-day and 3-day, the second premolar at 7-day and the canine at 14-day intervals. Any enamel pieces remaining were removed on day 14 (Fig. 1). Before detachment, each piece was rinsed gently with distilled water. Finally, the residual composite resin was removed and a thorough prophylaxis performed.

Specimen preparation

Immediately after detachment, the enamel pieces were placed for a minimum of 24 hrs in a plastic chamber containing a fixative of 25% glutaraldehyde and 10% paraformaldehyde in 0.2 M cacodylate buffer (adjusted to pH 7.4) (Karnovsky, 1965). The pieces were then rinsed four times in 0.1 M cacodylate buffer (pH 7.4) and immersed in 1% osmic acid in 0.1 M cacodylate buffer for 30 min, followed by four rinses in cacodylate buffer. Subsequently, the pieces were dehydrated by serial transfer in ascending concentrations of acetone (50–100%), critical point-dried in CPD 020 (Balzers Union, Liechtenstein), mounted on copper stubs and sputter-coated with gold to a thickness of approx. 15 nm (Fine Coat Ion Sputter JFC-1100; JEOL Ltd, Tokyo, Japan). All the samples were examined with a scanning electron microscope (JXA-840; JEOL Ltd) at 5–10 kV. Micrographs were taken at 100 \times to investigate the general pattern of plaque development and up to 5000 \times for specific morphological features.

RESULTS

Plaque growth rates

The mean PI of the 49 volunteers after 3 days of no oral hygiene ranged from 0.84 to 2.63. For the

five 'rapid' plaque formers, the mean PI was 2.5 with a range of 2.41 to 2.63; the mean PI of the six 'slow' plaque formers was 1.02 with a range of 0.84 to 1.16. The mean PI of the remaining unselected volunteers was 1.74 with a range of 1.19 to 2.31. All 11 selected participants showed clinically healthy gingiva with a minimal amount of crevicular fluid detected before the start of the 14-day period.

Diet

During the 14 days, all the participants maintained a basic Chinese diet with rice as the staple carbohydrate, supplemented by vegetable and protein in relatively smaller amounts with few or no sugary snacks between meals.

Enamel blocks

Sixty-five enamel blocks were retrieved from the 11 participants. In one of the 'slow' plaque formers, three enamel blocks became detached within 1 day and hence no 14-day sample was available for this individual.

Morphological features: 3-hr–1-day plaque

'Slow' plaque formers. Granular material was observed on six enamel blocks recovered from the 'slow' plaque formers after 3 hrs (Fig. 2a). This material was particularly evident on scratch marks possibly created by the pre-experimental prophylaxis (Fig. 2a). Whilst a sparse scattering of bacteria was found in three of the blocks, no bacteria were found on the others.

Similar granular material could be found on the 6-hr enamel blocks but increased numbers of organisms were noticeable, especially in pits and grooves (Fig. 2b). The vast majority of bacteria colonizing the blocks at this stage were coccid.

Variation in both the structure and the quantity of bacteria was noticeable on the 1-day-old blocks. These ranged from a surface covered with a very few bacteria, particularly colonizing the pits and grooves (Fig. 2c), to localized and confluent microcolonies of mixed coccid and rod-shaped bacteria (Fig. 2d). Despite the 24-hr period of exposure, individual bacteria were readily discernible in most samples in these 'slow' plaque formers (Fig. 2e).

'Rapid' plaque formers. The ultrastructural features of early plaque observed in the 3 to 6-hr enamel blocks obtained from the 'rapid' plaque formers were remarkably similar to those of the 'slow' plaque formers. Epithelial cells were found in some blocks (Fig. 2f) and, as in the 'slow' group, few bacteria were found scattered on the surface, especially in pits and grooves (Fig. 2g). However, the frequency of detection of bacteria was higher in these blocks than in those of 'slow' plaque formers.

The most significant morphological variations in plaque between the two groups of volunteers were noted after 1 day of plaque development when the

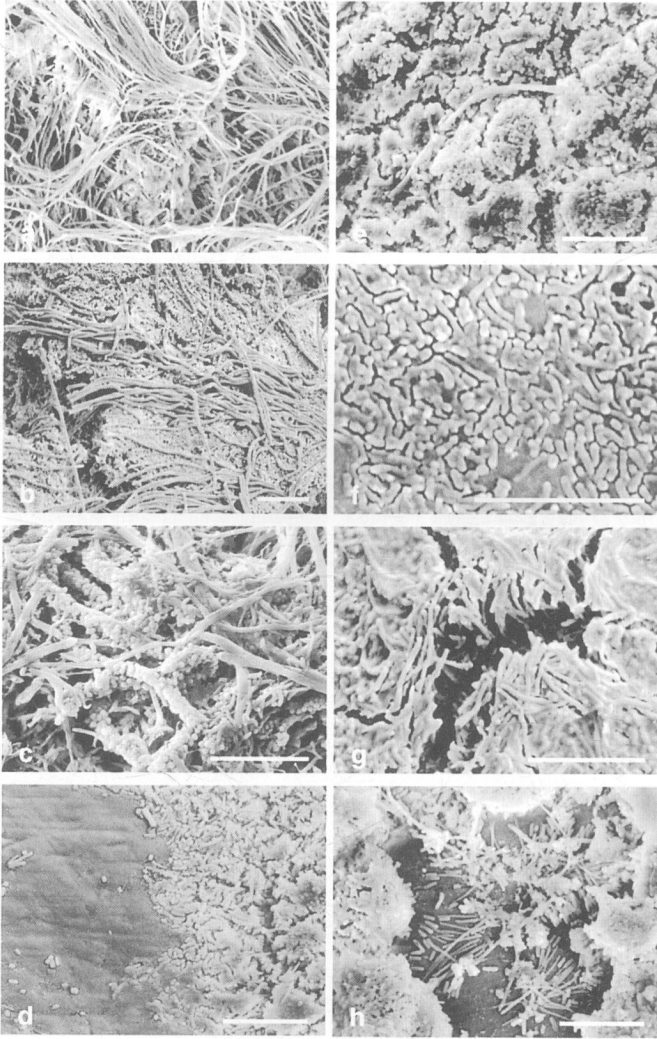


Fig. 3. Scanning electron micrographs of enamel blocks retrieved from 'slow' (a-d) and 'rapid' (e-h) plaque formers during 3 to 14 days of undisturbed plaque development. All bars represent $10 \mu\text{m}$. (a) Day-3 block: filamentous bacteria organized in a complex structure (original magnification: $\times 1000$). (b) Day-7 block: filamentous bacteria running parallel to the surface (original magnification: $\times 1500$). (c) Day-14 block: 'corn-cob' arrangement of bacteria (original magnification: $\times 3500$). (d) Day-3 block: bacteria spreading across the surface as a monolayer (original magnification: $\times 2500$). (e) Day-3 block: complex bacterial flora with intermicrobial matrix obscuring detailed individual features (original magnification: $\times 2500$). (f) Day-7 block: coccoid and rod-shaped bacteria embedded in a intermicrobial matrix (original magnification: $\times 5000$). (g) Day-14 block: a multilayered bacterial mass covered by an intermicrobial matrix, structure of the superficial organisms is visible; cracks are due to preparation artefacts (original magnification: $\times 3500$). (h) Day-14 block: aggregation of bacteria with, in between, a monolayer of bacteria developing (original magnification: $\times 2500$).

enamel surfaces from the 'rapid' group were totally covered with a multilayer of bacteria whereas the 'slow' group had relatively sparse bacterial colonization (Fig. 2c,h). The bacteria were closely aggregated, thus losing individual features, and covered by a surface coating possibly derived from saliva (Fig. 2h). Occasionally, filamentous organisms were found adhering to this surface layer and merging with the surrounding bacterial matrix (Fig. 2i) while individual bacteria were more readily discernible in the 'slow' plaque formers (Fig. 2d). Interbacterial granular material was more frequently observed and more well-formed in all day-1 enamel blocks of the 'rapid' plaque formers (Fig. 2j) when compared with those of 'slow' formers (Fig. 2e).

Morphological features: 3-14-day plaque

'Slow' plaque formers. The enamel blocks showed maturation of plaque structure after 3-14 days of undisturbed growth. Filamentous organisms were frequently detected, running both parallel and perpendicular to the surface (Fig. 3a, b). 'Corn-cob' formations were occasionally observed (Fig. 3c). The overall colonization pattern was variable, especially in the thickness of the bacterial mass; there were areas with no, or only a monolayer of, bacteria while voluminous and multilayered bacterial masses were found in adjacent areas (Fig. 3d).

'Rapid' plaque formers. All the enamel blocks retrieved on day 3 showed mature plaque development. The bacterial deposits were embedded in an intermicrobial matrix and organized into a complex structure (Fig. 3e). The day-7 and day-14 blocks had features similar to those of the day-3. Multilayered bacterial masses were composed of a prominent intermicrobial matrix obscuring the features of individual bacteria (Fig. 3f, g). In areas where individual features were visible, a complex microbial community consisting of filamentous, rod and coccoid micro-organisms could be detected (Fig. 3h). In most of the enamel blocks, intermicrobial matrix was more prominent in the 'rapid' plaque formers.

DISCUSSION

This morphological study shows that 'rapid' plaque formers exhibit a relatively complex plaque morphology earlier than 'slow' plaque formers after 24 hrs of undisturbed plaque formation. However, the morphological features of day-3 to day-14 plaque in both groups were remarkably similar except for the fact that 'rapid' formers had a more prominent intermicrobial matrix. This indicates that the differences in the rate of plaque formation in these two groups arose mainly as a result of early rather than late plaque development. Nevertheless, to obtain more objective data on this feature, a quantitative study of plaque development should be undertaken.

Our group recently reported a study using the same group of 'rapid' and 'slow' plaque formers to investigate predominant cultivable organisms in supragingival plaque (Zee *et al.*, 1996). Those results demonstrated statistically significant differences in the mean relative proportion of the Gram-positive and Gram-negative bacteria between the 'rapid' and 'slow' plaque formers. The 'slow' formers had a lower proportion of Gram-positive bacteria than the 'rapid' formers on day 1 but this proportion became greater than that of the 'rapid' formers from day 3 to 14. The proportion of Gram-negative bacteria was higher in the 'slow' than the 'rapid' plaque formers at day 1, but as the proportion of Gram-negative rods increased in the 'rapid' formers the overall proportion of Gram-negative bacteria became higher than that of Gram-positives at day 14. In the present study, we also observed that at day 1 the 'rapid' formers showed a more mature plaque structure than the 'slow' formers, while from day 3 onwards both groups showed a relatively similar complexity of plaque. There was, however, an obvious difference in PI after 3 days between 'rapid' and 'slow' plaque formers. This discrepancy may reflect the difficulty of comparing a quantitative measure of plaque with qualitative data on superficial ultrastructural features.

In general, in both groups of volunteers the bacteria colonized the enamel surface via a surface coating material likely to be salivary pellicle, first, as individual organisms retained in pits and grooves, and then forming microcolonies (Fig. 2d). These coalesced to form larger colonies and then spread out as a monolayer (Fig. 3d). Similar observations have been reported in other electron-microscopic studies (Lie, 1979; Lie and Gusberti, 1979; Nyvad and Fejerskov, 1987a).

Differences in the rate of plaque formation between individuals have been reported by some as purely clinical observations (Theilade *et al.*, 1966; Ritz, 1970; Bergström, 1981; Simonsson *et al.*, 1987a,b; Quirynen *et al.*, 1985; Quirynen and van Steenberghe, 1989) and by others at the ultrastructural level (Listgarten *et al.*, 1975; Lie 1977; Berthold 1979; Nyvad and Fejerskov, 1987a). However, to the best of our knowledge only Simonsson and co-workers (1987a, b) have identified 'heavy' and 'light' plaque formers by screening a large population, and attempted to investigate clinical and other variables appertaining to the observed differences in the colonization potential of plaque bacteria. They observed that among a total of 12 clinical and salivary variables, only three were significantly different between 'heavy' and 'light' plaque formers: the saliva-induced aggregation of certain oral streptococci, the amino acid composition of the acquired pellicle and the retention depth of the dentogingival area. However, due to the large individual variations, they concluded that

none of the investigated variables could alone explain the differences in the amount of plaque (formed after 3 days) between the 'heavy' and 'light' formers. Nevertheless, they surmised that combinations of some of the variables might have an impact on the rate of plaque formation. In the present study, the term 'slow' and 'rapid' were used instead of 'light' and 'heavy' as we believe that the differences in plaque growth in these groups are better expressed as a function of time rather than of the quantity of plaque.

The lack of a retention area on bare enamel surfaces may affect early plaque formation as plaque growth normally starts at the gingival margin and in interdental spaces and spreads in a coronal direction (Mierau and Singer, 1978; Mierau, 1984; Quirynen and van Steenberghe, 1989). Thus, when interpreting the morphological features of the developing supragingival plaque in the present study, it should be borne in mind that all the enamel blocks were attached to the buccal surfaces coronal to the gingival margin. Furthermore, as the deposition and retention of pioneer plaque bacteria is highly dependent on the microstructure of the surface (Lie, 1977; Nyvad and Fejerskov, 1987a,b), it has been suggested that natural tooth surfaces should not be replaced by artificial substrates with an unknown surface morphology in studies on early microbial colonization (Nyvad and Fejerskov, 1987a). On the other hand, there are various surface irregularities on the enamel blocks that enhance initial bacterial attachment (Lie, 1977; Nyvad and Fejerskov, 1987a) and surface roughness has been shown to have an effect on bacterial adhesion (for review see Quirynen and Bollen, 1995). For instance, in an *in vitro* experiment, Swartz and Phillips (1957) reported a 10-fold increase in colony-forming units after roughening of the enamel surfaces. Therefore the observed quantitative and qualitative differences in early supragingival plaque formation between the 'rapid' and 'slow' formers may have been influenced to some extent by these confounders. Nonetheless, as great care was taken to randomize the enamel blocks between the two groups, the differences in plaque formation observed are likely to be authentic. Further quantitative studies, possibly using planimetry or image-analysis systems, or quantitative microbiology, in a larger cohort of individuals are essential to confirm the current data. Moreover, the implications of such a difference in the structure of early supragingival plaque under conditions of experimental gingivitis on the initiation of periodontal disease may need to be further investigated.

In conclusion, the results of this morphological SEM study on the development of supragingival plaque in 'rapid' and 'slow' plaque formers tend to suggest that there are quantitative and qualitative differences in the rate of early (3 hr-1 day) plaque development between the two groups.

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An in vivo replica study of microbial colonization in "rapid" and "slow" dental plaque formers

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Zee K-Y, Samaranayake LP & Attström R. An in vivo replica study of microbial colonization in "rapid" and "slow" dental plaque formers. *APMIS* 2000;108:113-21.

The aim of the present study was to investigate the morphological features of supragingival plaque development in "rapid" and "slow" plaque formers using the replica technique. Forty-nine healthy volunteers were screened for their plaque formation rate after 3 days of oral hygiene abstinence using the plaque index (PI). Five subjects with the highest mean PI and six with the lowest mean PI were selected as "rapid" and "slow" plaque formers, respectively. After a series of thorough prophylaxis and oral hygiene instruction, all subjects went through a 14-day period of no oral hygiene to allow undisturbed plaque formation. Light body silicone elastomeric impressions were taken for the upper right central incisor of each subject on day-0, 1, 3, 7, and 14. Positive replicas were then poured from the impressions using epoxy resin and observed under a scanning electron microscope. A more complex supragingival plaque structure was seen in the day-1 and day-3 replicas of the "rapid" group compared to the "slow" group. From day-7 to day-14, during the maturation period of supragingival plaque, no discernible differences were noted between the two groups. Quantitatively, the percentage of plaque coverage on the tooth surfaces was higher in the "rapid" group than in the "slow" group in day-1 and day-3 replicas. These observations indicate that the morphological and topographical features of supragingival plaque in "slow" and "rapid" plaque formers differ, especially in the early developmental phase.

Key words: SEM; replica; supragingival plaque; rate of plaque formation; experimental gingivitis.

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Sequential supragingival plaque development has been studied extensively either using culture techniques (Theilade & Theilade 1970; Syed & Loesche 1978; Socransky et al. 1977; Moore et al. 1982; Moore et al. 1987) or by light and/or transmission electron microscopy (Theilade & Theilade 1970; Listgarten et al. 1975; Brex et al. 1981; Nyvad & Fejerskov 1987b). Other workers have used scanning electron microscopy (Berthold et al. 1971; Eastcott & Stallard 1973; Lie 1974; Nyvad & Fejerskov 1987a; Walsh et al. 1991) or this together with a replica

technique (Saxton 1973; Lie & Gusberti 1979; Lambrechts et al. 1982). Differences in the rate of plaque formation between individuals have also been documented by some as clinical observations (Theilade et al. 1966; Ritz 1970; Bergström 1981; Simonsson et al. 1987a, 1987b; Quirynen et al. 1985; Quirynen & van Steenberghe 1989) and others at the ultrastructural level (Listgarten et al. 1975; Lie 1977; Berthold 1979; Nyvad & Fejerskov 1987a). Despite the availability of such voluminous data, information on the structural and microbiological differences of supragingival plaque in subjects with different rates of plaque formation is scarce.

Simonsson et al. (1987a & b), in a pioneering

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study, characterized biochemical, biophysical and microbiological features of "heavy" and "light" plaque formers and the related clinical findings in the respective subject groups. The authors concluded that several of these factors might be involved, although none of the studied variables could by itself explain the large differences in the amount of plaque formed. However, they did not investigate the detailed composition and structure of the supragingival plaque in these subjects.

In a previous study we used enamel blocks to investigate the morphological features of supragingival plaque development in "rapid" and "slow" plaque formers (Zee *et al.* 1997). The enamel blocks were attached onto the buccal surfaces of teeth in a group of volunteers and were sequentially removed and observed using scanning electron microscopy. As plaque growth normally starts at the gingival margin and the interdental spaces, and spreads in a coronal direction (Mierau & Singer 1978; Mierau 1984; Quirynen & van Steenberghe 1989), the enamel blocks which could only be attached onto the buccal surfaces coronal to the gingival margin lacked a marginal retention area and, hence, poorly simulated plaque formation in humans. Moreover, it was impossible to observe the sequential changes of plaque development on the same surface using this method.

The indirect replicating method is widely used for studying dental plaque morphology (Saxton 1971, 1973 & 1976; Lie & Gusberti 1978 & 1979; Lambrechts *et al.* 1982). It is superior to other techniques as it permits detailed visualization of microbial structures on natural tooth surfaces and, more importantly, the sequential changes during plaque formation in longitudinal studies. Hence, the aim of the present study was to investigate the morphological features of supragingival plaque development in "rapid" and "slow" plaque formers using the replica technique.

MATERIAL AND METHODS

Selection of "rapid" and "slow" plaque formers

The selection of the "rapid" and "slow" plaque formers has been described in detail in a previous study (Zee *et al.* 1996). In brief, a total of 49 ethnic Chinese dental students, dental surgery assistants

and dental hygienists from the Prince Philip Dental Hospital, Hong Kong, volunteered for the study. The subjects were screened to exclude those with extensive interproximal or buccal restorations, untreated caries or periodontal diseases, and recent treatment with antibiotics; none of the subjects smoked. After this preliminary screening, suitable subjects entered a 3-week oral hygiene period, with professional cleaning and oral hygiene instruction once a week. At the start of the plaque accumulation periods, the gingival health of the subjects was confirmed by measurements of gingival cervicular fluid (GCF) according to Löe & Holm-Pedersen (1965). The plaque formation rate was then assessed using the plaque index (PI) (Silness & Löe 1964) after 3 days of undisturbed plaque accumulation. PI on the buccal surfaces of all canines, premolars and first molars was registered, yielding a total of 16 sites per individual. To ascertain the screening results, the above procedure was repeated after 1 month and mean plaque scores were recalculated for each individual. The collated data from the first and second plaque accumulation periods were used to select the five "rapid" plaque formers with the highest mean PI scores and the six "slow" plaque formers with the lowest mean PI scores (Simonsson *et al.* 1987a).

Dietary sheets were given to all subjects to record their daily food and drink intake during the study period. Permission to conduct the study was sought and granted by the Ethics Committee of the Faculty of Dentistry, University of Hong Kong. Informed consent was obtained from all the subjects.

Replica preparation

Before the start of the 14-day experimental gingivitis period, the selected 11 subjects received prophylaxis and oral hygiene instruction once a week for 3 weeks to ensure gingival health. At day 0, GCF was taken to confirm the gingival health of all the subjects and thorough prophylaxis removed observable plaque deposits on tooth surfaces. During the 14-day experimental gingivitis period, undisturbed plaque accumulation was facilitated by refraining from all oral hygiene measures although the subjects were advised to maintain their routine dietary habits.

The buccal surface of the upper right central incisor of each subject was replicated using the method of Lambrechts *et al.* (1982). A light body silicone elastomeric impression material (President, Coltène® AG, Altstätten, Switzerland) was used throughout the study. Impressions were taken on day 0, 1, 3, 7 and 14 after the tooth surface was thoroughly rinsed with distilled water and gently dried by air.

When the polymerized impression was removed from the tooth surface, it was rinsed in running tap water and then distilled water. After air drying, epoxy resin (Taab 812, Taab Laboratory Equipment, England) was poured onto the impression to produce a

positive replica within 3 h. The resin replica was polymerized for 18 h at 60°C in an oven and subsequently sputter coated with gold for 8 min in an ion sputter coater (JFC-1100, JEOL Ltd., Tokyo, Japan). All the replicas were examined with a scanning electron microscope (JXA-840, JEOL Ltd.) at 5–10 kV. Micrographs were taken at 500× to 4000× magnifications at eight sites in each replica, as shown in Fig. 1, so that similar areas could be compared within and between different subjects. Micrographs of other areas were also taken whenever appropriate.

The extent of the plaque colonization on each tooth surface was quantified using the replicas with the help of an image analyzer (IBAS2000, Lecia, Germany) and expressed as the percentage of the tooth surface covered by at least a monolayer of plaque. Image analysis was performed in a blind manner by a single operator who was unaware of the specimen groups.

RESULTS

All the subjects had clinically healthy gingivae with minimal GCF detected before the start of the oral hygiene abstinence period. The mean PI of the 49 subjects after 3 days of oral hygiene abstinence ranged from 0.84 to 2.63. For the five “rapid” plaque formers the mean PI was 2.5 (range 2.41 to 2.63). The mean PI of the five “slow” plaque formers was 1.02 (range 0.84 to 1.16). The mean PI of the remaining unselected subjects was 1.74 (range 1.19 to 2.31).

Diet

All the subjects maintained a basic Chinese diet with rice as the staple carbohydrate, supplemented by vegetable and proteins in relatively smaller amounts, and with little or no sugary snacks in between meals during the 14-day experimental period.

Morphological features: day-0 – day-3 replicas

“Slow” plaque formers. At day-0 immediately after prophylaxis, all the replicas failed to show any microbes or deposits. Scratch marks, running in haphazard directions, were visible in the replicas, possibly due to the prophylactic polishing procedure. At day-1, microbial deposits were found on two of six replicas in the “slow” plaque formers, mainly in the mesial and distal aspects along the gingival margin or within grooves or pits of the tooth surface (Fig. 2a). A general pattern of plaque development was

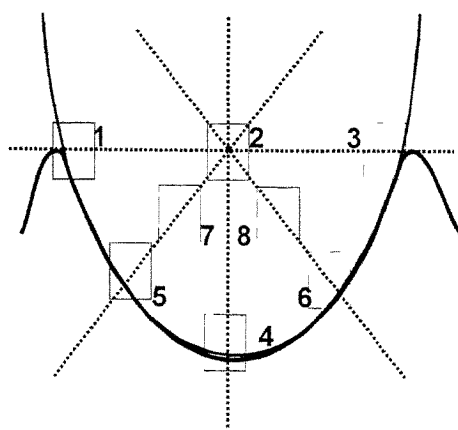
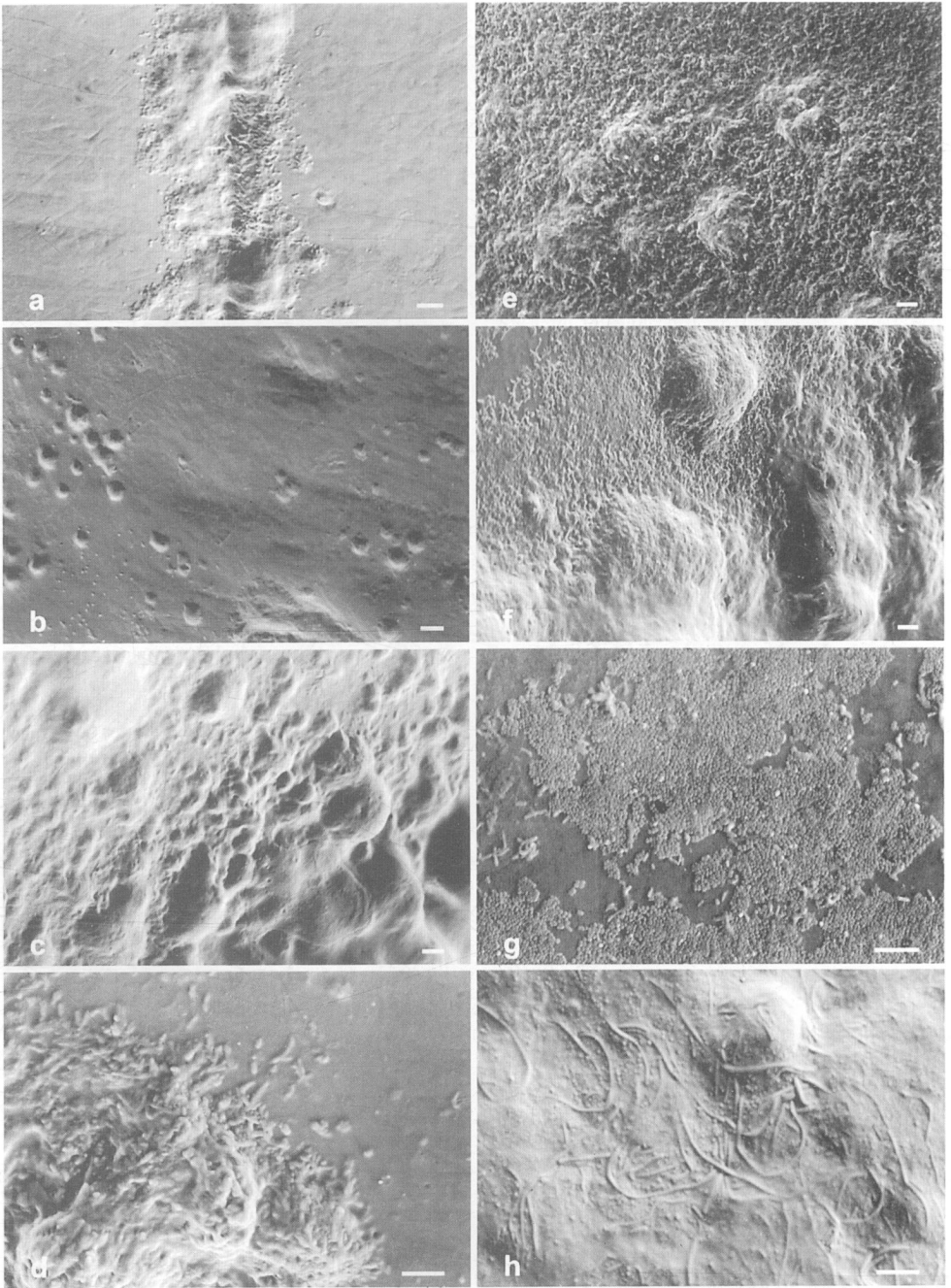


Fig. 1. A schematic diagram of the eight regions on the tooth surface sampled using the replica technique and scanning electron microscopy.

discernible at this stage: the tooth surfaces were first covered by an amorphous film and bacterial deposits were organized into discrete aggregates spreading on the tooth surfaces (Fig. 2b). Occasionally, globular deposits ranging in size from 5 to 20 μm close to the gingival margins could be identified (Fig. 2c). At day-3, plaque was found in all the replicas along the gingival margin and spreading coronally towards the incisal edge. The globular deposits were again obvious and higher magnifications showed coccoid bacteria and short rods, especially at the margins of the globular deposits where a monolayer of bacteria was discernible (Fig. 2d).

“Rapid” plaque formers. The day-0 replicas also showed no plaque or deposits on the tooth surfaces after professional prophylaxis. In contrast to day-1 samples of the “slow” plaque formers, deposits could be found in all five replicas in the “rapid” group. Again, plaque colonization was seen along the gingival sulcus and the mesial and distal aspects of the tooth surfaces. Rough textured globular deposits were observed which coalesced to form a monolayer of confluent bacterial growth, eventually developing into a continuous undulating surface (Fig. 2e). On day-3, bacterial colonization was accentuated with further development of globular deposits on the tooth surfaces (Fig. 2f). Besides



the coccoid and short rod forms (Fig. 2g) seen in the "slow" plaque formers, filamentous bacteria were readily discernible on the surfaces of a few replicas (Fig. 2h), suggesting the early development of a more complex and mature flora.

Morphological features: day-7 – day-14 replicas

The morphological features of the plaque development in both groups were very similar during this period. On day-7, the undisturbed plaque covered approximately 65% of the tooth surfaces in both groups. The features were complex with a multitude of rod-shaped bacteria criss-crossing in many directions on the pioneer plaque community (Fig. 3a). The bacteria were embedded in an intermicrobial matrix (Fig. 3b) comprising many irregularly scattered globular deposits (Fig. 3c). These features were also found in the day-14 replicas; but, in addition, the surface features were characterized by an amorphous nondescript surface coating together with filamentous and long rod-shaped bacteria sometimes arranged in palisades or in an irregular manner (Figs. 3d, e & f). When the surface features were not obscured by the superficial coating, bacteria could be seen cohabiting within a well-formed intermicrobial matrix (Fig. 3g). Surprisingly, even on day-14, an advancing front of plaque could be seen in some areas where a monolayer of bacteria resembling day-3 plaque (Figs. 2d & e) was visible (Fig. 3h).

Extension of plaque

The mean percentage plaque-covered tooth area during the 14-day period of oral hygiene abstinence is shown in Fig. 4. The most obvious difference in the plaque coverage was observed on day-1 when the area of microbial growth in the "rapid" plaque formers was almost 24 times that of the "slow" group (9.5% "rapid" group versus 0.4% "slow" group, $p < 0.01$). This differ-

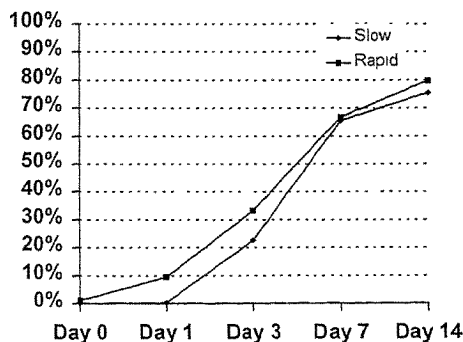


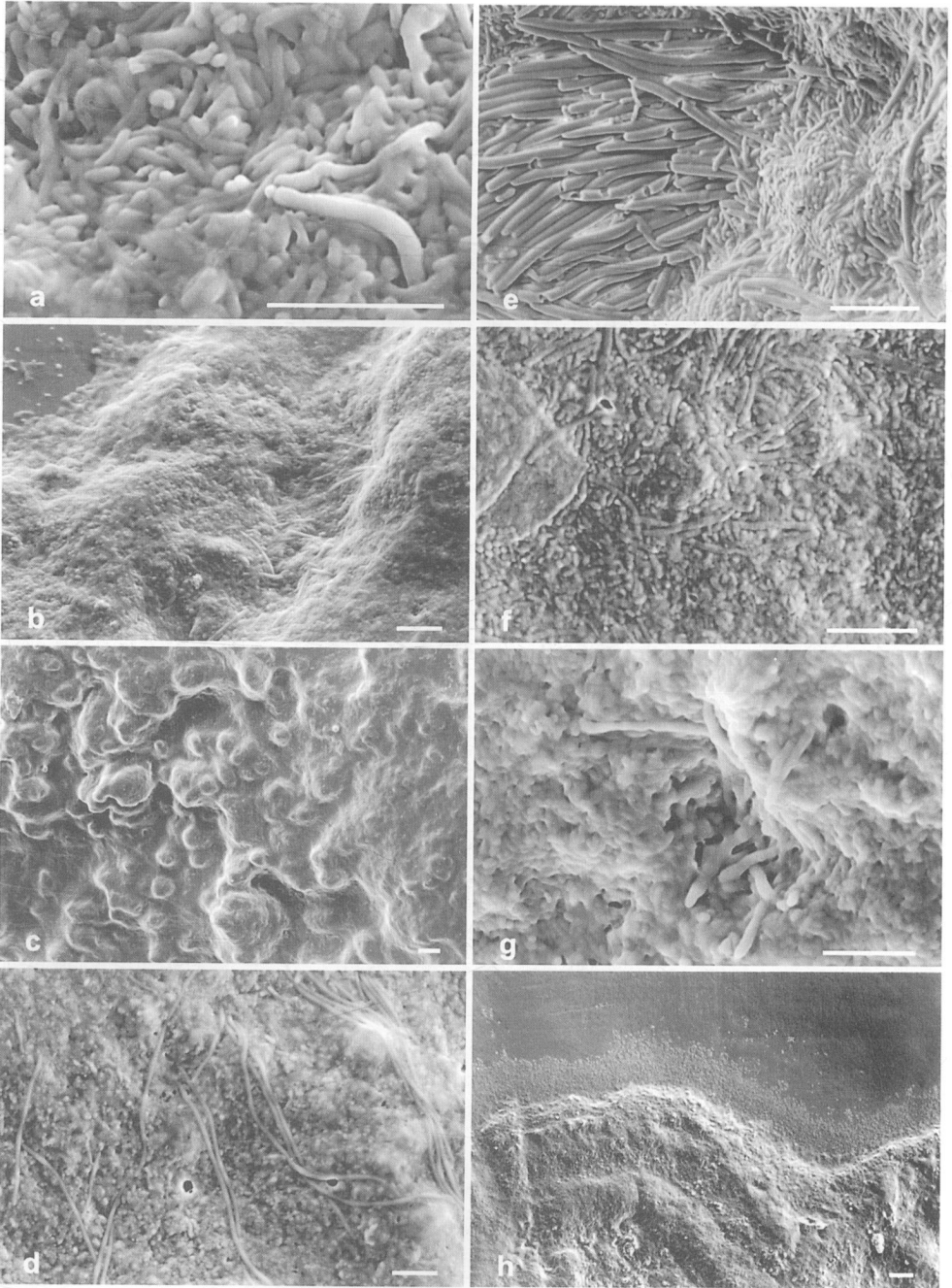
Fig. 4. The relative percentage plaque coverage detected on the surfaces of the replicas taken from the "slow" and "rapid" plaque formers during the 14-day period.

ence between the "slow" and "rapid" plaque formers gradually decreased from then onwards, and on day-7 and day-14 a similar percentage coverage could be observed in both groups.

DISCUSSION

The results of the present study indicate that the morphological features of the supragingival plaque formed in "rapid" plaque formers are relatively more complex than in the "slow" plaque formers, especially during the first 3 days of undisturbed plaque formation. The complex morphological features were characterized by filamentous bacteria found in the replicas of the "rapid" plaque formers in addition to the cocci and rods which also appeared in "slow" plaque formers. Quantitatively, the most significant differences were found on day-1. Thereafter, both the morphological features and the percentage of plaque coverage were very

Fig. 2. Representative scanning electron micrographs of replicas obtained from "slow" (a-d) and "rapid" (e-h) plaque formers on day-1 and day-3 of the undisturbed plaque development period. All bars represent 10 μ m. (a) Day-1 replica: bacteria colonizing and proliferating within an enamel groove. (b) Day-1 replica: discrete bacterial aggregates colonizing the tooth surface. (c) Day-1 replica: globular deposits ranging in size from 5–20 μ m. (d) Day-3 replica: higher magnification of the globular deposits showing the surfaces colonized by bacteria; individual bacterial imprints are visible at the edges of the deposits. (e) Day-1 replica: globular deposits coalescing with monolayers of bacteria forming an undulating surface. (f) Day-3 replica: monolayers of bacteria and globular deposits further colonizing the surface. (g) Day-3 replica: a monolayer of bacteria composed of coccoid and short rod shapes. (h) Day-3 replica: filamentous bacteria running in different directions on a surface covered by plaque and apparently homogeneous surface matrix.



similar between the two groups. These results further confirm our previous findings derived using *in situ* enamel blocks (Zee et al. 1997) which showed that differences in the rate of plaque formation in these two groups arise mainly as a result of early, rather than late, plaque development. In general, the plaque development pattern was in agreement with that reported in previous scanning electron microscopic studies (Lie 1979; Lie & Gusberti 1979; Nyvad & Fejerskov 1987a).

The major advantage of using replicas rather than enamel blocks is the possibility of observing successional microbial structures on natural tooth surfaces and the sequential changes that occur during undisturbed plaque development *in vivo*. The only drawback is that the impression material is rather sensitive to moisture and this may affect the setting period and the sensitivity of the technique. For this reason, Lie & Gusberti (1978) recommended repeating the procedure a number of times to improve the reproduction details of the colonization pattern. The good quality scanning electron microscopy images obtained by us in the present study imply that with care and correct manipulation of the materials, this is an acceptable technique for investigating plaque development *in vivo*.

The most prominent feature reported by us here is the presence of globular deposits throughout the 14-day study period in both the "slow" and "rapid" plaque formers. Although this phenomenon was reported by previous workers using the replica method (Saxton 1971, 1973 & 1976; Lie & Gusberti 1978; Lambrechts et al. 1982), others who used only scanning electron microscope did not observe such deposits (Berthold et al. 1971; Eastcott & Stallard 1973; Nyvad & Fejerskov 1987a; Walsh et al. 1991; Zee et al. 1997). Saxton (1973) suggested that the globular deposits are likely to be bacterial aggregates attached to the tooth surfaces, while

Lie & Gusberti (1978) surmised that they were artifacts due to air bubbles or moisture derived from saliva or gingival exudate. According to our observations, the surfaces of these globular structures were always covered by bacteria (Figs. 2c, 2e & 2f) and, under higher magnification, it was apparent that they comprised bacteria embedded in an intermicrobial matrix. We are unable to agree with Saxton (1973) that such globular deposits are randomly distributed focal bacterial aggregates. On the contrary, we propose that the globular deposits are first formed by bacteria which attach onto pits and grooves of the enamel in the initial plaque formation stage. These pioneer bacteria appear to proliferate and develop into microcolonies and eventually coalesce with the monolayers of neighboring bacteria on the subjacent tooth surfaces (Figs. 2e & 3b). Similar observations have been reported by Björn & Carlsson (1964) in very early studies using clinical photography. They observed focal bacterial aggregates with a hemispherical appearance in the early phase of dental plaque development within a matrix of organic material, possibly polysaccharide in nature, and subsequently covered by salivary components.

We previously studied the same groups of "rapid" and "slow" plaque formers to investigate the predominantly cultivable bacteria in supragingival plaque (Zee et al. 1996). The "slow" plaque formers showed a statistically significant higher relative proportion of gram-positive bacteria and a lower relative proportion of gram-negative bacteria when compared with the "rapid" plaque formers. This suggested that the "rapid" plaque formers tend to attain a complex flora earlier than the "slow" plaque formers. The morphological features as well as the quantitative data derived from the replicas of the present study support these findings. These results taken together suggest that the ini-

Fig. 3. Representative scanning electron micrographs of replicas obtained from "slow" and "rapid" plaque formers on day-7 and day-14 of the undisturbed plaque development period. Figs. 3a, c, e & g are from replicas of "slow" plaque formers, while b, d, f & h are from replicas of "rapid" plaque formers. All bars represent 10 μ m, except h (bar=100 μ m). (a) Day-7 replica: rod-shaped and filamentous bacteria running in different directions. (b) Day-7 replica: bacteria embedded in an intermicrobial matrix. (c) Day-7 replica: prominent globular deposits. (d)–(f) Day-14 replicas: filamentous bacteria running in different directions on the surfaces of the pioneer bacteria deposits already formed. (g) Day-14 replica: coccoid and rod-shaped bacteria aggregated in an intermicrobial matrix. (h) Day-14 replica: a monolayer of coccoid forms at the advancing front of the plaque development.

tial enamel colonization by the pioneer bacterial species may be a major factor contributing to the dynamics of plaque formation. However, to demystify the enigma of differential rates of plaque formation in varying population cohorts further investigations into the micro-ecological as well as host factors contributing to this process are needed.

In clinical terms, a complex supragingival plaque flora has been shown to be associated with inflammatory changes in the adjacent gingival margin (Theilade *et al.* 1966; Moore *et al.* 1982; Moore *et al.* 1987; Syed & Loesche 1978). The observation of a more complex flora early in the "rapid" plaque formers in the present study indicates that they might be more prone to develop inflammatory changes in the gingivae compared to the "slow" plaque formers. However, so far no study has shown differences between rapid and slow plaque formers in the propensity to develop gingival inflammation. A refined methodology for studying the inflammatory processes in the gingival margin is required for the demonstration of such biological differences in the inflammatory processes in the gingivae of individuals with different rates of plaque formation.

In conclusion, there are qualitative and quantitative differences in the early supragingival plaque development between the "rapid" and "slow" plaque formers. The indirect replication used in this study seems to be an acceptable method to investigate the morphological features of supragingival plaque formation *in vivo*.

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IV

Short Article

Salivary immunoglobulin A levels in "rapid" and "slow" plaque formers: A pilot study.

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OBJECTIVE: To investigate the salivary immunoglobulin A (IgA) concentration in "rapid" and "slow" plaque formers.

SUBJECTS AND METHODS: After 3 days of oral hygiene abstinence, 49 healthy volunteers were screened using the plaque index (PI) to assess their plaque formation rates. Five subjects with the highest, and five with the lowest mean PI were selected as "rapid" and "slow" plaque formers, respectively. Unstimulated whole saliva was collected from each of these subjects and the salivary IgA levels assessed using a conventional ELISA technique.

RESULTS: When the differences between the two groups were compared, almost a twofold increase in the mean salivary IgA concentration in the "slow" ($17\mu\text{g/ml} \pm 6$) as compared with the "rapid" ($9\mu\text{g/ml} \pm 2$) plaque formers was noted ($p < 0.05$).

CONCLUSION: These findings, reported for the first time, imply that salivary IgA may play a crucial role in regulating the pioneer plaque development on enamel surfaces.

Key words: supragingival plaque, immunoglobulin, salivary IgA, rate of plaque formation

1977, Berthold, 1979, Nyvad and Fejerskov, 1987). However, only a few have attempted to investigate parameters associated with differences in the rate of plaque formation. Simonsson *et al* (1987a&b) in a pioneering study, characterized "heavy" and "light" plaque formers from clinical, biochemical, biophysical and microbiological aspects. These authors concluded that several of the investigated factors might be involved in plaque formation although none of the studied variables alone could explain the large differences in the amount of plaque formed between the "heavy" and "light" plaque formers.

It has been suggested that antimicrobial factors in saliva such as lysozyme (Salton, 1961, Brandtzaeg and Mann, 1964), lactoferrin (Kochan, 1973, Adamson and Carlsson, 1982), glycoproteins (Hay *et al*, 1971, Ericson and Magnusson, 1976) and secretory IgA (Williams and Gibbons, 1972) may influence plaque development. IgA is the predominant salivary immunoglobulin which, under normal conditions, is the only immunoglobulin that is actively secreted into the oral cavity. Previous work has indicated that salivary IgA exerts a direct inhibitory effect on the adherence of microorganisms to host mucosal epithelial cells (Abraham and Beachey 1985) and to saliva coated hydroxyapatite (Reinholdt and Kilian, 1987). However, most of these investigations were done *in vitro*. As it is important to confirm whether IgA mediated suppression of bacterial adherence and colonization

Introduction

Variations in the rate of plaque formation between individuals have been reported previously, both in clinical terms (Theilade *et al*, 1966, Ritz 1970, Bergström, 1981, Simonsson *et al*, 1987a, Simonsson *et al*, 1987b, Quirynen *et al*, 1985, Quirynen and van Steenberghe, 1989) and at the ultrastructural level (Listgarten *et al*, 1975, Lie,

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can reduce the rate of plaque formation in vivo, the aim of the present study was to compare the salivary IgA levels in two cohorts of "rapid" and "slow" plaque formers.

Material and Methods

As there is no data on the salivary IgA levels in Chinese subjects, a pilot study was performed. Eight ethnic Chinese dental students (3 male and 5 female) aged 21 to 25 were enrolled into a 14-day experimental gingivitis study (Løe *et al*, 1965). Prophylaxis with pumice and flossing were performed for each subject once every 2 days for 10 days before the experimental gingivitis period to ensure gingival health. All subjects then started a 14-day period of oral hygiene abstinence during which, saliva samples were collected from the 8 subjects at day 0, 2, 4, 6, 8, 10 and 14. A thorough prophylaxis was performed on each subject at day 14 and additional saliva samples were collected after 3 days.

Saliva collection

The subjects were asked to expectorate unstimulated saliva into an ice-chilled beaker at least one hour after meal. After collection, the saliva was immediately sent to the laboratory for centrifugation at 10,000g for 15 min., and the supernatants were stored in Eppendorf tubes at -70°C. Before analysis, the samples were thawed at room temperature.

Determination of salivary IgA

Salivary IgA levels were determined using the ELISA method as previously described (Sandholm *et al*, 1987). Polystyrene microtiter plates were coated with goat anti-human IgA (Sigma BioSciences, St. Louis, USA) by adding 200µl of antiserum diluted 1:10000 in phosphate buffered saline (PBS; pH 7.3) per well. The plates were then wrapped in plastic sheets and kept at room temperature overnight. Before use, the plates were washed 3 times in water containing 0.15M NaCl, 0.1% (v/v) Tween 20 and 0.02% (w/v) sodium azide in an automatic ELISA washing machine (Tri-Continent Scientific Inc., Grass Valley, USA).

Reference curves for IgA were established by testing serial dilutions of purified human colostrum

IgA (Sigma BioSciences). The IgA was diluted with PBS containing 5mg/ml bovine serum albumin (PBS/BSA) plus 0.02% sodium azide, and 200µl was applied per well. The plates were incubated at 37°C overnight.

Test samples of whole saliva were diluted 1:10, and 200µl was applied per well. After overnight incubation as described above, the plates were washed and 200µl of goat anti-human IgA alkaline phosphatase conjugate (Sigma BioSciences) diluted 1:28000 in PBS/BSA was applied per well. The plates were incubated at 37°C for 2 hours, washed 5 times and incubated with 1.0 mg/ml *p*-nitrophenyl phosphate (*p*NPP, Sigma BioSciences) in 10% diethanolamine buffer, pH 9.8 with 0.5 mM MgCl₂ in the dark for 30 minutes at room temperature. The color change was then read in a spectrophotometer (SpectraMax 340, Molecular Devices Corporation, California, USA) at 405nm. Each plate included duplicate negative controls (coated wells reacted with PBS/BSA), duplicate references (coated wells reacted with standard IgA), duplicated standard IgA in uncoated wells and triplicate test samples. Results of the concentration of salivary IgA were calculated from the reference curve and expressed as µg/ml.

Selection of "rapid" and "slow" plaque formers

A total of 49 ethnic Chinese subjects volunteered for the study. They were dental students, dental surgery assistants and dental hygienists from the Prince Philip Dental Hospital, Hong Kong. The subjects were screened to exclude those with extensive interproximal or buccal restorations, untreated caries or periodontal diseases, and recent treatment with antibiotics; none of the subjects were smokers. After this preliminary screening, suitable subjects entered a 3-week oral hygiene period, comprising once a week professional cleaning and oral hygiene instruction. The plaque formation rate was then assessed using the plaque index (PI, Silness and Løe, 1964) after 3 days of undisturbed plaque accumulation. PI on the buccal surfaces of all canines, premolars and 1st molars were registered yielding a total of 16 sites per individual. To ascertain the screening results, the above procedure was repeated after 1 month and mean plaque scores recalculated for each individual. The collated data from the first and second plaque accumulation pe-

riods were utilized to select five "rapid" plaque formers with highest mean PI scores and six "slow" plaque formers with lowest mean PI scores. One subject in the "slow" plaque former group was not available for saliva sampling, therefore a total of 10 individuals (7 males, 3 females) were used in the final study.

Permission for the study was sought and granted by the Ethics Committee of the Faculty of Dentistry, The University of Hong Kong. Informed consent was obtained from all the subjects.

The saliva collection and determination of salivary IgA levels followed the same protocol as described above except only one saliva sample was collected for each of the "rapid" and "slow" plaque formers.

Statistical Analysis

The plaque forming capacities of the "rapid" and "slow" plaque formers in the first and second 3-day plaque accumulation period were compared using the Wilcoxon Matched Pairs Signed Ranks Test. The analysis of the salivary IgA was repeated after 1 week in order to test the reproducibility. Differences between the results of the two analyses were tested using paired *t*-test while differences in the mean concentration of the salivary IgA between the two groups of plaque formers were compared using unpaired *t*-test with Welch correction.

Results

Range of Salivary IgA levels

The salivary IgA levels of the 8 subjects in the pilot study ranged from 2.0 to 49.1 µg/ml during the 14-day experimental gingivitis period. The mean IgA level was 12.6 µg/ml three days after they resumed normal oral hygiene practice.

Selection of subjects

The mean PI of the 49 subjects after 3 days of no oral hygiene ranged from 0.84 to 2.63. For the five "rapid" plaque formers, the mean PI was 2.5 with a range of 2.41 to 2.63. The mean PI of the five "slow" plaque formers was 1.02 with a range of 0.84 to 1.16. Differences in the mean PI of the first, and second 3-day plaque accumulation period of the 10 subjects were not statistically significant ($p=0.3552$). The mean PI of the remaining unselected subjects was 1.74 with a range of 1.19 to 2.31.

Salivary IgA

Results of the mean salivary IgA concentration with standard deviation of the two groups of plaque formers measured on two separate occasions are shown in Table 1. "Rapid" plaque formers showed a statistically significant, 46% lower mean concentration of salivary IgA (9.0µg/ml) than the "slow" plaque formers (16.8µg/ml; $p<0.05$). There was no significant difference in the IgA levels between the first and the repeat analysis.

Discussion

Salivary IgA is known to inhibit bacterial adherence to oral structures by blocking adherence determinants, reducing hydrophobicity, and agglutination of bacteria (Kilian and Bratthall 1986). Such properties have been confirmed in vitro (Williams and Gibbons, 1972, Kilian *et al*, 1981, Reinholdt and Kilian, 1987). Results of the present pilot study indicated that subjects with a slow rate of plaque formation have significantly higher salivary IgA levels than individuals with rapid plaque formation rates. Although this appears to be the first time that salivary IgA levels in subjects with extreme

Table 1 Mean Salivary IgA concentrations (µg/ml ± S.D.) of the "rapid" and "slow" plaque formers as measured by ELISA on two different occasions. (n.s. = not statistically significant)

	1 st Measurement	2 nd Measurement	<i>p</i> value
"Rapid" group	9.20±2.59	9.00±1.87	0.3974 (n.s.)
"Slow" group	15.60±4.04	16.80±6.30	
<i>p</i> value	0.0175		

plaque formation rates are shown to correlate with undisturbed plaque formation, it is important to bear in mind that our results were obtained from two groups with very few subjects. Hence, the results should be interpreted with caution.

The only comparable study that could be found in the literature was that by Simonsson *et al.* (1987a), who investigated clinical and biochemical characteristics and the rate of plaque formation in a group of "heavy" and "light" plaque formers. Although they were unable to show differences in salivary IgA, lactoferrin, lactoperoxidase and lysozyme levels between the "heavy" and "light" plaque formers, the saliva-induced aggregation of certain oral streptococci was found to be significantly different between the two groups of subjects. The differences between this study and ours might be due to the variations in the methodology used for detection of salivary IgA, and/or the ethnic differences between the investigated cohorts. All subjects in the current study were Chinese as opposed to a Scandinavian cohort used by Simonsson and co-workers.

We used the identical groups of "rapid" and "slow" plaque formers to investigate the predominantly cultivable bacteria in supragingival plaque in a recent study (Zee *et al.*, 1996). The results demonstrated statistically significant differences in the mean relative proportion of gram-positive/gram-negative bacteria between the "rapid" and "slow" plaque formers. The "slow" plaque formers had a lower proportion of gram-positive bacteria than the "rapid" plaque formers on day 1 of undisturbed plaque formation. Kilian *et al.* (1981) investigating the interactions of salivary IgA with sorption of oral bacteria to hydroxyapatite showed the ability of this immunoglobulin component to influence the initial sorption of oral bacteria, especially the early colonizer species, to hydroxyapatite. This, perhaps may be a factor which contributed to a lower proportion of gram-positive bacteria we observed in the day-1 supragingival plaque flora of the "slow" plaque formers.

In conclusion, our results indicate that salivary IgA might be an important factor contributing to the differences in the rate of early plaque formation. Further studies using more subjects with extreme plaque formation rates are necessary before definite conclusions can be drawn.

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Effect of delmopinol hydrochloride mouthrinse on plaque formation and gingivitis in "rapid" and "slow" plaque formers

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Abstract. The aim of the present study was to investigate differences in the plaque and gingivitis inhibiting effect of delmopinol rinsing between "rapid" and "slow" plaque formers. 23 subjects (12 "rapid" and 11 "slow" plaque formers) were selected from 71 healthy young adults. The selection was based on the plaque index on the buccal surfaces of all premolars and 1st molars after 3-days without plaque control. The 23 subjects were randomly assigned into 3 groups with different mouthrinses, i.e., 0.1% delmopinol, 0.2% delmopinol, and placebo. The study was double-blind with parallel design between the "rapid" and "slow" plaque formers and cross-over design between 2 active periods and a placebo period. Each rinsing period lasted for 5 days. During the 3 test periods, the subjects refrained from all oral hygiene and rinsed 2× daily with either one of the 3 solutions. Gingival crevicular fluid (GCF) was collected from buccal surfaces of upper canines and premolars and bleeding on probing (BOP) recorded at 6 sites around each tooth before and after each test period. Plaque assessment, including plaque index (PI) and standardized color slides for planimetric analyses obtained from the canines and premolars, were only recorded after each test period. Results showed that the mean PI and planimetry values for both the "rapid" and "slow" plaque formers were lower than the placebo, for either the 0.1% or the 0.2% delmopinol mouthrinse. The differences between the "rapid" and "slow" plaque formers were not statistically significant. There was a small reduction in BOP in both groups for the delmopinol periods, as against a slight increase in the placebo period; the difference between the placebo group and the 2 groups of plaque formers was not statistically significant ($p > 0.6$ for both 0.1% and 0.2% delmopinol). Results suggested that both 0.1% and 0.2% delmopinol reduce plaque formation and gingivitis to a similar extent in subjects with extreme rates of plaque formation.

Key words: delmopinol; dental plaque; gingivitis; mouthrinse; rate of plaque formation

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Mechanical plaque control by toothbrushing and/or regularly repeated professional tooth cleaning has been shown to bring about resolution, and prevent recurrence of gingival inflammation both in children (Axelsson & Lindhe 1974) and in adults (Lövdal et al. 1961,

Glavind 1977). However, these results were obtained from studies under experimental conditions. In industrialized countries, gingival inflammation is still very prevalent among the adolescents though most of them have acquired a daily toothbrushing habit (Pilot & Mi-

yazaki 1991). The gingival inflammation may not necessarily progress to measurable periodontal breakdown in most people (Ramfjord 1987, Baelum et al. 1988), but it is not possible to diagnose which gingivitis will progress and which will not (Page 1986). Therefore,

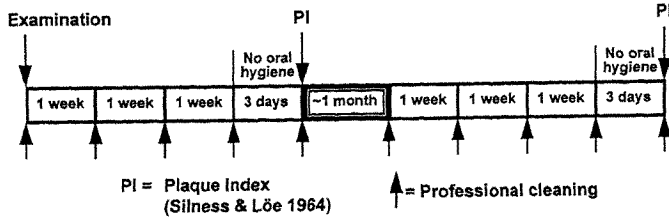


Fig. 1 Clinical schedule for the selection of "rapid" and "slow" plaque former.

numerous chemical agents have been tested for clinical use as an adjunct, or to replace supragingival plaque control in an attempt to obviate gingivitis (for reviews: see Van der Ouderra 1991).

Of the many agents that were shown to have a beneficial effect on gingival health, chlorhexidine has consistently proven its superior clinical efficacy (Lang & Brex 1986). As an antiseptic, chlorhexidine's major mode of action is related to its antibacterial properties. Delmopinol hydrochloride is a surface active agent with an antimicrobial effect falling within a range of 5–125× lower than that of chlorhexidine against both oral and non-oral bacterial species (Simonsson et al. 1991). This low antimicrobial activity has also been confirmed in an *in vivo* study of cell vitality of dental plaque after 4 days of mouth-rinsing with the substances (Rundegren et al. 1992). Further, in a recent study (Collaert et al. 1993), no change in the salivary microbiological counts was observed after rinsing with 10 ml 0.2% delmopinol for 2 weeks. Nevertheless, a number of studies (Simonsson et al. 1991b, Collaert et al. 1992, Moran et al. 1992) have shown its effectiveness against plaque formation both *in vitro* and *in vivo* as well as against development of gingivitis.

In a pioneering study of 133 healthy subjects, Simonsson et al. (1987) demonstrated considerable variation in the rate of plaque formation assessed by recording plaque index (Silness & Løe 1964), after 3 days of plaque accumulation with no oral hygiene measures. In addition, in a delmopinol dose response study (Collaert et al. 1992), the values for plaque planimetry obtained after 2 weeks of rinsing with the placebo solution were found to vary between 16% to 90%. The response to delmopinol, in subjects with high plaque values after placebo rinsing, showed relatively less plaque extension

after delmopinol rinsing than subjects with low placebo plaque values. However, this comparison was based on subjects not particularly categorized according to their plaque formation rate. Hence, the aim of the present study was to investigate differences in the plaque inhibiting effect of delmopinol rinsing for 5 days between a group of "rapid" and "slow" plaque formers, and also to evaluate the gingivitis-inhibiting effect of the drug in the 2 groups.

Material and Methods

Selection of subjects

A total of 71 subjects volunteered for the study. They were dental students, postgraduate students and personnel at the Center for Oral Health Sciences in Malmö. The subjects were screened according to their plaque forming capacity based on the plaque indices (PI, Silness & Løe 1964) after 3 days of plaque accumulation. Before the screening period, the subjects were examined to exclude those with extensive interproximal or buccal restorations, untreated caries or periodontal diseases and treatment with antibiotics, anticholinergics or antiphlogistics. After the examination, the subjects entered a 3-week oral hygiene period, comprising 1× a week visits for professional cleaning and oral hygiene instructions. At the start of the plaque accumulation periods, gingival health of the subjects was confirmed by the measurements of gingival crevicular fluid (GCF) according to Løe & Holm-Pedersen (1965). PI was registered on the buccal surfaces of all premolars and 1st molars. The number of registered surfaces for each individual was 12. The mean plaque scores were calculated for each individual. After the recordings, 12 subjects with highest mean plaque scores ("rapid" plaque formers) and 11 with lowest mean plaque scores ("slow" plaque for-

mers) were selected for subsequent study (Simonsson et al. 1987). Fig. 1 shows the study design for the selection period.

Study design

The study was double-blind with parallel design between the "rapid" and "slow" plaque formers and cross-over design between 2 active treatment periods and a placebo period. Each test period was 5 days as illustrated in Fig. 2. 1× a week, professional prophylaxis was provided 3 weeks before the start of the test periods. During the test periods, the subjects were asked to stop their normal oral hygiene procedures. Unsupervised rinsing with 10 ml of the mouthwash was performed for 60 seconds in the morning and in the evening. The placebo solution was an aqueous solution containing a herb flavor (eucamint) 0.02%, sodium saccharine 0.01% and ethanol 1.5%. The 2 active solutions were similar to the placebo with either 0.1% or 0.2% delmopinol and sodium hydroxide ad pH=5.7 added. The subjects were instructed not to eat within 30 min after rinsing. The test solutions were supplied in identical 200-ml glass bottles with only subject codes marked. All the remaining test solutions in the bottles were measured to monitor the compliance of the subjects. Neither the investigator nor the subjects knew whether the test solutions were active or placebo. The subjects were assigned to treatment groups according to a computer-generated randomized list.

Clinical assessments

Plaque assessment. Supragingival plaque was disclosed with erythrosine at the end of each rinsing period (Fig. 2). PI was scored for buccal and lingual surfaces of all teeth, except 3rd molars. Standardized color slides for planimetric analyses were taken from the canines and premolars (Carlsson & Egelberg 1965). The slides were enlarged 6.5× and projected onto white paper. The extension of the stained plaque on the canines and premolars were measured with computerized digitization and expressed as relative area units.

Gingivitis assessment. Gingival crevicular fluid (GCF) was collected with a standardized filter paper strip at the start and the end of the each rinsing period according to Løe & Holm-Pedersen (1964). All the buccal surfaces of

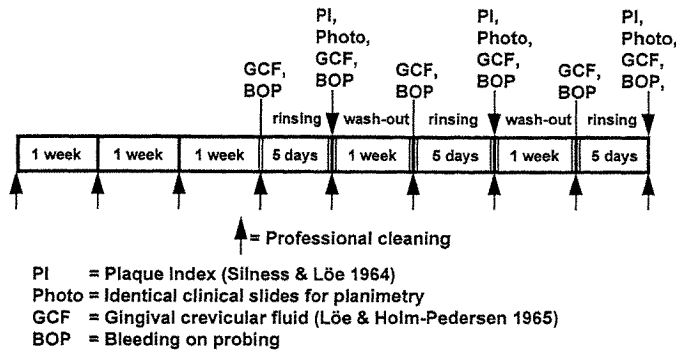


Fig. 2. Experimental design for the rinsing period.

upper canines and premolars were sampled. After collection, the filter paper strips were mounted on glass slides and stained with ninhydrine. The stained length on the paper strips was then measured with computer digitization. Bleeding on probing (BOP) was recorded after gentle probing with a WHO-probe to the bottom of the gingival crevice at 6 sites around each tooth viz: mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual (Fig. 2). All clinical assessments were made by the same examiner (ZKY).

The study was approved by the local Ethics Committee at the University of Lund and by the Swedish National Board of Health and Welfare. Each subject was given full verbal and written information regarding the objective, the procedure of the study, and the possible risks and benefits involved prior to inclusion in the study. Informed consent was obtained from all the subjects.

Adverse experiences

The subjects were questioned about the occurrence of any adverse experiences and the duration and severity of a possible anesthetic sensation in the mouth at the end of the 3 rinsing periods.

Statistical analyses

All statistical analyses were performed with non-parametric tests. Wilcoxon rank sum test was used to test the difference between "rapid" and "slow" plaque formers using the percentage of placebo response. No adjustment for multiple comparisons of the calculated *p*-values was performed.

Results

Selection of subjects

The mean PI score obtained from the two 3-day plaque accumulation periods ranged from 0.08 to 2.88. The mean PI score of the 11 "slow" plaque formers was <0.96 (range: 0.08–0.96) while that of the 12 "rapid" plaque formers was >1.75 (range: 1.75–2.88).

Plaque index (PI)

For the total 23 subjects in the main study the mean PI for all sites was 60% lower for 0.2% delmopinol compared to placebo. In the two groups of plaque formers who rinsed with 0.2% delmopinol solution, PI values were 60% and

59% lower for the "slow" and "rapid" plaque formers respectively when compared with the placebo. However, 0.1% delmopinol solution gave 46% and 35% lower values for "slow" and "rapid" plaque formers respectively (Fig. 3). The delmopinol/placebo PI ratios were not statistically different between the two groups for the 0.2% and 0.1% solutions (*p*=0.9999 and 0.3401, respectively).

Planimetry

Compared to placebo the mean plaque planimetry value for 0.2% delmopinol was 71% lower for the "slow" plaque formers and 77% lower for the "rapid" plaque formers (Fig. 4). The respective values of the 0.1% delmopinol were 52% for the "slow" group and 50% for the "rapid" group. The difference between the "rapid" and "slow" plaque formers was not statistically different (*p*=0.9264 for both 0.1% and 0.2% delmopinol).

Gingival crevicular fluid (GCF)

A marginal increase in the mean values of GCF is evident for all subjects after the various treatment periods (Fig. 5). Although the difference in GCF value between delmopinol and placebo was less for the "rapid" plaque formers compared to the "slow" plaque formers (Fig. 5), no statistical difference was observed between the groups (*p*=0.7818).

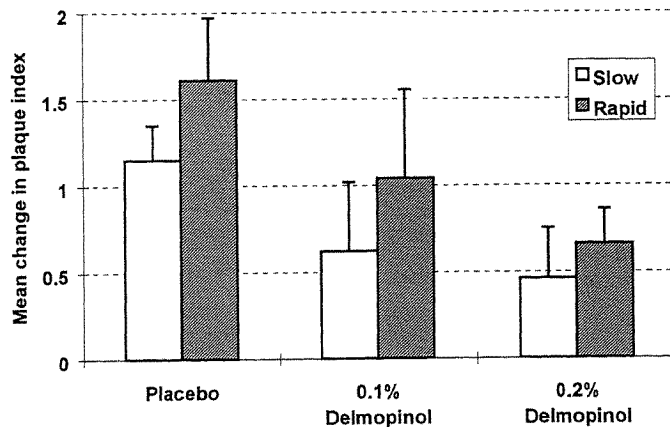


Fig. 3. Mean change in plaque index of all sites (+standard deviation) after 5 days of rinsing with delmopinol 0.1%, 0.2% and placebo.

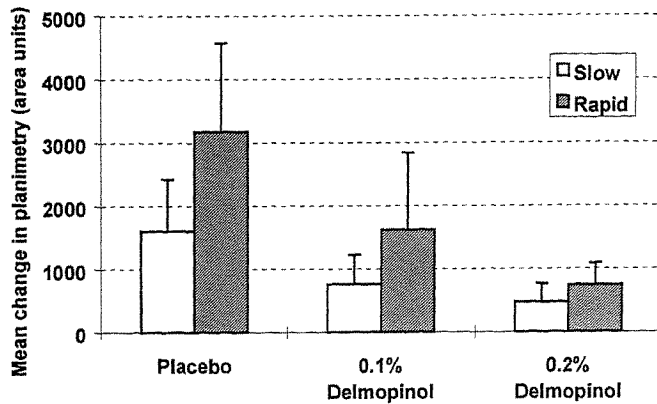


Fig. 4. Mean change in buccal plaque planimetry (+standard deviation) in arbitrary area units after 5 days of rinsing with delmopinol 0.1%, 0.2 % and placebo.

Bleeding on probing (BOP)

A small reduction in BOP was evident for the delmopinol periods, contrary to the increased GCF value observed (Fig. 6); but a slight increase in BOP for both "slow" and "rapid" plaque formers was seen in the placebo. However, the differences between the placebo groups and the two delmopinol groups of plaque formers were not statistically significant ($p > 0.6$ for both 0.1% and 0.2% delmopinol). When the "slow" and "rapid" groups were compared in isolation, there was a significant difference between the latter and placebo groups for 0.1% delmopinol ($p = 0.0244$) and close to significant for 0.2% delmopinol ($p = 0.0522$). Similarly, the difference for the "slow" groups was significant for 0.2% delmopinol ($p = 0.0029$) but not for 0.1% delmopinol ($p = 0.0830$).

Adverse experiences

The only adverse event was localized anesthesia of the oral mucosa during delmopinol rinsing which was transient and mostly localized to the tongue. The intensity of the anesthesia was characterized from mild to moderate. No adverse experience was reported in the placebo period.

Discussion

The results of PI, planimetry and BOP all showed a positive response to both 0.1% and 0.2% of delmopinol mouthrinses when compared with placebo. These results are in broad agreement

with previous studies related to delmopinol (Collaert et al. 1992, 1993, 1994). When compared with chlorhexidine, delmopinol shows less reduction in the amount of supragingival plaque (Collaert et al. 1993) even though there was no significant difference in the reduction of bleeding and GCF between the two treatment regimens. This may be due to the fact that delmopinol has a lower antimicrobial effect than chlorhexidine (Simonsson et al. 1991b, Rundegren et al. 1992a). Although the exact mode of action of delmopinol is not known in detail, it has been shown that it may interfere with bacterial glucosyltransferase mediating glucan synthesis, which may play a role in bacterial colonization and in the formation of plaque matrix (Steinberg et al. 1992). It

may also reduce the viscosity of extracellular glucan (Rundegren & Arnebrant 1992, Rundegren et al. 1992b) and hence reduce the cohesion within dental plaque and facilitate its mechanical removal (Simonsson et al. 1991a).

On considering the clinical parameters evaluated (PI, planimetry, GCF and BOP), the "rapid" plaque formers showed higher % reduction in plaque due to the delmopinol than the "slow" plaque formers, when compared with the placebo. However, this was not statistically significant. This may suggest that the clinical effect of delmopinol mouthrinse is similar in subjects with different rates of plaque formation. In a previous delmopinol dose response study (Collaert et al. 1992), the response to delmopinol treatment in subjects with high plaque values after placebo rinsing showed relatively higher reduction in plaque when measured by planimetry than subjects with low placebo plaque values. However, the subjects in the study by Collaert and co-workers (1992) were not specifically screened for their rate of plaque formation while the 12 "rapid" and 11 "slow" plaque formers in the present study represented extreme groups from a population of 71 subjects. On the other hand, the rinsing period for each treatment was only 5 days in contrast to 28 days in the Collaert study. Further studies with longer treatment period may be necessary to confirm the present findings.

The current results indicated a marginal increase in GCF value after the various treatment periods with delmopinol in contrast to a small reduction in

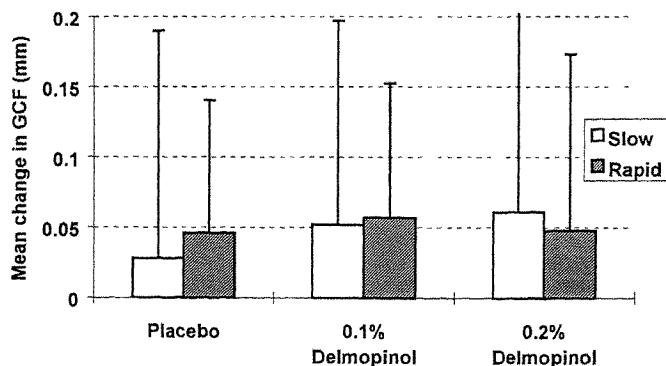


Fig. 5. Mean change in gingival cervicular fluid (GCF) (+standard deviation) in mm after 5 days of rinsing with delmopinol 0.1%, 0.2 % and placebo.

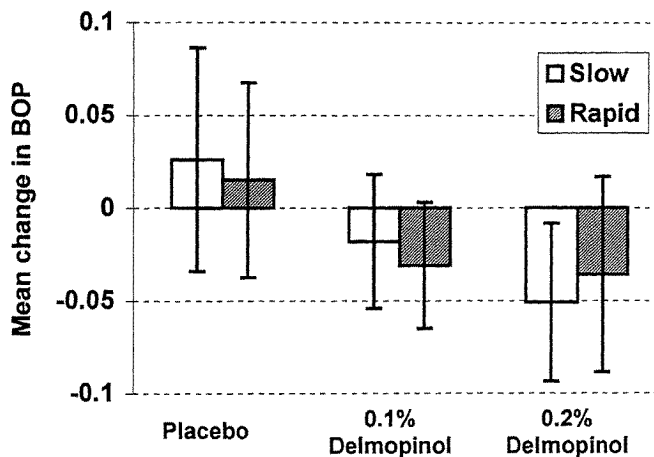


Fig. 6. Mean change in bleeding on probing (BOP) of all sites (+standard deviation) after 5 days of rinsing with delmopinol 0.1%, 0.2% and placebo.

BOP. This may reflect the insensitivity of the method used to record GCF to detect any observable changes in the small number of subjects during each rinsing regimen or that delmopinol has no effect on this parameter. Nonetheless, five days of treatment may not be adequate to show detectable changes in gingival inflammation as reflected by GCF since it has been shown that during an experimental gingivitis period, clinically observable gingival inflammation develops, at first, between 9 and 21 days (Theilade et al. 1966). Though more sensitive methods such as the use of the Periotron® machine (ProFlow, Amityville, NY, USA) may help to improve the sensitivity of these studies.

In conclusion, the results of the present short term study indicated a positive effect of 0.1% and 0.2% delmopinol mouthrinses in reducing supragingival plaque formation and bleeding on probing. Both the "rapid" and "slow" plaque formers showed similar positive response to the 0.1% and 0.2% delmopinol mouthrinse with no statistically significant difference between the two groups of plaque formers. Thus, it appears that there is no difference of the anti-plaque and anti-gingivitis effect of either 0.1% or the 0.2% delmopinol mouthrinse in subjects with extreme plaque formation rates. However, due to the limited number of subjects and the short period of rinsing utilized in the present study, further investigations

are required to confirm the current findings.

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Zusammenfassung

Der Einfluß der Mundspülung mit Delmopinol Hydrochlorid auf Plaquebildung und Gingivitis von Patienten mit "schneller" und "langsamer" Plaquebildung

Das Anliegen der vorliegenden Studie bestand in der Untersuchung der Unterschiede zwischen der plaque- und gingivitis-hemmenden Wirkung von Delmopinol-Mundspülungen bei Patienten mit "schneller" und "langsamer" Plaquebildung. Aus einer Gruppe von 71 gesunden Jugendlichen wurden 23 (12 "schnell" und 11 "langsam" plaquebildende) ausgewählt. Die Auswahl ging von einem, nach 3 Tage lang eingestellter Plaquekontrolle erhobenen, Plaqueindex der bukkalen Oberflächen aller Prämolaren und 1. Molaren aus. Die 23 Versuchspersonen wurden zufällig 3 Gruppen zugeteilt, die den Mund mit verschiedenen Spüllösungen, wie 0.1%igem Delmopinol, 0.2%igem Delmopinol und einer Placebolösung, spülten. Die Studie war doppelblind angelegt mit paralleler Versuchsanlage zwischen Versuchspersonen mit "schneller" und "langsamer" Plaquebildung

und Überkreuzanlage (cross-over design) zwischen zwei aktiven Zeitabschnitten und einer Placeboperiode. Jede Spülperiode dauerte 5 Tage. Während der 3 Testperioden stellten die Versuchspersonen jegliche Mundhygiene ein und spülten den Mund zweimal täglich mit einer der 3 Lösungen. Vor und nach jeder Testperiode wurde an den bukkalen Oberflächen der oberen Eckzähne und Prämolaren Sulkussektret (GCF) entnommen und das Bluten nach dem Sondieren (BOP) an 6 Stellen rund um jeden Zahn registriert. Eine Beurteilung der Plaque, bestehend aus Plaqueindex (PI) und standardisierten Farbaufnahmen für planimetrische Analysen an Eckzähnen und Prämolaren, wurde nur nach jeder Testperiode vorgenommen. Die Ergebnisse zeigten, daß der mittlere PI und die planimetrischen Werte nach Spülung mit sowohl 0.1%igem als auch 0.2%igem Delmopinol bei "schnell" und "langsam" plaquebildenden Versuchspersonen niedriger waren als bei den mit Placebo spülenden. Die Unterschiede zwischen Versuchspersonen mit "schneller" und "langsamer" Plaquebildung waren statistisch nicht abgesichert. Während der Delmopinolperioden kam es bei beiden Gruppen zu einer geringen Reduktion des BOP. Während der Placeboperiode wurde indessen eine leichte Erhöhung beobachtet. Der Unterschied zwischen der Placebogruppe einerseits und den beiden Gruppen der Versuchspersonen mit zeitlich unterschiedlicher (schnell/langsam) Plaquebildung andererseits, war statistisch nicht signifikant ($p > 0.6$ bei sowohl 0.1%igem als auch 0.2%igem Delmopinol). Die Ergebnisse lassen deutlich werden, daß sowohl 0.1%iges als auch 0.2%iges Delmopinol Plaquebildung und Gingivitis reduziert – und das auch bei Personen mit extremer Plaque Neubildung.

Résumé

Effet du bain de bouche à l'hydrochlorure delmopinol sur la formation de la plaque dentaire et la gingivite chez les formeurs de plaque rapides et lents

Le but de l'étude présente a été d'analyser les différences d'inhibition de la plaque et de la gingivite suite au rinçage au delmopinol chez les formeurs de plaque dentaire rapides et lents. 23 sujets (12 rapides et 11 lents) ont été sélectionnés à partir de 71 jeunes adultes sains. La sélection a été basée sur l'indice de plaque des surfaces vestibulaires de toutes les prémolaires et premières molaires après 3 jours sans contrôle de plaque dentaire. Les 23 sujets ont été partagés au hasard en 3 groupes avec différents bains de bouche c.-à-d. delmopinol 0.1%, delmopinol 0.2% et placebo. Cette étude était en double aveugle avec un modèle parallèle entre les formeurs de plaque dentaire rapides et lents, et un modèle croisé entre 2 périodes actives et 1 période placebo. Chaque période de rinçage durait 5 jours. Durant les 3 périodes test, les sujets ont cessé toute hygiène buccale et se sont rincés 2x par jour avec une de ces 3 solutions. Le fluide crémulaire gingival (GCF) a été collecté des surfaces vestibulaires

laïres des canines et prémolaires supérieures, et le saignement au sondage (BOP) a été évalué au niveau de 6 sites autour de chaque dent avant et après chaque période test. L'évaluation de la plaque dentaire comprenait l'indice de plaque (PI) et des analyses planimétriques à partir de diapositives couleurs standardisées obtenues des canines et des prémolaires, et qui n'ont été enregistrées qu'après chaque période test. Les résultats ont montré que l'indice de plaque moyen et les valeurs planimétriques tant pour les formeurs de plaque dentaire rapides que lents étaient plus bas que pour le placebo, ce qui soit avec le bain de bouche à 0.1 ou à 0.2% de delmopinol. Les différences entre formeurs rapides et lents n'étaient pas statistiquement significatives. Il y avait une faible réduction du BOP dans les deux groupes pour les périodes de delmopinol tandis qu'il y avait une petite augmentation durant la période placebo; la différence entre le groupe placebo et les 2 groupes de formeurs de plaque n'était pas statistiquement significative ($p > 0.6$ tant pour le delmopinol 0.1 que 0.2%). Ces résultats suggèrent que tant le delmopinol 0.1% que le 0.2% réduisent la formation de plaque dentaire et la gingivite d'une manière semblable chez les sujets avec des taux extrêmes de formation de plaque dentaire.

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