Kinetics of Adherence of *Actinomyces viscosus* to Saliva-coated Silica and Hydroxyapatite Beads

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Adherence of ^{14}C -labelled strains of *Actinomyces viscosus* to uncoated and saliva-coated silica and hydroxyapatite beads had both loose and firm components, probably reflecting different subpopulations of bacteria within a single culture. Adherence was characterized by the proportion of bacteria available for each type of adherence and a constant (K_b) for each combination of bacterial strain and bead surface. Loose adherence, which was greater with silica than with hydroxyapatite beads, always involved many more bacteria than firm adherence. Firm adherence was greater with *A. viscosus* WVU627 than *A. viscosus* TF11. The association rate constants (K_a) for loose and firm adherence were similar, indicating simultaneous processes, but the dissociation rate constant (K_d) was lower for loose adherence than for firm adherence. Removal of loosely adhering bacteria by washing may only reflect their distance from the bead surface. Silica beads were convenient for studying bacterial adherence and formed an acceptable coating of salivary glycoprotein.

INTRODUCTION

In terms of the ecology of microorganisms, adherence is very important. By definition, it will lead to an increased concentration of the microorganisms, and their effects, at a particular site. The adherence of oral microorganisms to tooth surfaces is an important aspect of this phenomenon. Tooth enamel is normally covered with a salivary pellicle and it is to this glycoprotein coating that microorganisms adhere (Embery & Hogg, 1981; Rolla, 1977). In order to study some adherence properties of oral microorganisms, hydroxyapatite beads have been widely used as a model system (Clark *et al.*, 1978; Gibbons & van Houte, 1975) and they are often treated with saliva in an attempt to simulate the salivary pellicle (Weerkamp & McBride, 1980; Wheeler *et al.*, 1979). The characteristics of adherence to saliva-coated beads are due to the combination of adherence to salivary glycoprotein and to uncoated bead surface (Stinson *et al.*, 1981), so that as the glycoprotein coating becomes nearer complete, the characteristics of adherence will approach those of adherence to glycoprotein. It is not possible to determine how completely the beads are coated by simply comparing adherence of bacteria to coated and uncoated beads.

Actinomyces viscosus is normally found in the oral cavities, particularly at the gingival margins, and can cause root surface caries (Jordan & Keyes, 1964; Sumney & Jordan, 1974). Strains of *A. viscosus* also adhere to saliva-treated HA (Wheeler *et al.*, 1979). It has been proposed that *A. viscosus* and *A. naeslundii* may not be separate species but that the organisms grouped within them may be serotypes of a single species. *Actinomyces viscosus* TF11 used in this work has also been designated *A. naeslundii* TF11 (Fillery *et al.*, 1978).

In the present study two strains of *A. viscosus* were used to compare the effectiveness of hydroxyapatite and silica, another inorganic surface, as supports for an *in vitro* salivary pellicle. An attempt was made to characterize the factors that determine adherence, and their relative contributions to the changes in adherence observed after treatment of the surfaces with saliva.

Abbreviations: HA, hydroxyapatite; S, silica.

METHODS

Cultures and culture conditions. Laboratory-maintained strains of *A. viscosus* WVU627 and TF11 were kindly provided by P. D. Marsh, London Hospital Medical College, London. All cultures were stored on beads at -40°C in nutrient broth containing 10% (v/v) glycerol (Feltham *et al.*, 1978). Cells of *A. viscosus* strains, labelled with ^{14}C or ^3H , were prepared freshly from frozen stocks for each experiment by growing the organisms in Casitone medium [1.5% (w/v) casitone, 1% (w/v) yeast extract, 0.5% (w/v) sodium chloride, pH 7] containing 18.5 KBq [^{14}C]glucose ml^{-1} or 18.5 KBq [^3H]thymidine ml^{-1} (obtained from Amersham). Organisms from 20 h cultures were harvested by centrifugation at 1860 *g* for 5 min at 4°C . The supernate was discarded and the cells washed three times with 0.14 M-NaCl containing 2 mM-KCl, 6.5 mM- Na_2HPO_4 and 1.5 mM- KH_2PO_4 , pH 7.3 (PBS). The cells were finally resuspended in PBS and the A_{600} and radioactivity of the suspension were determined. A graph relating A_{600} to bacterial concentration was then used to estimate the specific activity of the bacteria.

Saliva coating of beads. Whole, unstimulated saliva was collected from five adult donors into tubes cooled in ice. The pooled saliva was heated at 60°C for 30 min to inactivate degradative enzymes and then centrifuged at 10000 *g* for 10 min to remove debris and denatured protein. The treated saliva was stored at -20°C until used and was not refrozen.

Florisol silica beads (S; 100–200 mesh, 74–149 μm ; Koch-Light), and hydroxyapatite beads (HA; 75–185 μm ; BDH) were left to stand with 1 ml of treated saliva in a bijou bottle for 16–18 h. The saliva was then removed and the beads were washed with PBS.

Analysis of salivary protein coating. The protein concentration of the saliva (prepared as described above) was determined by the Lowry method. The corresponding hexose concentrations were also determined (Shetlar & Masters, 1957). After removal of the saliva, the beads were washed with PBS until the washings were protein-free as indicated by A_{280} values. The beads were then assayed for the amount of protein and hexose adsorbed.

Estimation of bacterial adherence. The assay used was based on that previously described by Clark *et al.* (1978). An appropriate weight of HA beads or S beads, in glass bijou bottles, was either pre-equilibrated in PBS for 16–18 h, or coated with saliva as described above. To each bottle was added 1.6 ml of a suspension of radioactively-labelled bacteria in PBS. The bottles were placed either on a rotating table in the case of S, or in a shaking waterbath for HA beads. Maximum (equilibrium) adherence was determined after 2 h incubation at room temperature, or as appropriate for kinetic studies. The beads were then allowed to settle under gravity for 1 min and the radioactivity in 50 μl samples of the supernatant bacterial suspension was determined. The remaining supernate was removed from the beads which were washed three times with PBS by allowing them to settle under gravity for 1 min on each occasion. The radioactivity associated with the beads was then determined. The decrease of radioactivity in the supernate was used as a measure of total bacterial adherence, and radioactivity attached to the beads after washing as a measure of firm adherence. Loose adherence was the difference between these values. Numbers of bacteria adhering were determined from the previously calculated specific activity of the bacteria. Adherence to the walls of the incubation vessels was determined from bacterial suspensions incubated without beads.

Scanning electron microscopy. Beads with firmly adhering bacteria were fixed for 1 h in 5% (v/v) glutaraldehyde in PBS, and then washed three times in PBS. They were dehydrated through 20, 50, 70, 90 and 100% alcohol (15 min in each solution) and amyl acetate was then added. A sample of treated beads was mounted on a stub that had been previously coated with silver paint, and critical point dried, using liquid CO_2 in a Polaron critical point dryer. The stub was then coated with gold in a Polaron diode sputter coater and examined using a scanning electron microscope.

RESULTS

Preparation of radioactively labelled bacteria

When *A. viscosus* WVU627 and *A. viscosus* TF11 were grown in the presence of [^3H]thymidine the specific activity of the cells was 55 d.p.m. per 10^6 cells. This was too low for the accurate determination of small numbers of bacteria. When grown in the presence of [^{14}C]glucose, the cells obtained had a specific activity of 1050 d.p.m. per 10^6 cells. Only small amounts of ^{14}C were released from the bacteria while they were suspended in buffer so that, after 4 h, 4% of the total radioactivity was not sedimented with the bacteria when centrifuged at 1860 *g* for 5 min. When bacteria were double-labelled with [^3H]thymidine and [^{14}C]glucose, ^3H and ^{14}C had the same distribution between the beads and supernate. This showed that the results were not affected by the choice of radioactive label. Therefore, *A. viscosus* WVU627 and *A. viscosus* TF11 were radioactively-labelled by incubation with [^{14}C]glucose for adherence studies.

Table 1. *Composition of glycoprotein coating on S and HA beads*

S or HA beads (20 mg) were treated with 1 ml saliva as described in Methods. The beads were washed with PBS to obtain a stable coating of glycoprotein and the amount of hexose associated with each type of bead was determined as described in Methods.

	Protein*	Hexose*	Protein/hexose ratio
S	0.05	0.14	1:2.8
HA	0.025	0.16	1:6.4
Saliva	2.1	2.05	1:1

* Numbers are in units of mg per 10 mg S or HA, and mg ml⁻¹ saliva.

Coating of S and HA beads with salivary protein

S beads adsorbed protein from saliva more efficiently than HA beads, but in both cases a large proportion of the adsorbed protein was lost in subsequent washing. When the coating was stable to washing, S beads had twice as much protein as HA beads (Table 1). However, the amount of hexose associated with both types of bead was similar. Therefore, S and HA beads adsorbed similar amounts of glycoprotein but the hexose/protein ratio was much greater for the glycoproteins adsorbed to HA than for those adsorbed to S. The ratio for S was much closer to that of the whole saliva whereas HA appeared to selectively adsorb glycoproteins of high carbohydrate content.

Appearance of adhering bacteria

The surface of HA as seen by scanning electron microscopy was so irregular that it was not possible to identify adhering bacteria. This difficulty has been noted previously (Staat *et al.*, 1980). However, the surface of S was much smoother and adhering bacteria could be seen on both S and saliva-coated S. Bacteria were more commonly seen in crevices in the S particles rather than on exposed surfaces, and all the particles were not uniformly coated with bacteria. However, some of these observations could have resulted from the loss of bacteria from more exposed positions during the preparation for scanning electron microscopy, since critical point drying was essential and no bacteria were seen after samples of S had been vacuum dried.

*Firm and loose adherence of *A. viscosus* to HA and S beads*

The adherence of *A. viscosus* TF11 and *A. viscosus* WVU627 was studied using two methods of measurement. The reduction in bacterial concentration in the supernate was taken to be an indication of the accumulation of bacteria on the beads due to an attractive force that could be considered adherence. Any medium trapped between the beads would not affect the concentration of bacteria in the supernate. Bacteria that remained attached to the beads after washing were described as 'firmly adhered' and bacteria that were removed by washing were said to be 'loosely adhered'. The number of bacteria adhering from a constant bacterial concentration (6×10^7 cells ml⁻¹) to 20 mg HA or S, with or without saliva treatment, is given in Table 2. There was more bacterial adherence to S than to HA although the degree of preference for S depended on the strain of *A. viscosus*. Saliva treatment of the beads reduced firm adherence of both strains to S by a similar proportion, but with HA, saliva treatment did not significantly affect firm adherence. With *A. viscosus* WVU627 more bacteria adhered to S than to HA, but less bacteria adhered to saliva-treated S than saliva-treated HA.

In this system loose adherence to 20 mg S was also much greater than to 20 mg HA, but varied much less with the bacterial strain than firm adherence.

During adherence studies bacteria were evenly distributed in the supernatant buffer in small aggregates of 3–5 cells that showed no tendency to auto-agglutinate and sediment with the beads.

Table 2. *Estimated number of bacteria adhering to 20 mg S or HA beads*

Adherence was determined after 2 h mixing with 1.6 ml of a suspension of ^{14}C -labelled bacteria (6×10^7 cells ml^{-1}). Loosely adhered bacteria were removed from the beads by three washings with PBS but those bacteria firmly adhered remained attached. Numbers of bacteria were determined from radioactive counts using the specific activity of each preparation of bacteria. This was estimated by measuring the A_{600} and radioactivity of each suspension and using a standard curve relating A_{600} to bacterial concentration. The mean numbers of adhering bacteria are given $\pm$ S.E.M. The differences in numbers of bacteria adhering to S and HA were significant in all cases ($P < 0.01$) except for loose adherence of strain WVU627 to saliva-coated beads. The differences in values for firm adherence to untreated S and saliva-treated S were also significant ($P < 0.01$). Differences in adherence to untreated HA and saliva-treated HA were not statistically significant.

		$10^{-6} \times \text{No. of bacteria adhering to:}$			
		S		HA	
Strain		Untreated	Saliva-treated	Untreated	Saliva-treated
Firm adherence	<i>A. viscosus</i> TF11	6.3 ± 0.5	2.4 ± 0.5	1.3 ± 0.3	1.1 ± 0.1
	<i>A. viscosus</i> WVU627	13.1 ± 1.7	3.7 ± 0.6	7.8 ± 1.0	5.9 ± 0.6
Loose adherence	<i>A. viscosus</i> TF11	39.0 ± 2.6	33.0 ± 4.7	14.4 ± 1.0	16.9 ± 2.9
	<i>A. viscosus</i> WVU627	28.2 ± 5.3	22.9 ± 4.0	18.6 ± 1.7	20.8 ± 1.5

Effect of the amount of beads used on bacterial adherence

When the amount of untreated or saliva-treated S or HA used for adherence was increased, the proportion of available bacteria that adhered to the beads, either firmly or loosely, increased to a maximum value. When 1.6 ml of a bacterial suspension of 6×10^7 cells ml^{-1} was used there was very little increase in bacterial adherence with more than 40 mg of beads and in all cases both loose and firm adherence were maximum with 80 mg of beads (Fig. 1). This maximum adherence was taken to be the maximum proportion of bacteria available for adherence and it was always much greater for loose adherence than for firm adherence. As the amount of beads used increased, the bacterial concentration in the medium at equilibrium decreased. To allow for this, bacterial adherence at equilibrium (2 h) was expressed as:

$$-dA/dB = K_b A \quad (1)$$

where dA was the change in bacterial concentration in the medium, dB was the change in amount of beads, A was the bacterial concentration in the medium and K_b a constant dependent on the properties of the interaction between the bacteria and the bead surface. On integration this expression gives:

$$\ln(A_T/A_B) = K_b B \quad (2)$$

where A_T was the total bacterial concentration that would adhere (firmly or loosely, as appropriate) i.e. the value for maximum adherence, and A_B was the concentration of bacteria potentially able to adhere but remaining in the medium with B mg of beads. Figure 2 shows that this equation describes both firm and loose adherence, and the values obtained for K_b are given in Table 3. In both cases this expression was not obeyed if it was assumed that for firm or loose adherence all of the bacteria in the medium were able to adhere in that particular way. K_b was equal to the equilibrium constant for 1 mg of beads since it was obtained at equilibrium adherence and related bacterial adherence (mg beads) $^{-1}$ to bacterial concentration in the medium.

Rate constants for adherence

The rate of adherence of bacteria to beads was first order with respect to the concentration of bacteria that was able to adhere (Fig. 3). The adherence rate constant (K_a) was determined from the equation:

$$\ln(A_T/A_t) = K_a t \quad (3)$$

Table 3. *Characteristics of adherence to S and HA beads before and after treatment with saliva*

Maximum adherence is the maximum proportion of bacteria from a culture that would adhere and was determined using 80 mg beads with 1.6×10^7 cells ml^{-1} . K_a is the first order rate constant for bacterial adherence to 1 mg beads. K_b was determined from bacterial adherence to different weights of beads and was similar to the equilibrium constant. The dissociation constant was determined from equation 4 in the text.

	Beads	<i>A. viscosus</i> WVU627				<i>A. viscosus</i> TF11			
		K_b (mg) $^{-1}$ *	K_a (min) $^{-1}$ †	K_d (min) $^{-1}$	Maximum adherence‡	K_b (mg) $^{-1}$ *	K_a (min) $^{-1}$ †	K_d (min) $^{-1}$	Maximum adherence‡
Firm adherence	S	0.025	0.0011	0.044	0.2 $\pm$ 0.021	0.02	0.00175	0.088	0.08 $\pm$ 0.03
	S + saliva	0.04	0.0008	0.020	0.14 $\pm$ 0.020	0.02	0.00125	0.063	0.09 $\pm$ 0.02
	HA	0.04	0.00035	0.0088	0.21 $\pm$ 0.025	0.04	0.00048	0.012	0.031 $\pm$ 0.011
Loose adherence	HA + saliva	0.04	0.00045	0.011	0.11 $\pm$ 0.016	0.04	0.00053	0.013	0.053 $\pm$ 0.028
	S	0.06	0.00175	0.029	0.71 $\pm$ 0.028	0.08	0.0013	0.016	0.84 $\pm$ 0.05
	S + saliva	0.04	0.0006	0.015	0.52 $\pm$ 0.048	0.05	0.0004	0.008	0.61 $\pm$ 0.08
HA	HA	0.07	0.00033	0.0043	0.31 $\pm$ 0.037	0.045	0.00053	0.012	0.37 $\pm$ 0.051
	HA + saliva	0.10	0.00038	0.0038	0.32 $\pm$ 0.03	0.04	0.00043	0.011	0.36 $\pm$ 0.03

* S.E.M. varied from 2% to 12% of the mean shown.

† K_a was determined using regression analysis and the S.E.M. of the slope varied from 2% to 12% of the value shown.

‡ Proportion of bacteria present $\pm$ S.E.M.

Fig. 1

Fig. 1. Variation of loose and firm adherence of *A. viscosus* TF11 to S (○, ●) and HA beads (□, ■) with increasing amounts of beads. Filled symbols, loose adherence; open symbols, firm adherence. The determination of loose and firm adherence is described in Methods. The bars represent S.E.M.

Fig. 2

Fig. 2. Determination of the constant, K_b , for loose and firm adherence of *A. viscosus* TF11 to S (●, ○) and HA beads (■, □) using equation 2 in the text. Filled symbols, loose adherence; open symbols, firm adherence. K_b was taken to be the slope of the straight line obtained. The data are taken from Fig. 1.

Fig. 3

Fig. 3. Determination of the association rate constant, K_a , for loose and firm adherence of *A. viscosus* WVU627 to S (●, ○) and HA beads (■, □) using equation 3 in the text. Filled symbols, loose adherence; open symbols, firm adherence. K_a was taken to be the slope of the straight line obtained.

Fig. 4

Fig. 4. Variation in the proportion of *A. viscosus* WVU627 that showed loose and firm adherence to S (●, ○) and saliva-coated S beads (■, □) as the total concentration of bacteria in the assay (total vol. 1.6 ml) was increased. Filled symbols, loose adherence; open symbols, firm adherence.

where A_T was the total concentration of bacteria that would adhere (firmly or loosely, as appropriate) and A_I was the concentration of bacteria potentially able to adhere but remaining in the medium at time t min. The value of K_a was directly related to the weight of beads used and is given in Table 3 as the value for 1 mg of beads in each case.

Since the bead constant, K_b , gave a measurement of the equilibrium constant, an assessment of the dissociation constant, K_d , was obtained from the equation:

$$K_b = K_a/K_d \quad (4)$$

using the value of K_a determined above. The values calculated for K_a are given in Table 3. In support of the concept of a dissociation constant, when beads with firmly adhering bacteria were placed in fresh buffer, 40–90% of the bacteria were released within 60 min. Loose adherence by the nature of its measurement was completely reversible. Dissociation of adhered bacteria has also been reported previously (Stinson *et al.*, 1981).

Effect of bacterial concentration on adherence

When adherence to 20 mg S or 40 mg HA with or without saliva treatment was determined with a range of bacterial concentrations, it was found that with *A. viscosus* WVU627 a constant proportion of bacteria adhered to the beads until high bacterial concentrations were achieved, when the proportion adhering was reduced (Fig. 4).

DISCUSSION

Since the characteristics of bacterial adherence to the *in vivo* glycoprotein coating on tooth surfaces is unclear, it is difficult to assess the validity of a model system. However, the effect of different glycoprotein coatings on bacterial adherence was investigated by coating two types of beads. HA beads appeared much more selective than S beads for the glycoproteins adsorbed. It has been reported that the high molecular weight salivary glycoproteins that are believed to be a major component of salivary pellicle have a hexose/protein ratio of 2:1 (Hogg & Embery, 1979) which is much closer to that found associated with S (Table 1).

Bacterial adherence to both native HA and saliva-coated HA has often been reported (Rolla, 1977; Gibbons & van Houte, 1975). The strains of *A. viscosus* used also adhered to S and saliva-coated S. Adherence did not appear to be a single process and different subpopulations of bacteria adhered either 'loosely' or 'firmly'. The terms 'loose' and 'firm' adherence have been suggested previously in connection with bacterial adherence (Russell & Jones, 1980). Although bacteria were removed from the beads by the final washing step, there were fewer removed than by the earlier washings, and three washings have previously been widely used (Clark *et al.*, 1978). Reported values for the adherence of other strains of *A. viscosus* ('firm adherence') to HA (Clark *et al.*, 1978; Wheeler *et al.*, 1979) are similar to those for *A. viscosus* WVU627 but much higher than for *A. viscosus* TF11. Saliva coating of HA has resulted in variable effects on adherence. The previously reported values for the adherence of strains of *A. viscosus* to saliva-coated HA have varied with the strain used but they have been generally higher than the values for *A. viscosus* WVU627 and *A. viscosus* TF11. Although firm adherence is most commonly studied, loose adherence involved many more bacteria and any process which leads to a localized concentration of bacteria at a surface is likely to be biologically important. Both loose and firm adherence increased as the amount of native or saliva-coated beads increased. There was no evidence that one type of adherence increased at the expense of the other with an excess of beads. When small amounts of beads were used the bacteria were in excess and both types of adherence increased in proportion to the amount of beads (Fig. 2). However, when the beads were in excess both types of adherence reached a maximum value which is given in Table 3. In some cases there was a large proportion of bacteria that would not adhere at all, especially with HA. This strongly suggests that subpopulations of bacteria with different adherence characteristics were present, even though bacteria for adherence experiments were grown from a single colony.

The difference in bacterial adherence to 20 mg S or HA pretreated with saliva (Table 2) suggested that the nature of the glycoprotein coating obtained on the beads had a great effect on the adherence properties. It may be useful to try to identify the factors that are responsible for this difference.

Both the affinity and capacity of the beads for the bacteria and the concentration of bacteria available affected the observed bacterial adherence. The concentration of bacteria available for adherence appeared to be less than the total concentration of bacteria, since even with an excess of beads only a fraction of bacteria adhered. Bacterial adherence to each type of bead surface

was related to the concentration of the appropriate subfraction of bacteria rather than to the total bacterial concentration. This was shown using first order kinetics and an analogous calculation for adherence to increasing amounts of beads.

The constant, K_b , was related to the affinity and capacity of the beads for bacteria independently of the size of the particular subfraction of bacteria that could adhere. In general, the observed adherence was at least as greatly affected by the concentration of the subfraction of bacteria available as by the affinity and capacity of the beads. This can be demonstrated in two cases. Firstly, HA had the same K_b (firm adherence) for *A. viscosus* TF11 and *A. viscosus* WVU627, but a much greater proportion of *A. viscosus* WVU627 was able to adhere, thus giving greater observed adherence (Tables 2 and 3). Secondly, the adherence of *A. viscosus* WVU627 to S and saliva-coated S with increasing initial bacterial concentrations showed that the proportion of bacteria adhering remained constant until the bacteria capable of adhering exceeded the bead capacity (Fig. 4). There was then a reduction in the proportion adhering and this point was related to K_b .

There was a much greater proportion of *A. viscosus* WVU627 cells available for firm adherence compared with *A. viscosus* TF11 cells on all bead surfaces. However, with each bacterial strain, the properties of adherence to S and HA, as indicated by K_b values, were different. The maximum proportion of bacteria available for loose adherence was much more closely related to the type of bead (Table 3). Saliva treatment of S reduced loose adherence of both strains of *A. viscosus* by 56% whereas saliva treatment of HA had the most consistent effect on firm adherence (Table 3). Even after saliva coating, the bead surface continued to have a large effect on the proportion of bacteria that could adhere. HA was least affected by the saliva coating.

Firm and loose adherence to S had the highest association rate constants (K_a). Although in most cases the K_a for adherence to S was reduced by saliva coating, it still remained higher than the K_a for adherence to HA which was affected only slightly by saliva coating. Since in most cases K_a values for loose and firm adherence were very similar, loose adherence was not a preliminary step to firm adherence. This was also supported by the lack of any increase in firmly adhering bacteria at the expense of loosely adhering bacteria when beads were in excess.

Most of the differences in values obtained for K_b appeared to reflect differences in the dissociation rate constant (K_d). In most of the cases where K_b for loose adherence was greater than K_b for firm adherence, this was due to a K_d for loose adherence which was lower than K_d for firm adherence rather than a change in K_a . Even when the values for K_b were the same, the values for K_a and K_d could each be different. A low K_d implies that a particular bacterial cell will spend a longer period adhered before it returns to the medium in exchange for another cell and may therefore be important in terms of bacterial ecology. The constants K_b , K_a and K_d are probably characteristic of a particular type of bacterial cell. The proportion of cells of a particular type, which was estimated by 'maximum adherence', probably depends on the conditions for growth.

The contribution of loose adherence to the overall effects of bacterial adherence may be greater than has been recognised because of the much larger number of bacterial cells involved and the low K_d for their adherence. Loosely adhering bacteria were probably present in the more distant liquid layers around the beads. These layers were removed by washing, together with the bacteria they contained. Since, at equilibrium, these bacteria appear to be adhering in a stable way, 'firm' and 'loose' may be inappropriate terms and, for example, 'proximal' and 'distant' may be better. The presence of bacteria in the supernate from the beads after three washings, and the almost total dissociation of 'firmly' adherent organisms in some cases over a short period, suggest that 'loose' and 'firm' adherence are not two clearly defined processes but represent an artificial segregation of a continuous spectrum of adherence. Nonetheless, the use of this separation into 'firm' and 'loose' with appropriately standardized conditions could increase understanding of bacterial adherence.

S was a convenient support for a glycoprotein coating which may be useful for studying adherence of oral bacteria.

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