

# Microbial adhesion to surfaces with different surface charges

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The adhesion behaviour of *Streptococcus sanguis* was studied on well-defined stearic and behenic acid multilayers which were prepared by depositing fatty acids on a solid substrate according to the Langmuir-Blodgett technique. Using this technique and by adjustment of the substrate pH it was possible to form outer surfaces consisting of either close-packed methyl or carboxyl end-groups. Thus adhesion was studied on non-polar surfaces formed by the methyl end-groups and on high density negatively charged surfaces formed by carboxyl groups. Under the experimental conditions the non-polar surfaces showed the same degree of adhesiveness as the reference (metal), whereas adhesion was completely inhibited on multilayers with a high negative surface charge. The significance of these results is discussed with regard to dental plaque formation.

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Adhesion of microorganisms to solid surfaces is involved in numerous phenomena, such as formation of dental plaque, marine fouling and bacterial contamination of food processing equipment. Many studies have been conducted on various types of cellular adhesion to materials with different surface properties (for a review see Manly (9)). A common problem recognized in these studies is the often limited knowledge about the surface microtopography and the nature of the exposed chemical groups. Conse-

quently specific information about the nature of the dominating interfacial interactions is often difficult to obtain from these applied studies.

In physical chemistry it has been known for some time that by transferring lipids from a monomolecular surface film on water to a solid, it is possible to prepare surfaces where the molecular structure is known. It was thought worth while to study microbial adhesion to surfaces given different types of surface charges with this technique.

## MATERIALS AND METHODS

Lipid multilayers were prepared according to the well-known Langmuir-Blodgett technique (3, 6, 9) using normal fatty acids, stearic acid and behenic acid, all of reagent grade. The relationship between the structure in monomolecular phases and in the three-dimensional state, as given by X-ray diffraction analysis, indicates that the deposited multilayers have the same bilayer unit structure as the crystalline state (4). When the hydrophobic solid was moved up and down through the condensed monolayer at the air/water interface, the monolayers were transferred to the solid. This allowed the carboxyl groups to form the multilayer surface in water and the methyl end groups formed the surface in air. To obtain a multilayer surface consisting of the carboxyl groups it was thus necessary to keep the multilayer under water all of the time after the deposition of the last monolayer. Further, it was necessary to have most of the carboxyl surface groups in the ionic state. The monolayer, however, could be transferred to a solid support only if it was close-packed enough. This was the case when the molecules were uncharged or counterions were present to reduce lateral electrostatic repulsion.

The multilayers were deposited on chromium plated spatulas from condensed monolayers of stearic acid and behenic acid formed on water. Triolein was used as a piston oil, and the monolayers were spread on a solution of 0.1 N hydrochloric acid in order to obtain undissociated fatty acid molecules. The lipid multilayers were deposited on the lowest 1 cm of the spatulas, which were also covered by a thin layer of crystalline paraffin to facilitate the transfer of the first monolayer. The multilayers with methyl end group surfaces consisted of 20 monomolecular fatty acid

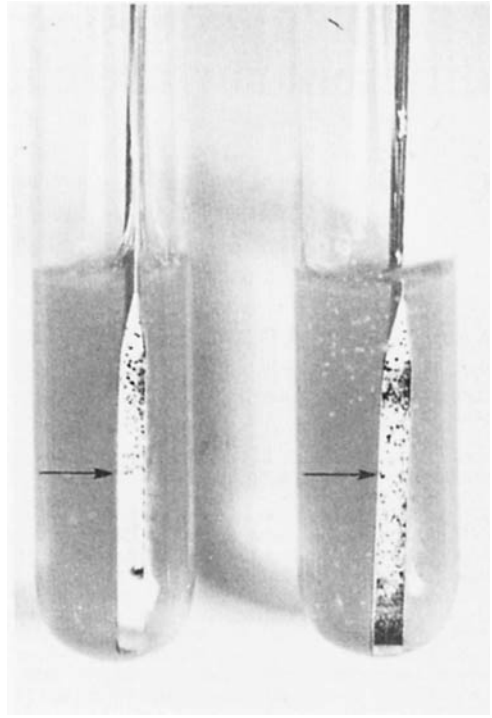


Fig. 1. Growth patterns of an oral strain of *S. sanguis* on metal spatula coated with behenic acid to give either exposed carboxyl (left) or methyl (right) groups. Arrows mark lines below which the left solid had not been and the right solid had been exposed to air between the adsorption of the fatty acid and exposure to the microorganisms.

layers, and those with carboxyl group surfaces of 19 monolayers.

An aqueous suspension of an oral strain of *Streptococcus sanguis*, kindly provided by professor B. Krasse, Univ. of Göteborg, was prepared by centrifugation from the nutrient medium described below (without agar) and repeated washing (four times) in distilled water. Each washing step involved shaking with distilled water for a few seconds, centrifugation for 10 minutes and removal of the washing water. By reculturing, these bacterial demonstrated no damage from the centrifugation and washing procedures. The spatula with the methyl group multilayer surface was dipped into a test tube of distilled water, whereas the liquid in the

tube with the carboxyl group multilayer surface was successively changed from 0.1 N hydrochloric acid to distilled water. A unit volume of the bacterial suspension was then added to each of the two test tubes. As an alternative, sodium chloride solution was used instead of distilled water. After 15 minutes the spatulas were removed and excess liquid present on their surfaces shaken off.

The number of adhering bacteria were determined in the following way. A nutrient medium was prepared consisting of tryptone (10 g), yeast extract (5 g),  $K_2HPO_4$  (5 g), sucrose (50 g) and water (1000 ml). A gel of this medium was formed around the spatulas by adding 1.5% (w/w) agar. After incubation at 37°C for about 30 hours the number of adhering bacteria (colony forming units) were counted by visual examination (Fig. 1). The effects studied here, however, require only a qualitative estimation of adhering bacteria.

## RESULTS AND DISCUSSION

A profound difference in adhesion of *Streptococcus sanguis* was observed between the behenic acid multilayers with the methyl end group surface and those with a dissociated carboxylic group surface (Fig. 1). The methyl group surface showed no significant difference in bacterial adhesiveness compared to the metal surface, whereas no adhesion at all was noticed on the carboxyl group surface. These results were reproduced many times after the experimental conditions had been standardized. Initial experiments were performed on stearic acid multilayers, where the same distinction was observed only occasionally. It was then, however, found that great care was required to

maintain the surface structure. The described methyl group surfaces are easily damaged when they are dipped through a water surface not covered by a film. Then a reversed process will occur from the build up of multilayers from existing surface monolayers. By moving the multilayers quickly through the water surface this loss of surface properties was prevented. A definite risk of losing the multilayer due to the solubility of the fatty acid molecules was also found to be present. Behenic acid was therefore preferred over stearic acid.

It seems reasonable to assume that the prevention of adhesion in the presence of lipid multilayers with carboxyl group surfaces, was due to electrostatic repulsion. The charge density is very high in these experiments, corresponding to one electron of about  $23 \text{ \AA}^2$ . Although the surface pH cannot be determined, it is probable that all carboxyl groups were ionized as the bulk pH was about two units above the dissociation constant ( $pK_s$ ) of the fatty acid.

The observed effects were not limited to the strain of *Streptococcus sanguis* studied but were also noticed when a gram negative organism, *E. coli*, was used under the same experimental conditions.

The effects of ions were tested by exchanging distilled water for sodium chloride solutions of various concentrations. Already at 0.5% (w/w) of sodium chloride a few colonies were observed on the carboxyl group surfaces. At a concentration of 2% sodium chloride there were no longer any significant differences in adhesion on methyl group surfaces as compared to carboxyl group surfaces.

It should be noted that the effects observed cannot be due to the antimicrobial activity of the fatty acids (7), as the comparison samples contain the same molecules, the only difference being

the molecular orientation at the interface.

It seems rather apparent that the demonstrated inhibition of adhesion was due to electrostatic repulsion between the bacteria, with a net negative surface charge (Olsson, Glantz & Krasse (11)), and the negatively charged surface groups on the fatty acid multilayers.

Many discussions of cell adhesion are based on a simplified model of cells as colloidal particles, the adhesion of which is determined by the balance between the electrostatic repulsive forces and the van der Waals attractive forces. The theory of attractive forces has been extended by Lifshitz (8) to macroscopic regions, and can therefore be applied in the theoretical treatment of cell adhesion. Such an analysis of the physical forces involved in cell adhesion has been reported by Parsegian & Gingell (10). In this comprehensive treatment of adhesion a distinction is made between, on one side, long-range forces of the attractive/repulsive type discussed above, and on the other side, short-range interaction, where specific cell surface groups can be involved. In the case of the formation of dental plaque it is, for example, quite obvious that specific interactions are involved. When the electrostatic repulsion is strong enough, the distance is too great for specific bindings to take place.

An increase of the net negative charge of the tooth surface should be expected to reduce bacterial adhesion and thus prevent or decrease the rate of plaque formation. Anionic surface active agents might give this effect provided that they can be adsorbed on the tooth surface in a surface concentration comparable to those of the multilayers described.

Cationic surface active agents should be expected to be strongly adsorbed to the tooth surface. Although they will re-

duce the electrostatic barrier preventing adhesion, they might on the other hand change the surface energy towards a more bioadhesive range or to a more 'biocompatible' surface with regard to the actual microorganisms (1). Furthermore such substances could be expected to alter the structure of the mucoprotein biofilm present on solid oral surfaces (2), and in that way obstruct the short range «specific» forces involved in the adhesion.

It is obvious that the mechanisms involved in bioadhesive events are complex. Therefore empirical approaches are necessary in studies where ionic surface-active substances are evaluated as a mechanism to influence the adhesiveness of the tooth surface.

#### REFERENCES

1. Baier, R.E. Surface properties influencing biological adhesion In: Manly, R.S., ed. Adhesion in biological systems, Academic Press, N.Y. 1970, pp. 15 - 48
2. Baier, R.E. & Glantz, P.-O. Characterization of oral in vivo films formed on different types of solid surfaces. *Acta Odontol. Scand.* 1978, 36, 289 - 301
3. Blodgett, K.B. Monomolecular Films of Fatty Acids on Glass. *J.Am. Chem. Soc.* 1934, 56, 495 - 496
4. Blodgett, K.B. Film Built by Depositing Successive Monomolecular Layers on a Solid Surface. *J.Am. Chem. Soc.* 1935, 57, 1007 - 1022
5. Gingell, D. & Parsegian, W. A. Computation of van der Waals Interaction in Aqueous System Using Reflectivity Data. *J. Theor. Biol.* 1972, 36, 41 - 52
6. Larsson, K. Lipid Multilayers. *Surface and Coll. Sci.* 1973, 6, 261 - 287
7. Larsson, K., Norén, B. & Odham, G. Antimicrobial Effect of Simple Lipids and its Relations to Surface Film Behaviour. *Biochem. Pharm.* 1972, 21, 947 - 962
8. Lifshitz, E.M. The theory of molecular attractive forces between solids. *Soviet Physiol. J.E. T.P.*, 1956, 2, 73 - 83
9. Manly, R.S. (ed.). Adhesion in Biological Systems, Academic Press, New York 1970
10. Parsegian, W.A. & Gingell, D. Some Features of Physical Forces Between Biological Membranes. *J. Adhesion* 1972, 4, 283 - 306
11. Olsson, J., Glantz, P.-O. & Krasse, B. Electrophoretic mobility of oral streptococci. *Arch. Oral Biol.* 1976, 21, 605 - 609