The Deposition of *Streptococcus sanguis* NCTC 7868 from a Flowing Suspension

By PAUL RUTTERT† AND ROGER LEECH*

Department of Colloid and Surface Science, Unilever Research, Port Sunlight Laboratory, Wirral, Merseyside L62 4XN

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Measurements were made of the rates at which continuously cultured *Streptococcus sanguis* NCTC 7868 cells accumulated on the inside surfaces of narrow glass capillaries from suspensions of the bacteria flowing down the capillaries at different velocities. Initially, the rate of accumulation of bacteria on the clean walls of the capillary was rapid. The deposition rate decreased with time, however, resulting in a saturation coverage of the glass surface which was considerably less than a monolayer. Multilayer coverage of the tube surface by bacteria was only achieved when fresh nutrient was pumped over deposited cells. This was attributed to cell growth.

Although theoretical considerations of the deposition of small particles on to the walls of a tube suggest that the initial deposition rate should increase with flow rate, this was not the case with cells grown at dilution rates of 0.2 and 0.5 h⁻¹. It is suggested that this can be explained by a polymer bridging mechanism of attachment.

**INTRODUCTION**

Bacteria appear to be able to accumulate on the surfaces of a wide variety of different materials. This important property has led to a number of studies designed to quantify bacterial deposition and adhesion. In general, these experiments have involved placing a defined collector surface, often glass, into a suitable environment for a known period of time and observing the surface of the collector after it has been withdrawn (Corpe, 1974). Alternatively, simple experiments *in vitro* have been carried out involving the sedimentation of bacteria on to the bottom of polystyrene Petri dishes (Fletcher, 1977), the adsorption of bacteria to suspended hydroxyapatite particles (Clark *et al.*, 1978) and the accumulation of bacteria on metal wires (McCabe *et al.*, 1967).

In addition to their ability to accumulate on surfaces in static systems, bacteria are also able to accumulate on surfaces over which liquid is flowing. This poses a number of interesting problems since it is not clear whether the rate at which bacteria accumulate on a surface will increase or decrease with the flow rate of the suspension.

The purpose of the following study was to observe and quantify the deposition of *Streptococcus sanguis* NCTC 7868 on to glass from suspensions flowing at different rates. *Streptococcus sanguis* was chosen because it adheres to glass and is representative of organisms that are often present in the bacterial deposits found on teeth (Gibbons & van Houte, 1973). The influence of growth rate on the rate at which cells accumulated on the glass surface was also observed since it has been suggested that growth rate has a considerable influence on bacterial adhesion (Ellwood *et al.*, 1974).

† Present address: B.P. Research Centre, Sunbury-on-Thames, Middlesex.
METHODS

Organism. *Streptococcus sanguis* NCTC 7868 was used in all the experiments. Cultures were maintained on blood agar (Oxoid); they were subcultured every 2 weeks by growing for 24 h aerobically at 37 °C and then stored at 4 °C. Similar cultures grown in 200 ml BH broth (Oxoid) were used to inoculate the chemostat.

Chemostat medium. Carlsson's M51 medium (Carlsson, 1970) supplemented with 0-1 % (w/v) Acidase (BBL) was used. Glucose (0.25 %, w/v) was the growth-limiting substrate and the chemostat was monitored daily to ensure that no glucose was left in the effluent. The bulk medium, minus the vitamins, glucose, salt solution and buffer, was autoclaved twice (24 h interval) at 121 °C for 20 min. The vitamins plus glucose were autoclaved at 115 °C for 20 min. The salts solution and buffer solution were individually filtered (Millipore, 0-45 μm pore size). All three solutions were added aseptically to the sterile bulk medium in a laminar flow hood.

Chemostat. A 3 l chemostat (LH Engineering, Stoke Poges, Bucks) was used. The temperature was maintained at 36 ± 1 °C, the stirrer speed was 300 rev. min⁻¹ and the pH was maintained at 6.8 by automatic addition of 2 M-NaOH. The head space above the culture was gassed with 10% CO₂/90% N₂ at 60 ml min⁻¹ to prevent foaming. The inoculum was grown in the chemostat under batch growth conditions until mid-exponential phase and then the chemostat was switched on to continuous growth at a dilution (specific growth) rate (μ) of 0.04, 0.2 or 0.5 h⁻¹. The culture was allowed to attain a steady state, monitored by the absorbance at 570 nm and by the rate of alkali addition, before any experiments were carried out. At steady state, samples taken from the effluent line gave single cell counts (as assessed in an Improved Neubauer Counting Chamber) of 1.1 ± 0.1 x 10⁸ cells ml⁻¹ and total counts of 2.4 ± 0.5 x 10⁹ cells ml⁻¹ for all the specific growth rates.

Microslide. The culture effluent, which was continually drawn from the chemostat, was pumped into a 150 ml flask containing a magnetic stirrer which acted as a constant head for the microslide (Fig. 1). Excess effluent flowed over a weir in the constant head and went to waste. The bacterial suspension was drawn from the constant head at a known flow rate through a glass microslide (0.2 x 2 x 30 mm; Camlab, Cambridge). At the maximum flow rate of 1.5 ml min⁻¹ the Reynolds number (Re = 12.5) was well within the range required for streamline flow (Re < 40). Streamline flow was assumed to be fully developed at the centre of the tube since the tube length was considerably greater than its half-width. A new slide was used for each experiment. All the slides were taken from the same batch and were used without further treatment, although each was checked microscopically for evidence of any particulate contamination. Two heat exchangers were incorporated into the line bearing the flowing suspension, on either side of the microslide, in an attempt to eliminate any thermal gradient down the tube and to maintain the central sampling area of the tube at approximately 36 °C.

The rates of deposition on the top and bottom surfaces of the microslide were obtained by counting the number of cells which had deposited after fixed intervals using a phase contrast microscope (Carl Zeiss Photomicroscope) at ×400 magnification. Two counts were made for each field. In the first all the cells were counted irrespective of whether they were deposited individually on the surface or as part of a group (deposited aggregate). The total deposition rates were calculated from these counts. In the second count only singly deposited cells were counted. These were quite easy to count especially at low surface coverages.

The deposition characteristics of *S. sanguis* grown at three different specific growth rates (0.04, 0.2 and
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Fig. 2. Total accumulation of cells (a) and accumulation of single cells (b) on to the top (○) and bottom (●) surfaces of the tube from a suspension of bacteria grown at a dilution rate of 0·04 h⁻¹ flowing at an average rate of 0·007 m s⁻¹.

0·5 h⁻¹) were observed at a variety of suspension flow rates varying from 0·007 to 0·063 m s⁻¹. Initial deposition rates were calculated from the slopes of curves plotted to show the increase in the number of deposited cells with time (e.g. Fig. 2) from at least two experiments involving each flow rate and growth rate.

To obtain growth of the cells in the microslide, 2 h deposition experiments were carried out after which the supply of cells from the constant head was stopped. Medium from the chemostat feed line was then fed directly to the microslide for up to 72 h.

Latex experiment. Latex particles (diam. 0·8 μm) were suspended in the chemostat medium to give 1·6×10⁸ particles ml⁻¹. The suspension was then pumped through a clean microslide and deposition was monitored at various flow rates on both the inside top and inside bottom surfaces of the capillary in the same way as for the bacteria.

RESULTS

Results of a typical experiment monitoring the increase with time in the number of bacteria attached to the walls of the capillary tube at a constant flow rate are shown in Fig. 2. The curves resemble those associated with the deposition of glass particles from flowing suspensions (Bowen & Epstein, 1979). Initially, the number of deposited cells increases rapidly and then a gradual decrease in the slope of the curve occurs until it becomes almost zero as it tends towards an apparent maximum coverage. The maximum number of deposited cells recorded in any of the experiments was 3×10⁷. This corresponds to a coverage of about 30% of the tube surface with bacteria, and this coverage was never exceeded even when deposition was allowed to continue for up to 70 h.

However, if the organisms were allowed to grow, multilayer coverage was obtained. Figure 3 shows a typical coverage obtained after 6 h continuous deposition at a flow rate of 0·007 m s⁻¹ on to the top inside surface of the capillary, and Fig. 4 shows the same surface after fresh medium had been pumped through the tube at the same rate for 53 h. A dense layer of organisms can be seen, and chains of cells extended away from the glass wall of the tube and into the fluid. The layers of cells were not uniform but exhibited a ‘patchiness’ as though fragments of the deposit had become suddenly detached, leaving areas of the tube surface free of cells. This phenomenon was not investigated in any detail.

The deposition rates of single cells identified within the heterogeneous suspensions showed a striking difference between the cells grown at different rates (Fig. 5). The single cells of the culture grown at a dilution rate of 0·04 h⁻¹ showed an apparent increase in deposition rate with an increase in flow rate, whereas single cells from the other two cultures showed a decrease in deposition rate with increasing flow rate.
Fig. 3. Bacteria accumulated, after 6 h, on the top inside surface of a capillary tube from a suspension of bacteria grown at a dilution rate of 0.04 h⁻¹ flowing at 0.007 m s⁻¹. Bar marker represents 10 µm.

Fig. 4. The same field as shown in Fig. 3 after fresh medium had been pumped through the tube at 0.007 m s⁻¹ for 53 h. Bar marker represents 10 µm.

Fig. 5. Variation with flow rate in the single cell deposition rate on to the top (○, □, △) and bottom (●, ■, ▲) surfaces of the tube. The bacteria had been grown at dilution rates of 0.04 h⁻¹ (○, ●), 0.20 h⁻¹ (□, ■) and 0.50 h⁻¹ (△, ▲).

Fig. 6. Variation with flow rate in the deposition rate for single cells of S. sanguis grown at a dilution rate of 0.04 h⁻¹ (○, □) and for polystyrene latex particles (●, ■) on to the top (○, ●) and bottom (□, ■) surfaces of the tube. (C₀ latex, 1.6 × 10⁶ ml⁻¹; C₀ bacteria, 1.2 × 10⁹ ml⁻¹.)

Figure 6 shows the deposition behaviour of single cells grown at a dilution rate of 0.04 h⁻¹ and of polystyrene latex spheres (diam. 0.8 µm) suspended in Carlsson’s medium. Both the bacteria and the latex particles show an increase in deposition rate with increasing flow rate.

DISCUSSION

The results show that the rate at which bacteria deposit on to the walls of a glass tube from a flowing suspension is time-dependent. Initially, deposition on to the clean glass
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surface is rapid but it decreases with time until an apparent saturation surface coverage is attained when the deposition rate is zero (Fig. 2). The saturation coverage does not represent a complete monolayer: the maximum surface coverage recorded in any of the experiments carried out was about 30% and it was usually much less than this. The low saturation surface coverage may be due to mutual repulsion between the cells resulting in the exclusion of depositing cells from the area of surface surrounding an attached cell. The presence of a few deposited cells would also disturb the fluid streamlines across the surface which might result in a decreasing deposition rate.

The deposition of fine particles on to the walls of a tube has been considered by Bowen & Epstein (1979) who concluded that the local particle deposition rate $J_d$ in a parallel plate channel is given by

$$J_d = \frac{DC_0}{b} \frac{(\frac{\gamma}{b})^\frac{1}{3}}{\Gamma(\frac{4}{3}) + (1/K)(\frac{\gamma}{b})^\frac{1}{3}}$$

where $\gamma = 2Dx/3V_m b^2$ is the dimensionless longitudinal distance from the channel inlet, $D = kT/6\pi\eta a$ is the Stokes–Einstein diffusion coefficient, $C_0$ is the concentration of particles at the channel inlet, $b$ the half-thickness of the channel, $x$ the distance down the channel, $V_m$ the mean velocity of the flowing suspension, $k$ the Boltzmann constant, $T$ the absolute temperature of the suspension, $\eta$ the viscosity of the aqueous medium, $a$ the average radius of the particles, $K$ the surface reaction rate constant, and $\Gamma(\frac{4}{3})$ is a constant. A similar result was obtained by Turitto & Baumgartner (1975) for the deposition of platelets on to subendothelium surfaces exposed to flowing blood.

The expression is composed of two parts, a diffusion term describing the transport of particles from the suspension to the tube walls and a surface interaction term describing the adhesion step during which a particle in close proximity to the wall becomes attached. In the case of small uniform particles and well-defined wall surfaces of known surface charge, $K$ can be evaluated by considering the electrostatic and London–van der Waal’s interactions between the particles and tube wall, although an attempt to do so by Bowen & Epstein (1979) showed only qualitative agreement between theory and experiment. In the absence of repulsion between the particles and tube walls, for example in the case of positive particles depositing on to a negatively charged surface, $K = \infty$. Consequently, the deposition rate is the same as the rate at which particles diffuse to the walls of the tube. This represents a maximum deposition rate which increases with $V_m^{\frac{1}{3}}$.

Since $K$ is independent of flow rate, the deposition rate of small diffusing particles in a tube must either be independent of $V_m$ in the case of a very slow surface reaction rate when $J_d = KC_0$ or increase as some function of $V_m$ approaching $V_m^{\frac{1}{3}}$. This is illustrated by the results obtained from experiments using polystyrene latex spheres of similar diameter to the bacteria. Although the scatter on the points (Fig. 6) is rather high it can be seen that the deposition rate does in fact increase with $V_m$. Comparison of the experimental deposition rates with the theoretical maximum deposition rate (assuming $K = \infty$) shows that about one-sixth of the particles which diffuse to the walls of the tube adhere.

Unfortunately, the bacterial suspensions used in the experiments were not homogeneous with respect to particle size. This was due to the presence of suspended aggregates as well as single cells. The presence of large suspended aggregates increases the complexity of the situation in two ways. Firstly, the aggregates tended to sediment to the bottom of the horizontal tube, leading to an increase in the local concentration of cells and hence a greater deposition rate on the bottom tube surface than on the top. Secondly, the aggregates appeared to be entrained in the fluid streamlines and could be seen rolling along, close to the bottom wall of the tube, under the influence of the shear gradient. This ‘sweeping’ of the surface by aggregates might have resulted in the removal of some of the deposited bacteria. However, this does not seem to have been the case since photographs showing
the positions of deposited cells at the beginning of an experimental run could be superimposed over those taken at the end of an extended deposition period. This also suggests that the fluid removal forces generated by the flowing suspension were not sufficient to remove particles once they were attached.

If deposited particles are not removed under the influence of fluid flow or collisions with aggregates, another mechanism must be proposed to explain the high initial deposition rates and the apparent decrease in deposition rate with increasing flow rate exhibited by single cells from the cultures grown at dilution rates of 0.2 and 0.5 h⁻¹. Although there are problems associated with the precise quantitative description of single particles depositing from a suspension that is heterogeneous with respect to particle size, the results shown in Fig. 5 indicate a definite reduction in deposition rate with increasing flow rate. This implies that $K$ is not independent of flow rate.

In the case of homogeneous spheres interacting with homogeneous surfaces under the influence of electrostatic and van der Waal's forces a lateral displacement during the interaction will not result in a change in the interaction energy. If, however, the particle is interacting with the surface by means of a polymer bridge, the rate of polymer adsorption must be greater than the rate of displacement of the particles or the bridges will not have time to form and the particle will not become attached. The formation of polymer bridges between bacteria and various substrates has been suggested by a number of authors both as a primary attachment mechanism (Rutter & Abbot, 1978; Mukasa & Slade, 1973) and as a method of consolidating weak, non-specific attachment (Marshall et al., 1971). It can be suggested, therefore, that the results shown in Fig. 5 indicate that the cells grown at 0.2 and 0.5 h⁻¹ readily attach to the glass walls of the tube from slowly moving suspensions but as the speed of suspension flow increases the residence time of each cell in the vicinity of a particular surface site decreases thus reducing the time available for the adsorption of cell surface bridging polymers to the tube walls.

The reason why the cells grown at 0.04 h⁻¹ did not show this behaviour is not clear. In addition to the slower dilution rate the cells were retained for some time in the constant head device prior to passing down the tube. This could have resulted in considerable changes to the cell surface and the general overall condition of the cells. Transmission electron micrographs showed that the cells were still intact, however, and of a similar size to the more rapidly grown cells.

Whilst it is perhaps unwise to draw any specific conclusions regarding the behaviour of cells grown at slow dilution rates on the basis of one experiment it does appear that the ‘flow tube’ technique is capable of demonstrating different types of adhesive behaviour between similarly sized particles and bacteria in a quantitative way. The method also has the advantage of the direct observation of the substrate surface during the deposition process and, if required, a subsequent growth stage. However, there are a number of problems associated with the formulation of suitable expressions to deal with the heterogeneous size distribution of many bacterial suspensions and with the surface reaction rate constant $K$.

REFERENCES


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