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Adherence of *Escherichia coli* to urinary catheter is a function of time, temperature and cell physiology

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Abstract

The in-vitro adhesion of *Escherichia coli* to urinary catheter was investigated by standard procedure. Adhesion depended on time and temperature: lower adhesion densities were observed when the contact time was only 2 h or 6 h, as compared to 24 h, or when the test was performed at refrigerated condition, as compared to 37°C. The effect of gentamicin on the adhesion was determined as a demonstration of the influence of cell physiology on the process. It was observed that a sub-minimum inhibitory concentration (3 µg /ml^{-1}) of gentamicin inhibited the adhesion. The reduction in adhesion densities thus observed by lowering the contact time or performing adhesion under unfavourable metabolic condition (refrigeration) or in the presence of gentamicin provides an additional evidence of the involvement of extracellular polymers in the adhesion of *E. coli* to inert surfaces.

Key words: *Escherichia coli,* in vitro adhesion, urinary catheter, gentamicin

Introduction

Bacteria adhere to a variety of surfaces, from inanimate surfaces, such as soil particles, rocks, pipes, heat exchangers and biotechnological reactors, to animate surfaces, such as other microbial cells, plant roots and leaves, and animal tissues (1). Bacterial adhesion and biofilm processes have important consequences (2, 3) which can be either beneficial, such as in biotechnology (waste water treatment, bioremediation, immobilized cells in reactors), or detrimental, such as in industrial systems (fouling, contamination) and medicine (accumulation on teeth, implants and prosthetic devices). Urinary tract infections (UTIs) are especially common in cases of catheterization; 100% of patients with in-dwelling catheters will acquire UTIs due to the establishment of biofilms on the catheter material (4). *Escherichia coli* is the most common bacterium found in biofilms that have developed on catheters introduced into the urinary tract (5).

Bacterial adhesion is governed by reversible physiochemical forces that include electrostatic, van der Waals, and hydrophobic interactions, followed by the establishment of irreversible interactions such as specific receptor ligand binding events through the production of extracellular polymeric substances and appendages, such as fimbriae, pili, flagella, lipopolysaccharides, or capsular polysaccharides (6, 7, 8). Marshall et al. (9) suggested that the production of adhesive polymers during the early adhesion process may result in irreversible adhesion, by bridging across the repulsion barrier. Polymer accumulation between bacteria and surfaces has been directly observed by atomic force microscopy (10). To date, bacterial polymers have been characterized after isolation from

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Culture supernatants or from the support surface; however, at the present time, the role of these important cell surface molecules in reversible physiochemical and specific binding interactions is poorly understood (11, 12). Our understanding of how bacterial cells attach and colonize solid surfaces is constantly evolving, and no one theory of adhesion encompasses the diversity of adhesion mechanisms reported.

Adhesion of microorganisms to surfaces is dependent on the species and the environment (13). In *E. coli,* fimbriae (14), flagella (15) and certain envelope components, such as capsule, slime, proteins and lipopolysaccharides (16, 17) have been shown to play key roles in its adhesion to surfaces-both inanimate and animate. However, little is known about how experimental conditions of time and temperature, and cell metabolism influence the adhesion of *E. Coli* to surfaces.

In the present study, adhesion of *E. Coli* to the inert surface of urinary catheter was investigated with the aim of better understanding the roles of time and temperature, and of envelope components produced by the cells on their adhesion. Given the important medical and economical consequences of adhesion, there is a strong need to understand the colonization process in order to discover means of interfering with it.

Materials and methods

Bacterial Culture

Escherichia coli NCTC 10418 used in this study was maintained on tryptic soy agar (Biolife Ltd., Italy) slants at refrigerated condition and sub-cultured on same agar overnight prior to use.

Inert support material

Urinary catheter (siliconized rubber) was cut into pieces (of size: 2 cm x 1 cm) and used as support material. Fresh pieces were used in each set of experiment.

Adhesion test

Adhesion was measured in static conditions in accordance with the protocol described previously (18) with certain modifications. Briefly, tryptic soy broth (E-Merck Ltd., Germany) was prepared and distributed in 15 ml volumes into twelve universal bottles (two bottles for each contact time and temperature) containing two pieces of the catheter and sterilized by autoclaving at 121°C (15 Psi pressure) for 15 min. After cooling, each bottle was then inoculated with 3 to 4 colonies from overnight agar culture. The twelve bottles were thereafter grouped into two groups of six bottles each namely A and B. Those in group A were kept in a refrigerator while those in B were incubated at 37°C. Two bottles each from both groups A and B were left for 2 h, 6 h and 24 h contact times. The catheter pieces were then transferred aseptically after the appropriate contact times into 10 ml of sterile normal saline and rinsed to remove loosely adhered cells. Each piece of the support from each bottle was then placed individually in universal bottles containing 9.9 ml of sterile normal saline (this gives a 1:100 dilution approximately) and vigorously mixed by vortexing for 2 min to dislodge adhering cells. The dislodged cells were then counted on tryptic soy agar plates after appropriate dilution in sterile normal saline.

The procedure was repeated, this time, in the presence of 3 $\mu g/ml^{-1}$ gentamicin, a sub-minimum inhibitory concentration, to determine the effect of the antibiotic on the adhesion

after 24 h incubation at 37°C. The minimum inhibitory concentration of gentamicin against the test strain earlier determined by macro-broth doubling dilution was 3 μg/ ml^{-1}. Similarly, count of cells in the catheter-free broth were determined after 2 h, 6 h and 24 h incubation at 37°C and refrigeration condition as well as after 24 h incubation at 37°C in antibiotic-containing broth to serve as controls. The percentage adhesion of bacteria in each experimental situation was then calculated using the formula: Adhesion (%) = (CFU of bacteria adhering to support / CFU of bacteria in the catheter-free broth after 2 h, 6 h and 24 h incubation at 37°C or refrigeration condition as well as after 24 h incubation at 37°C in antibiotic-containing broth) x 100.

Results

Adhesion as a function of contact time and temperature

The percent adhesion was lower when the contact time is only 2 h or 6 h compared to 24 h, or when adhesion is performed at refrigeration temperature compared to 37°C (Table 1).

Table 1: Adhesion of ***Escherichia coli*** to 2 cm ^{2} surface of urinary catheter as a function of contact time, temperature and cell physiology

Contact time	*		
	37°C	4°C	37°C, Gentamicin $^{-1}$)
24 h			
6 h			ND
2 h			ND

*Percent adhesion is determined from the relation: a / b x 100, where a = CFU of bacteria adhering to surface of support, and b = CFU of bacteria in the catheter-free broth after 2 h, 6 h and 24 h incubation at 37°C or refrigeration condition as well as after 24 h incubation at 37°C in antibiotic-containing broth. ND: not determined.

Effect of the antibiotic on the adhesion

Adhesion (%) was much lower (0.02 ± 0.00) in the presence of sub-minimal inhibitory concentration (3 μg/ml^{-1}) of gentamicin, an antibiotic known to inhibit protein synthesis (Table 1).

Discussion and conclusion

The dependence of the adhesion of *E. Coli* to catheter surface on contact time and on temperature could be attributed to two reasons: slow kinetics and cell physiology particularly, the synthesis of adhesive macromolecules. It is expected that as the cells

approach the support, they encounter an important potential energy barrier due to electrostatic repulsion between the two surfaces (19). Therefore, an increase in time and temperature should increase the number of cells which cross the potential barrier. Marshall et al. (9) had before now, shown that the adhesion of marine bacteria is a two-stage process, an instantaneous reversible step being followed by a time-dependent irreversible step.

The fact that adhesion was reduced in the presence of sub-minimal inhibitory concentration of gentamicin, an antibiotic known to inhibit protein synthesis, indicates that protein synthesis is required. Gentamicin may inhibit either the synthesis of attachment proteins or the production of enzymes necessary for the synthesis of macromolecules, like polysaccharides involved in adhesion. Fletcher (20) demonstrated, by using a range of metabolic inhibitors, that both energy production and protein synthesis may be required for attachment of marine bacteria to solid surfaces. Sub-lethal concentrations of gentamicin was found to reduce fimbriation and cell surface hydrophobicity of growing cultures of *Actinomyces viscosus* and *Bacteroides gingivalis* (21), as well as their attachment to experimental salivary pellicles (22). Furthermore, the anchoring of *Azospirillum brasilense* to polystyrene and wheat roots was reported to be reduced by several bacterial inhibitory substances (23). The dependence on time, temperature and cell metabolism, of the adhesion of *A. brasilense* to glass and polystyrene, has been demonstrated not long ago (12).

In conclusion, adhesion of *E. Coli* to the inert surfaces of urinary catheter is seen to depend on time, temperature and cell metabolism. Lower adhesion was observed when the contact time was only 2 h or 6 h, as compared to 24 h, or when the test is performed at refrigeration temperature as compared to 37°C. Cell physiology was also observed to influence the adhesion process as demonstrated by the inhibition of adhesion in the presence of gentamicin.

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