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# The effects of surface properties on *Escherichia coli* adhesion are modulated by shear stress

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ABSTRACT

The adhesion of *Escherichia coli* to glass and polydimethylsiloxane (PDMS) at different flow rates (between 1 and 10 ml $s^{-1}$) was monitored in a parallel plate flow chamber in order to understand the effect of surface properties and hydrodynamic conditions on adhesion. Computational fluid dynamics was used to assess the applicability of this flow chamber in the simulation of the hydrodynamics of relevant biomedical systems. Wall shear stresses between 0.005 and 0.07 Pa were obtained and these are similar to those found in the circulatory, reproductive and urinary systems. Results demonstrate that *E. coli* adhesion to hydrophobic PDMS and hydrophilic glass surfaces is modulated by shear stress with surface properties having a stronger effect at the lower and highest flow rates tested and with negligible effects at intermediate flow rates. These findings suggest that when expensive materials or coatings are selected to produce biomedical devices, this choice should take into account the physiological hydrodynamic conditions that will occur during the utilization of those devices.

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## 1. Introduction

Bacteria often adhere to surfaces and form biological communities called biofilms [1] that develop in almost all types of biomedical devices [2]. These sessile cells are typically more resistant to antimicrobial agents than planktonic ones, have a decreased susceptibility to host defense systems and function as a source of resistant microorganisms responsible for many hospital acquired infections [3]. Moreover, biofilm spreading on the surface upon prolonged use of the biomedical device can cause material biodegradation, changes in surface properties and deterioration of the medical functionality [1,2].

Different polymers are commonly employed in biomedical devices. These materials should be biocompatible and have to be stable, resistant against different body fluids and display anti adhesive properties toward microorganisms [1–3]. Polydimethylsiloxane (PDMS) is a polymer that has been widely used in biomedical devices like contact lenses, breast implants, catheters, and used in the correction of vesico ureteric reflux in the bladder [1,4]. These devices are often colonized by single bacterial species like *Escherichia coli* [5]. *E. coli* is responsible for 80% of the urinary tract infections and it was observed that even after antibiotic therapy it can persist and re-emerge in the bladder and in associated urinary tract biomedical devices (*e.g.* urinary catheters) [3,6,7]. *E. coli* has also been found in breast implants, being responsible for 1.5% of associated infections, and contact lenses [3,8]. It has been reported that 60–70% of the hospital acquired infections are associated with medical devices and cost $5 billion annually in the US [9,10]. Additionally, the costs associated with the replacement of infected implants during revision surgery may triple the cost of the primary implant procedure [11]. Moreover, secondary implants and devices have a higher infection incidence because antibiotic resistant bacteria residing in the surrounding tissue can proliferate and colonize the recently implanted device [11]. Therefore, owing to the problems associated with the increasing use of these devices, a preventive strategy must be adopted [3]. Understanding biofilm formation mechanisms and the factors that influence cell attachment to a surface is essential to prevent and to treat biofilm related diseases. The properties of microbial cells and environmental factors such as surface properties of the biomaterials as well as associated flow conditions affect the process of biofilm formation [12].

*In vitro* systems have been employed to test the effect of different surfaces on the biofilm formation process under different environmental conditions [13]. Barton et al. [14] have used a parallel plate flow chamber (PPFC) at a shear rate of 1.9 $s^{-1}$ to observe the adhesion of *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *E. coli* to orthopedic implant polymers. These authors verified

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that *P. aeruginosa* adhered more than *S. epidermidis* and that the estimated values of the free energy of adhesion correlated with the amount of adherent cells. Pratt-Terpstra et al. [15] developed a flow cell system to study the adhesion of three strains of oral streptococci to glass, cellulose acetate and a fluorethylenepropylene copolymer at a shear rate of 21 $s^{-1}$. They verified that a linear correlation was found between the number of bacteria adhering to those surfaces and the free energy of adhesion. Bruinsma et al. [16] used a PPFC at a shear rate of 10 $s^{-1}$ to study the adhesion of a hydrophobic *P. aeruginosa* and hydrophilic *Staphylococcus aureus* to hydrophobic and hydrophilic hydrogel contact lenses (CL) with and without an adsorbed tear film. The authors observed that adhesion of *P. aeruginosa* was more extensive than *S. aureus* although no difference between hydrophobic and hydrophilic CL was found. Millsap et al. [17] studied the effect of a hydrophobic silicone rubber and a hydrophilic glass in the adhesion of six *Lactobacillus* strains using a PPFC at a shear rate of 15 $s^{-1}$. These authors have also concluded that adhesion to the tested surfaces was not dependent on the hydrophobicity of the materials. These studies revealed that bacterial adhesion is not always correlated with surface properties. It is also apparent that studies performed under different hydrodynamic conditions have led to different conclusions. Thus, the effects of surface properties on bacterial adhesion should be evaluated in different hydrodynamic conditions according to the intended use of that material.

In this study, the adhesion of *E. coli* to glass and PDMS under different flow rates was monitored in a PPFC in order to understand the combined effect of the hydrodynamic conditions and surface properties on initial bacterial adhesion. A better understanding of the factors affecting the initial bacterial adhesion is important in the development of strategies to delay the onset of bacterial biofilms in biomedical devices.

## 2. Materials and methods

### 2.1. Numerical simulations

The PPFC used in the present work has a rectangular cross section of 0.8 × 1.6 cm and a length of 25.42 cm. The inlet and outlet tubes have a diameter ($D_{in}$) of 0.2 cm. The flow regime was defined using the Reynolds number calculated using the diameter and the velocity ($V_{in}$) of the inlet:

$$Re_{\mathrm{in}} = \frac{\rho V_{\mathrm{in}} D_{\mathrm{in}}}{\mu}$$

Here $\rho$ and $\mu$ are the density and viscosity of water, respectively.

A laminar regime in the inlet was considered for the flow rates of 1 and 2 ml $s^{-1}$ ($Re_{\mathrm{in}} < 2000$), and a turbulent regime was assumed for the flow rates of 4, 6, 8 and 10 ml $s^{-1}$ ($Re_{\mathrm{in}} > 3500$).

Numerical simulations were made in Ansys Fluent CFD package (version 14.5). A model of the PPFC was built in Design Modeler 14.5 and was discretized into a grid of 1,694,960 hexahedral cells by Meshing 14.5. The properties of water (density and viscosity) at 37 °C were used for the fluid.

Results in the laminar regime were obtained by solving the Navier–Stokes equations. The velocity–pressure coupled equations were solved by the PISO algorithm [18], the QUICK scheme [19] was used for the discretization of the momentum equations and the PRESTO! scheme was chosen for pressure discretization. The no slip boundary condition was considered for all the walls. Results for the turbulent regime were obtained by solving the SST $k$-$\omega$ model [20] with low Reynolds corrections.

Simulations were made in transient mode, to assure convergence and to capture transient flow structures. For each case, 2 s of physical time were simulated with a fixed time step of $10^{-4}$ s. Observation of the trajectories of tracer PVC particles circulating in the PPFC at different flow rates (as described in Teodósio et al. [21]) confirmed the flow pathlines predicted by CFD (not shown). A mesh independence analysis was performed by using a mesh with 690,475 cells and a 4.9% variation was obtained in the wall shear stress. Despite the small variation, the more refined mesh was used in the simulations to increase numerical accuracy.

### 2.2. Bacteria and culture conditions

*Escherichia coli* JM109(DE3) was used since this strain had already demonstrated a good biofilm formation capacity [22]. A starter culture was prepared as described by Teodósio et al. [23] and incubated overnight. A volume of 60 mL from this culture was centrifuged (for 10 min at 3202 × *g*) and the cells were washed twice with citrate buffer 0.05 M [24], pH 5.0. The pellet was then resuspended and diluted in the same buffer to obtain a cell concentration of $7.6 \times 10^7$ cell $mL^{-1}$.

### 2.3. Surface preparation and flow chamber experiments

The PPFC was coupled to a jacketed tank connected to a centrifugal pump by a tubing system. The PPFC contained a bottom and a top opening for the introduction of the test surfaces of glass and PDMS. Glass slides were firstly washed by immersion in a glass beaker containing 60 ml of a 0.5% solution of detergent (Sonasol Pril, Henkel Ibérica S A) for 30 min. After this, the slides were rinsed (with a squeezing bottle) with distilled water (10 ml) to remove the detergent and then they were immersed in other beaker containing sodium hypochlorite (60 ml at 3%) for an additional 30 min. After rinsing again with 10 ml of distilled water, half of the slides were coated with PDMS.

The PDMS (Sylgard 184 Part A, Dow Corning) was submitted to a 30 min ultrasound treatment in order to eliminate all the bubbles. The curing agent (Sylgard 184 Part B, Dow Corning) was added to the PDMS (at a 1:10 ratio). PDMS was deposited as a thin layer (with a uniform thickness of 10 μm) on top of the glass slides by spin coating (Spin150 PolosTM) at 2000 rpm for 60 s.

The PPFC was mounted in a microscope (Nikon Eclipse LV100, Japan) to monitor cell attachment. The cellular suspension was circulated through the PPFC at 1, 2, 4, 6, 8 or 10 ml $s^{-1}$ for 30 min. Images were acquired every 60 s with a camera (Nikon DS-RI 1, Japan) connected to the microscope. Temperature was kept constant at 37 °C using a recirculating water bath connected to the tank jacket. Three independent experiments were performed for each surface and flow rate.

### 2.4. Surface hydrophobicity and free energy of adhesion

Bacterial and surface hydrophobicity ($\Delta G$) and the free energy of adhesion ($\Delta G^{\mathrm{Adh}}$) were determined as described in van Oss [25]. Contact angles were measured at 25 ± 2 °C in a contact angle meter (Dataphysics OCA 15 Plus, Germany) using water, formamide and α-bromonaphtalene (Sigma) as reference liquids. One *E. coli* suspension was prepared as described for the adhesion assay and its physicochemical properties were also determined by contact angle measurement as described by Busscher et al. [26].

The Lifshitz-van der Waals components ($\gamma^{\mathrm{LW}}$) and Lewis acid-base components ($\gamma^{\mathrm{AB}}$) which comprises the electron acceptor $\gamma^{+}$ and electron donor $\gamma^{-}$ parameters were determined as described in van Oss [25] enabling the determination of $\Delta G$ and $\Delta G^{\mathrm{Adh}}$, using the equations:

$$\Delta G = -2\left(\sqrt{\gamma_{\mathrm{s}}^{\mathrm{LW}}} - \sqrt{\gamma_{\mathrm{w}}^{\mathrm{LW}}}\right)^2 + 4\left(\sqrt{\gamma_{\mathrm{s}}^{+}\gamma_{\mathrm{w}}^{-}} + \sqrt{\gamma_{\mathrm{s}}^{-}\gamma_{\mathrm{w}}^{+}} - \sqrt{\gamma_{\mathrm{s}}^{+}\gamma_{\mathrm{s}}^{-}} - \sqrt{\gamma_{\mathrm{w}}^{+}\gamma_{\mathrm{w}}^{-}}\right) \quad (1)$$

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