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Micro- and macro-flow systems to study *Escherichia coli* adhesion to biomedical materials

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HIGHLIGHTS

- *E. coli* adhesion was monitored in a microchannel and in parallel plate flow chamber.
- Different adhesion results were obtained using different biomedical materials.
- Similar results were obtained in the microchannel and parallel plate flow chamber.
- Average wall shear stress may be a good scale-up factor between different systems.

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ABSTRACT

Micro- and macro-flow systems have been used as *in vitro* platforms to study bacterial adhesion under physiological conditions. The decision of which platform to use has been dictated by the dimensions of the *in vivo* systems that they are supposed to mimic and by the available resources in each laboratory. In this work, a microchannel and a parallel plate flow chamber were operated in order to observe the adhesion of *Escherichia coli* to different materials that are commonly used to construct biomedical devices for the urinary and reproductive systems. The surface properties of cellulose acetate, glass, poly-L-lactide, and polydimethylsiloxane were thermodynamically characterized by contact angle measurement and the flow along the platforms was simulated by computational fluid dynamics. The results presented in this study demonstrate that different adhesion rates were obtained on different materials but similar values were obtained in the micro- and macro-platforms for each material under the same shear stress (0.022 Pa). This suggests that despite the scale factor (80 ×) both platforms may be equally used to mimic the same biomedical biofilms for a specified shear stress. Thus, depending on the expertise and equipment availability in different labs, micro-flow systems can be used taking advantage of lower hold-up volumes or macro-flow systems can be selected in order to obtain a higher biofilm mass which can be used for further biochemical analysis.

1. Introduction

Biofilms are communities of microorganisms adhered to living or inert surfaces, surrounded by self-produced extracellular polymeric substances (Stoodley et al., 2002). Microbial adhesion to surfaces is dictated by a set of important variables, including cell transport and the imposed shear stress, which are affected by the flow conditions, and by physicochemical interactions between cells and surfaces (Pace et al., 2006).

Hospital-acquired infections are the fourth leading cause of death in the US and 65% of this infections are caused by biofilms (Robert and Salek, 2010). Most cases of infection in critically ill patients are associated with medical devices. Infection rates in medical devices comprise dental implants and fracture fixation devices (5–10%), bladder catheters (10–30%) and heart assistant devices (25–50%) (Weinstein and Darouiche, 2001). *Escherichia coli* has been documented as the major cause for infection of these devices (Castonguay et al., 2006). This bacteria is responsible for 80% of the urinary tract infections, 1.5% of infections in breast implants and it has also been found in pacemakers and contact lenses (Shunmugaperumal, 2010; Trautner and Darouiche, 2004; Wood, 1999). Bacterial adhesion and biofilm development on the surface of these medical devices can compromise their function and increase the health risk (Weinstein and Darouiche, 2001).

The scientific community has been trying to understand how to control biofilms in order to reduce their effects. Bacterial adherence to a surface is one of the first steps in biofilm formation (Nikolaev and Plakunov, 2007) and controlling this step is one of the most

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promising biofilm control strategies (Campoccia et al., 2013; Chen and Zhu, 2005; Gallardo-Moreno et al., 2011; Petrova and Sauer, 2012). Shumi et al. (2013) used a microfluidic device in order to investigate the influence of flow shear stress and sucrose concentration in the adhesion of *Streptococcus mutans* aggregates. With this platform they simulated the space between adjacent teeth in order to understand the process of dental caries formation by *S. mutans*. They observed that sucrose-dependent aggregates (larger than 50 μm in diameter) are more tolerant to shear stress than sucrose-independent aggregates. Bruinsma et al. (2001) investigated the effect of physicochemical surface interactions between seven different bacterial strains isolated from ophthalmic infections and hydrophilic and hydrophobic contact lenses (CL) with and without an adsorbed tear film. They used a parallel plate flow chamber (PPFC) for bacterial adhesion assays in order to mimic the natural eye environment and understand the process of microbial keratitis development. They concluded that CL hydrophobicity dictates the composition of the adsorbed tear film and thus the extension of bacterial adhesion to the lens. Andersen et al. (2010) have used a flow chamber operated at hydrodynamic flow conditions similar to those found in implanted devices in order to observe the effect of surface chemistry and temperature in adhesion and biofilm formation by *E. coli* strains on two types of silicone rubber. They observed that surface chemistry influenced surface colonization and that temperature was also a critical factor.

PPFCs and microchannels are two of the most widely used flow devices for adhesion/biofilm studies (Busscher and van der Mei, 2006; Gottenbos et al., 1999; Rivet et al., 2011). Both systems enable real-time visualization of bacterial adhesion/biofilm development in conditions which mimic *in vivo* environments (Barros et al., 2013; Kim et al., 2012). They enable control of the hydrodynamic conditions (*e.g.* shear stress), temperature, testing different materials and they can be used as high-throughput platforms (Bakker et al., 2003; Barros et al., 2013; Salta et al., 2013; Situma et al., 2006). Microfluidic systems have some advantages such as low volume requirements (*e.g.* reagents) which may lead to reduced operational costs (Situma et al., 2006), they mimic phenomena occurring at a microscale, such as in microfluidic drug delivery systems (Gerecht et al., 2013), and due to their small dimensions they are easy to handle (Aimee et al., 2013). On the other hand, this platform is not accessible to many labs due to the unique requirements of micro-fabrication processes, liquid handling and sampling. These techniques are often time-consuming, labor-intensive and expensive since in most cases microchannels cannot be reused (Situma et al., 2006). Fabrication of a common PPFC can be more straightforward for some labs with the added advantage that after fabrication it can be used indefinitely. Additionally, several materials can be tested at the same time or consecutively and the amount of produced biofilm is higher enabling further biochemical analysis. This platform is often used to mimic systems with dimensions larger than few centimeters (Teodósio et al., 2013). The selection of a platform for bacterial adhesion studies can be an intricate issue. Both systems have their relative advantages and disadvantages and their selection is usually dictated by the equipment/expertise existing in the lab as well by the similarity to the physiological system that is supposed to be mimicked (*e.g.* size similarity) (Aimee et al., 2013; Bakker et al., 2003; Barros et al., 2013; Gerecht et al., 2013; Teodósio et al., 2013).

In this work, *E. coli* adhesion was visualized in a microchannel and in a PPFC in order to compare two platforms commonly used in adhesion studies. The same average wall shear stress (0.022 ± 0.002 Pa) was used on both systems and similar shear stress values can be found in the urinary (Aprikian et al., 2011) or reproductive systems (Nauman et al., 2007). Three materials, cellulose acetate (CA), poly-L-lactide (PLLA), and polydimethylsiloxane (PDMS), which are currently used to fabricate biomedical devices that are inserted in these body locations and a glass (used as a control) were tested (Abbasi et al., 2001; Andersson, 2006; Grewe et al., 2011; Multanen et al., 2000). The main objective of this work was to evaluate if the size similarity between the *in vitro* formation platform and the *in vivo* scenario is a relevant issue in the selection of the most adequate biofilm formation platform.

2. Materials and methods

2.1. Numerical simulations

Numerical simulations were made in Ansys Fluent CFD package (version 14.5). A model of each system was built in Design Modeller 14.5 and was discretized by Meshing 14.5.

The mesh for the PPFC (1,694,960 hexahedral cells) was refined near the walls, where velocity gradients are higher. A refined cylindrical core was also introduced to improve the accuracy of the calculation of the jet flow that forms at the inlet of the PPFC. Results were obtained by solving the SST k–ω turbulent model (Menter, 1994) with low Reynolds corrections. The velocity–pressure coupled equations were solved by the PISO algorithm (Issa, 1986), the QUICK scheme (Leonard, 1979) was used for the discretization of the momentum equations and the PRESTO! scheme for pressure equation discretization. The no slip boundary condition was considered for all the bounded walls. The SST k–ω turbulent model with low Reynolds corrections was selected because the flow conditions in the PPFC indicate the presence of regions with low Reynolds turbulence. While the Reynolds number in the inlet is 3600 (and therefore turbulence can develop in this region) the Reynolds number in the viewing area is 346. Turbulence decreases along the chamber, and the flow can even become laminar. Simulations using the SST k–ω model were compared to those obtained by solving the Navier–Stokes equations for the laminar regime and the results in the viewing area were similar. Shear stresses of 0.024 and 0.021 Pa were obtained with the SST k–ω model and for the laminar case, respectively.

The mesh for the microfluidic channel was divided into two parts, an inlet region with 124,154 hexahedral cells and the microchannel with a mesh of 94,374 hexahedral cells uniformly distributed. Results were obtained by solving the Navier–Stokes equations for the laminar regime using the PISO algorithm, the QUICK scheme and PRESTO!

For the simulations, the initial velocity field was set to zero, a uniform velocity profile was set at the inlet and the pressure was set to zero at the outlet. The properties of water (density and viscosity) at 37 °C were used for the fluid. 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.

2.2. Bacteria and culture conditions

E. coli JM109(DE3) was used since this strain had already demonstrated a good biofilm formation capacity (Teodósio et al., 2012). A starter culture was obtained by inoculation of 500 μL of a glycerol stock (kept at −80 °C) to a total volume of 0.2 L of inoculation media with 5.5 g L^{-1} glucose, 2.5 g L^{-1} peptone, 1.25 g L^{-1} yeast extract in phosphate buffer (1.88 g L^{-1} KH_2PO_4 and 2.60 g L^{-1} Na_2HPO_4) at pH 7.0, as described by Teodósio et al. (2011). This culture was grown in a 1 L shake-flask, incubated overnight at 37 °C with orbital agitation (120 rpm). A volume of 60 mL from the overnight grown culture was used to harvest cells by centrifugation (for 10 min at 3202*g*). Cells were washed twice with citrate buffer 0.05 M (Simões et al., 2008), pH 5.0 and finally the pellet was resuspended and diluted in the same buffer in order to reach a cell concentration of 7.6×10^7 cells mL^{-1}.