HYDRODYNAMICS ON CATHETER BIOFILMS

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The design of urinary catheters has not changed significantly since it was introduced by Foley in 1930s, and it has a number of drawbacks including a propensity for blockage due to formation of bacterial biofilm-mediated encrustations, and the promotion of aggressive infections of the urinary tract. Efforts at reducing catheter blockage have so far focussed either on improved materials typically impregnated with antimicrobials to reduce biofilm formation or on pH control measures to reduce biofilm-mediated encrustations. In both cases, results have been disappointing.

An extensive survey of literature indicates that the causal link between catheter blockage and biofilm-mediated encrustations have only been demonstrated qualitatively. Therefore, experimental investigation is necessary to obtain quantitative values for building computational models.

Six strains of *Proteus mirabilis* were cultured in artificial urine medium (initial pH = 6.5) at 37°C (Brooks & Keevil, 1997). Biofilm development was assessed on polydimethylsiloxane (PDMS), the typical material that popular urinary catheters are made of, and glass plates under three different shear regimes over 48 to 72 hours. Biofilm development was followed in real time by automated microscopic image acquisition every 15 minutes.

The acquired images were processed using ImageJ and MATLAB in order to remove the background intensities from each image and so measure the pixel intensity related to accumulation within the microfluidic channels. The average intensity of the pixels in the images acquired at different time points were computed and used to plot a graph of biofilm accumulation over time. These data were also used to calculate average accumulation rates over the first 12-14 hours and average intensities over 24-72 hours.

In addition to the time-lapse images themselves, these data were then compared between strains and flow rates in order to ascertain whether:

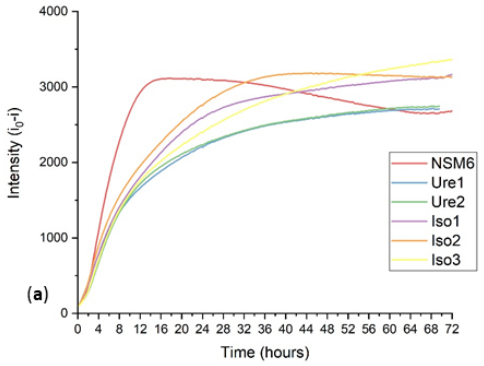
i) there is any significant difference between the six strains of Proteus mirabilis used, particularly with regards to biofilm accumulation
ii) increasing shear stress from a physiologically relevant 15mPa to a higher 50/100mPa has a noticeable effect on biofilm accumulation over 72 hours
iii) the surface properties of PDMS and glass affect the development of P. mirabilis biofilms

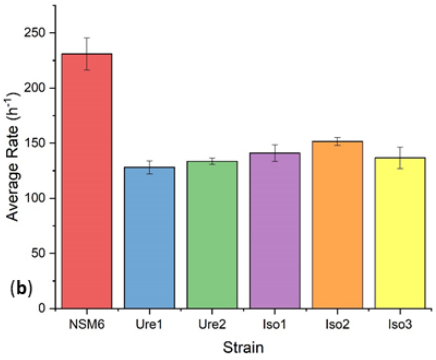
Key findings

The type strain NSM6 displays a similar biofilm accumulation profile to the other strains tested, indicating that it is a good model organism for this work (Figure 1a). Its biofilm accumulation rate is generally higher than all other strains (Figure 1b), but seems to achieve the same absolute biofilm amount as the final intensity reached by all strains are not significantly different (Figure 1c).

The biofilm morphology of *Proteus mirabilis* under flow in artificial urine is markedly different in glass vs PDMS microfluidic reactors (Figure 2).

On glass, urease-positive strains appear to undergo two phases of accumulation; the first seems mostly bacterial with little mineral deposition, the second is marked by high levels of precipitation (Figure 3).





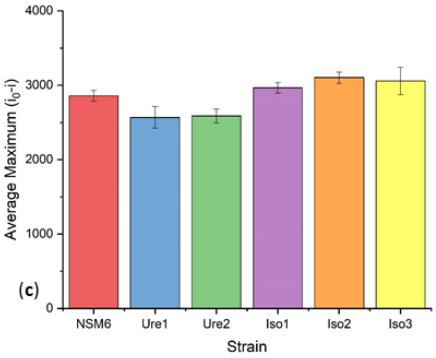


Figure 1: comparisons of six strains of *Proteus mirabilis* growing at 15mPa shear stress in artificial urine. (**a**) Intensity curves for each strain over 72hrs. (**b**) Average rate of accumulation over the first 12hrs of growth. (**c**) Maximum intensity measured as an average of intensity over 24-72hrs for each strain.

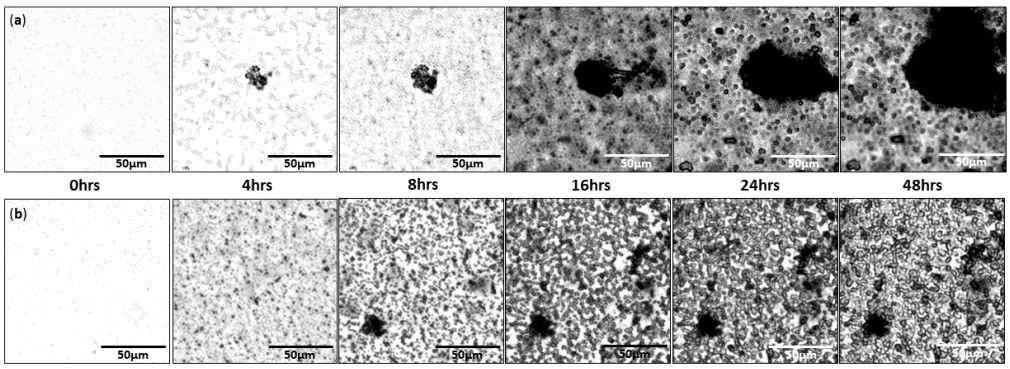


Figure 2: comparisons of *Proteus mirabilis* NSM6 growing at 15mPa shear stress in artificial urine on (**a**) glass or (**b**) PDMS. Flow direction is left to right. Images were taken at 200X magnification using the brightfield mode of the AxioObserver Z1.

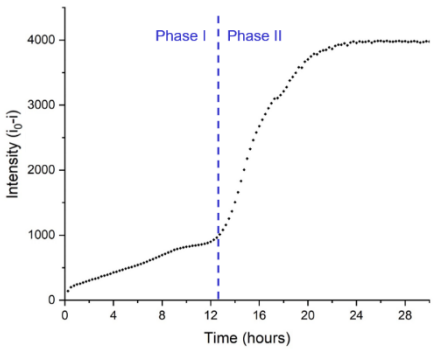


Figure 3: a representative intensity curve for Iso3 growing on glass at 15mPa shear stress in artificial urine. The two phases of accumulation are indicated.

On PDMS, there is generally no significant difference between urease-positive and urease-negative strain growth over 72 hours (Figure 4). However, NSM6 does have a much higher initial biofilm accumulation rate than all other strains (Figure 4a).

Increasing the shear stress from the physiologically relevant 15mPa to higher stresses of 50 and 100mPa results in higher biofilm accumulation rates and higher intensities for all six strains (Figure 4). At higher shear stress, the increased rate of biofilm accumulation may be due to a higher rate of supply of substrates such as urea, and better removal of metabolic by-products.

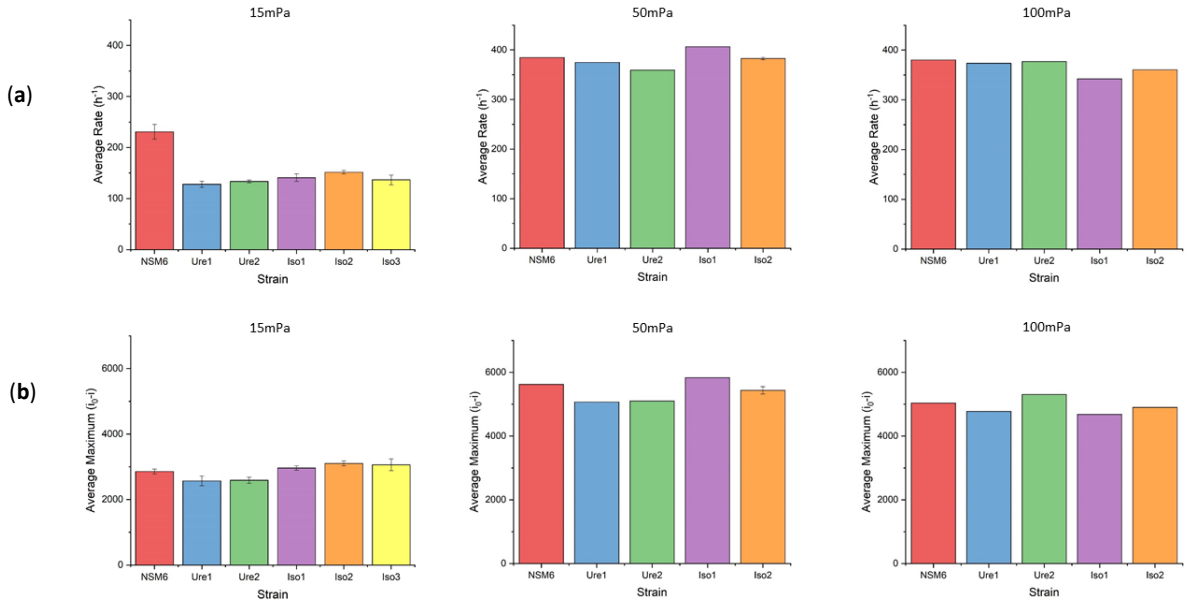


Figure 4: comparisons of six strains of *Proteus mirabilis* growing on PDMS at 15mPa, 50mPa or 100mPa shear stress in artificial urine. (**a**) Average rate of accumulation over hours 0-13. (**b**) Average intensity over hours 24-72. Results are not available for Iso3 growing at 50/100mPa due to issues in the experimental setup.

Link to videos from this work https://www.youtube.com/channel/UCk0xK-LbvTjVa26TX4XZUEw