395

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THE USE OF X-RAY MICRO COMPUTED TOMOGRAPHY (M-CT) TO UNDERSTAND CRYSTALLINE BIOFILM BLOCKAGE IN URINARY CATHETERS

Hypothesis / aims of study

The almost permanent bacterial colonisation of urine in long-term indwelling urinary catheter users leads to high levels of infection. In addition, up to 50% of users will also experience encrustations and blockages, leading to additional trauma and discomfort, and to high healthcare demands (1). Encrustations are often due to the presence of the urease-producing *Proteus mirabilis,* which causes the urine *pH* to rapidly rise, resulting in crystal precipitation. A recent study used episcopic differential interference contrast (EDIC) microscopy to track the development of *P. mirabilis* crystalline biofilms on urinary catheters over 24 days, identifying four distinct stages of formation (2) from initial, rapid colonisation of the catheter surface, to microcolony formation and subsequent crystalline formation and deposition, with the bacteria found through out the complex crystalline environment. The presence of multi-drug resistant strains of *P. mirabilis* has also been reported (3), including extended spectrum beta lactamase and AmpC beta-lactamase producing isolates, which can lead to serious outbreaks.

Our understanding of this complex crystalline biofilm remains limited and attempts to produce anti-microbial catheter materials have not been successful. In this study, we exploit advanced 2D and volume imaging techniques to improve our understanding of biofilm development and use this information to identify potential new candidate materials and designs.

Study design, materials and methods

Using a simple flow model, silicone (medical grade) tubes and urinary catheters have been colonised by *P. mirabilis* under controlled laboratory conditions. An overnight culture of *P. mirabilis* was used to inoculate the samples by adding with no flow and leaving at 37°C for 1 hr. Following this exposure time, an artificial urine medium was allowed to flow through the tube/catheter at a constant rate of 50 ml hr^{-1}. The medium was kept at a constant temperature of 37°C. At set times points, samples were removed and used for microscopy analysis. Using EDIC microscopy, the samples were cut longitudinally and examined under long working distance, high magnification objectives. Additionally, sections of the same sample were kept intact and imaged by means of laboratory (ZEISS Xradia 510 Versa) and synchrotron (TOMCAT beamline, SLS at PSI, Villigen, Switzerland) X-Ray µ-CT.

Results

EDIC microscopy permitted rapid, non-contact examination of the sample lumens, and could identify four stages in crystalline biofilm formation, leading to the development of complex crystalline biofilms (Figure 1). This replicated the findings of work described previously (2).

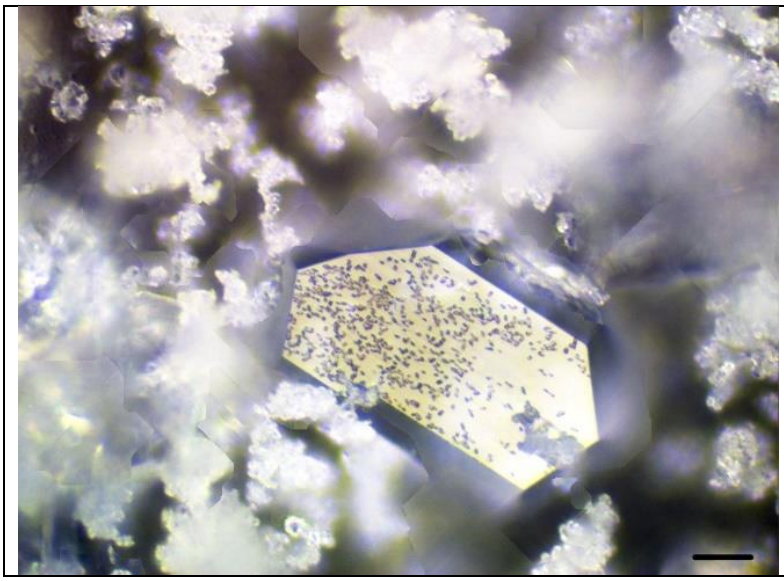

Figure 1. EDIC composite image of 19 optical slices through a 22 day old crystalline biofilm on a silicone catheter, showing a single struvite crystal surrounded by diffuse apatite and covered with highly motile *P. mirabilis*. (Magnification x 500, bar = 20 µm)

µ-CT imaging revealed the 3D structure of the crystalline biofilm *in situ*, showing the development of layers of microcrystalline material adjacent to the catheter's inner wall (Figure 2) and subsequent complete catheter blockage with large amounts of material obstracting the flow of the artificial urine medium (Figure 3). EDIC microscopy indicated that, even in a laboratory model system, a conditioning layer rapidly formed, covering the entire catheter surface. This was also observed with high-resolution µ-CT scanning (Figure 3) where the layer adjacent to the sample was seen as dense material upon which the more heterogeneous, diffuse crystalline deposits were found.

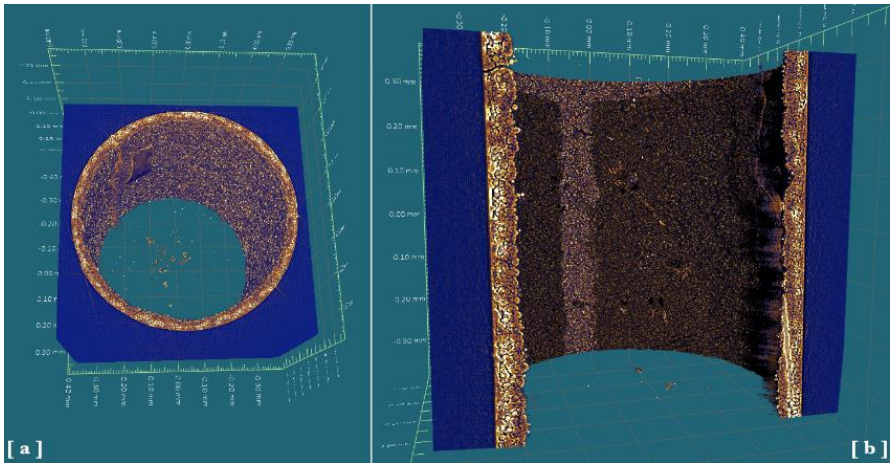

Figure 2. 3D renderings of synchrotron µ-CT scan of a crystalline biofilm developed after two days of artificial urine medium flow inoculated with *P. mirabilis* on a 0.6 mm lumen diameter silicone tube using a simple flow; [a] horizontal cross-section looking down the tube lumen, [b] vertical cross-section showing development on tube surface; bounding box at 800 µm.

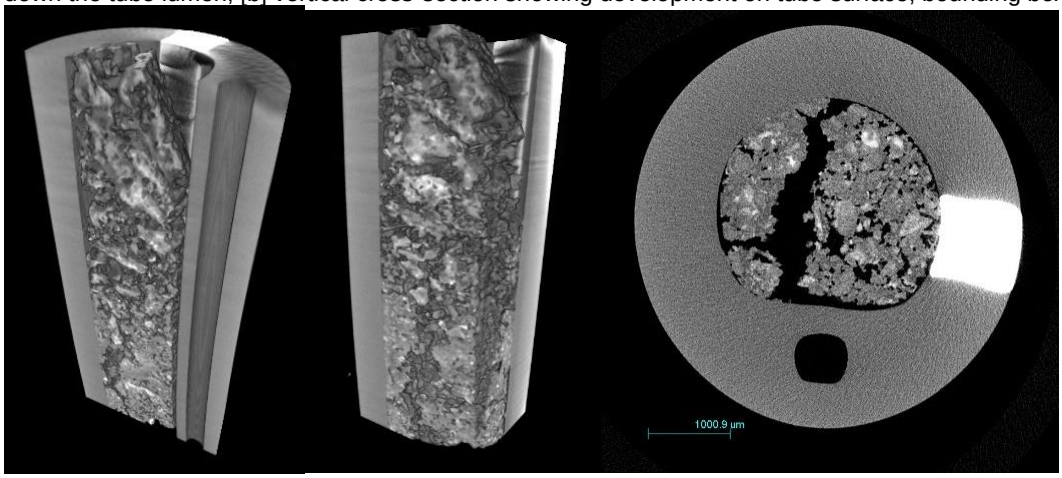

Figure 3. Laboratory µ-CT scan of a blocked silicone urinary catheter. As before, the catheter was blocked using a laboratory flow model containing artificial urine and *P. mirabilis*. The scan shows the heterogeneous nature of the deposits filling the lumen of the catheter; scale bar at 1mm.

Interpretation of results

EDIC microscopy has been found to be a useful analysis tool for understanding f crystalline biofilms on urinary catheters. The use of non-destructive volume imaging techniques, such as high-resolution µ-CT, shows great potential in expanding our understanding of the dynamics of the development of such encrustations in this complex environment, with this technique showing the highly heterogeneous nature of the crystalline biofilms and the interaction with the catheter surface. Importantly, such results can be used to inform *in-silico* experiments and further investigate the effect of crystalline encrustations on the flow dynamics of the system and provide information on the critical time points where crystalline encrustations start causing a clinical problem.

Concluding message

Using these advanced imaging techniques, we are improving our understanding of crystalline biofilm formation and this will aid in the development of anti-microbial materials, resistant to colonisation. Additionally, a detailed understanding of the stages of development could lead to the production of a sensor system able to indicate when encrustation formation begins, thus enabling early intervention and reducing the clinical burden caused by catheter blockages.

References

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Disclosures

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