

UNRAVELLING CATHETER BIOFILMS: A NEW APPROACH

Hypothesis / aims of study

The risk of infections and blockages in patients with indwelling catheters is high, with long-term use of catheterisation resulting in an almost permanent bacterial colonisation of urine (1). The attachment of such bacteria to catheter surfaces and subsequent biofilm formation are not well understood and their role in persistent infections and causing encrustations requires further research. Here we report on a toolbox of methods which can be used to track biofilm development and persistence, in particular, the use of an advanced live cell imaging microscopy technique to examine biofilms directly on catheter surfaces.

Study design, materials and methods

Using a simple 6-well plate method, biofilms of selected urinary pathogens (uropathogenic *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*) were allowed to develop on silicone, hydrogel-latex and silver impregnated hydrogel-latex catheter sections. A previously defined artificial urine medium (2) was used throughout. At set time points, sections were removed and examined using direct or indirect methods. Indirect analyses involved removal and disruption of the biofilm prior to culture and quantitative microscopy, using a cell elongation method combined with fluorescent staining. For direct examination, episcopic differential interference (EDIC)/epifluorescence (EF) microscopy (3) was used. EDIC microscopy requires no sample preparation and automated x-y-z scanning combined with long working distance but high magnification objectives permits direct examination of curved catheter surfaces. Sections were also stained with the LIVE/DEAD BacLight™ fluorescent labelling system and DAPI to show the location of viable and non-viable populations..

Results

EDIC microscopy could be successfully used to examine catheter surfaces directly, revealing information on surface topography and bacterial attachment with no disruption of any biofilm structures (Figure 1a - c). For all material types, the surface was not smooth, with areas of pitting, striations and an undulating structure (Figure 1a). All bacteria tested (*E. coli*, *Ps. aeruginosa* and *P. mirabilis*) rapidly attached (within 15 min) to all three catheter materials in the presence of artificial urine. Within 6 h, the surfaces were covered with a dense layer of bacteria (Figure 1b) with often copious amounts of extrapolymeric substances (EPS) which are an important component of the biofilm architecture. Analysis of resuspended biofilm samples found no significant differences in either bacterial attachment rates or number between any of the catheter materials tested. With the exception of *P. mirabilis*, at longer time periods it was possible to observe the biofilm lifecycle of maturation followed by detachment, leaving the typical heterogenous mosaic pattern of areas of dense biofilm interspersed with areas of exposed of catheter surface, permitting re-colonisation (Figure 1c).

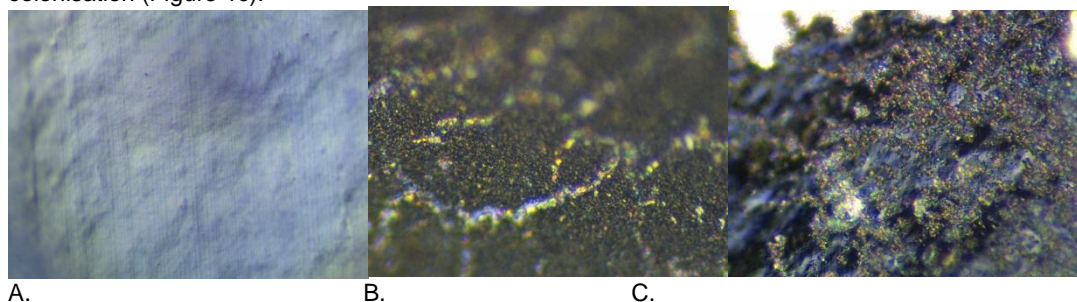


Figure 1. EDIC images showing A. clean, unused silicone catheter surface, and exposed to *Ps. aeruginosa* for B. 6 h showing a thick coverage of bacteria, with water channels running throughout allowing for the transportation of nutrients, and C. 24 h where the mosaic pattern typical of biofilm detachment is seen, showing areas of catheter surface which are exposed for re-colonisation. (Magnification x 1000).

For *P. mirabilis*, there was rapid formation of a complex crystalline biofilm with accompanying strong alkaline conditions in the artificial urine medium. This can be seen in Figure 2 where a composite, extended depth of field EDIC image through the encrusted biofilm is shown, with large amounts of diffuse crystalline material surrounding a single struvite crystal covered in swarming *P. mirabilis*.

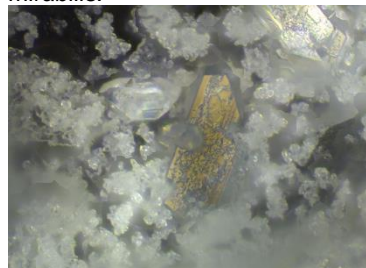


Figure 2. Composite, extended depth of field EDIC image of *P. mirabilis* after 10 days on a silicone catheter showing diffuse crystalline material surrounding a single struvite crystal with swarming *P. mirabilis* on the surface. (Magnification x 1000).

Interpretation of results

Using a mixture of indirect and direct analysis methods, the current study has demonstrated inadequacies of current catheter materials in preventing bacterial attachment and subsequent biofilm formation. Standard methods, such as culture analysis, show the rapid attachment and retention of viability of high numbers of bacteria entering the biofilm state. This indicates that the resulting biofilm is not leading to stressed bacteria but rather that the biofilm is a preferred environment. The use of EDIC microscopy has revealed new information regarding both the surface topography of catheter surfaces and the biofilm architecture. For all materials, the surface topography showed a high degree of roughness, creating numerous attachment sites for bacteria, indicating that the development of new materials could provide an effective anti-biofilm strategy. The pseudo-3D EDIC images have also demonstrated the close relationship between the material surface and the developing biofilm and are increasing our knowledge on the structure of catheter-associated biofilms. EDIC microscopy has been particularly useful in the non-destructive imaging of the complex nature of the crystalline biofilm formed by *P. mirabilis*, providing many advantages over conventional techniques such as scanning electron microscopy.

Concluding message

Biofilms have been implicated in catheter blockages and infections for a considerable amount of time but there remains a lack of understanding around their development and structure. EDIC microscopy provides a non-destructive approach for examining biofilms *in situ*, directly on the catheter, thus giving important information about the interactions between bacterial cell attachment, biofilm maturation and the surface material. Such information will expand our knowledge of catheter-associated biofilms, aid in the production of more appropriate control and prevention regimes, and could ultimately be used in the development of new materials.

References

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Disclosures

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