

A MULTI-TECHNIQUE APPROACH TO UNDERSTANDING THE IMPACT OF BIOFILMS ON URETERIC/URETERAL STENTS

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HYPOTHESIS / AIMS OF STUDY

Ureteric/ureteral stents and urinary catheters are widely used as a measure to maintain urine drainage in patients undergoing investigation and exploration of long-term treatments. Like all devices entering the body, they are susceptible to microbial colonisation and the subsequent formation of biofilms. Biofilms, structured communities of microorganisms encased in a self-produced matrix formed of extra-polymeric substances (EPS), affect both the rate of infection and the formation of blockages caused by crystalline deposits. These polymicrobial structured communities also exhibit greater tolerance to antibiotics and high levels of antimicrobial resistance development. On stents, the presence of biofilms can lead to increased frequency of infections and development of crystalline deposits. This can rapidly block drainage side-holes leading to stent failure and serious implications for a patient's quality of life, which also impacts on healthcare resources.

Within the urinary system, a diverse range of microorganisms can be found and our understanding of the complexity of these communities is increasing but knowledge remains limited on how biofilms form and how best to control them. In this study, we are using a multi-technique approach combining specific culture methods, advanced imaging, and a combination of laboratory modelling and patient samples to forward our understanding of biofilm formation and structure in stents.

STUDY DESIGN, MATERIALS AND METHODS

A combination of laboratory modelling and analysis of patient samples has been used to understand biofilm development and composition on stents. Laboratory work involved the use of a controlled stent environment with a physiologically correct artificial urine medium (AUM) (1) being inoculated with key uropathogenic bacteria, specifically *Proteus mirabilis*, a urease-producing bacterium known to be a main causative agent of crystalline biofilms and subsequent encrustations. The system was then kept at 37°C with AUM being replenished at a constant and controlled flow rate to replicate the flow dynamic environment to which stent are exposed in vivo. To understand biofilm and encrustation development both episcopic differential interference (EDIC) (2) and micro-computed tomography (μ CT) were used.

A second stage involved the collection of stents which were being surgically removed from patients at two clinical sites. All patients had a stent in situ for either kidney stone or cancer diagnoses. On removal, the stent and a urine sample were collected and returned to the microbiology laboratory for immediate analysis. Culture techniques (general and specific agar) were used to identify key culturable species including *P. mirabilis*, *Escherichia coli*, and *Candida* species, amongst others, on both urine samples and removed biofilm samples from three sections along each stent. Additionally, imaging techniques including EDIC, μ CT, and scanning electron microscopy (SEM) were used to examine how and where biofilms formed. For EDIC and SEM, sections of stent were cut longitudinally, and the lumen and eyehole regions imaged. EDIC imaging allows non-destructive, high magnification imaging with no need for sample preparation and provides additional information on the interactions with material surfaces. In contrast, μ CT scanning permits intact visualisation across and along the stent.

To further understand community dynamics and diversity, removed biofilm samples were investigated using 16S sequencing.

RESULTS

Initial studies using the controlled laboratory model system demonstrated how bacterial attachment and subsequent biofilm formation could be tracked using EDIC and μ CT imaging. These imaging techniques provided information on how and where biofilms form on the stents and the development of encrustations. This has added knowledge to previous particle deposition studies and will inform future stent design (3).

Analysis of patient samples has shown the diversity and complexity of microbial colonisation of the upper urinary tract and stent surfaces. By using a combination of techniques, a more complete picture of colonisation has been obtained, providing information on quantitative culturable numbers of bacteria and diversity, along with qualitative visualisation of biofilm development on stent surfaces and around eyeholes. Figures 1 and 2 show the culture data and example EDIC imaging results for two separate participants.

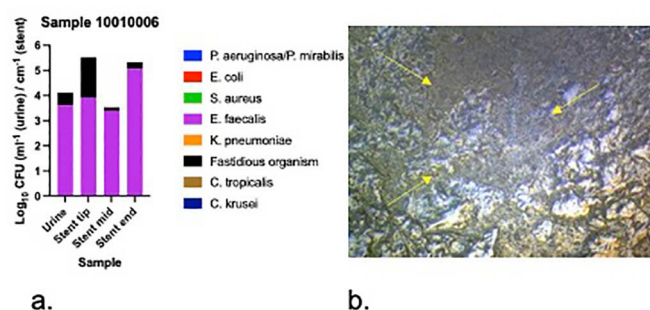
INTERPRETATION OF RESULTS

The combined use of several techniques from culture and sequencing analyses to biofilm imaging which is non-destructive and allows assessment of structural characteristics and relationship to the stent material surface, is expanding our knowledge of biofilm development on stents. The data is providing information on the community dynamics and population found in the urine and stent biofilm, but also indicating that a proportion of these microorganisms may go undetected in routine culture only based tests. The imaging techniques are highlighting the heterogeneous nature of crystalline biofilms and the interactions with surface materials. Comparisons between urine and biofilm are also revealing information on what species are likely to form biofilms as primary colonisers, use biofilms as a protective niche, or be unlikely to be a component of the biofilm community, all important in understanding infection risk and antibiotic choice.

CONCLUDING MESSAGE

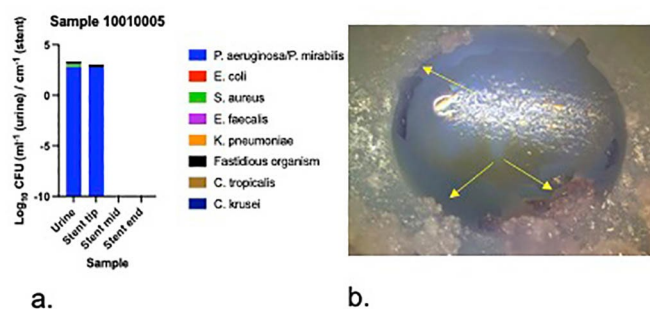
Using the combination of culture, molecular and imaging techniques, we are increasing our knowledge of microbial community dynamics and biofilm development on stents. Understanding how biofilms develop and the structure of the polymicrobial populations forming them could directly impact on future stent design, choice of stent materials, and use of antibiotic treatments.

FIGURE 1



Results from one participant a) colony forming units with *Enterococcus* dominant, b) EDIC image taken at a magnification of x 500, with arrows indicating areas of extensive biofilm development.

FIGURE 2



Results from one participant a) colony forming units with *Proteus/Pseudomonas* dominant, b) EDIC image taken at a magnification of x 100, with arrows showing crystalline biofilm around eyehole.

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