CSIRO PUBLISHING

IN FOCUS
https://doi.org/10.1071/MA23024

Microbiology Australia

Diversity and dynamics of clinical biofilms in ventricular assist device driveline infections and *in vitro* modelling

Yue Qu[A,B], David McGiffin[C] and Anton Y. Peleg[A,B,D,*]

For full list of author affiliations and declarations see end of paper

***Correspondence to:**
Anton Y. Peleg
Infection Program, Monash Biomedicine Discovery Institute, Department of Microbiology, Monash University, Clayton, Vic. 3800, Australia
Email: anton.peleg@monash.edu

Received: 11 April 2023
Accepted: 4 May 2023
Published: 18 May 2023

Cite this:
Qu Y *et al.* (2023)
Microbiology Australia
44(2), 83–87. doi:10.1071/MA23024

ABSTRACT

The important role of microbial biofilms in medical device-related infections is well established. Intervention strategies developed from *in vitro* biofilm studies often fail to prevent or cure device-related infections, possibly due to limited relevance of the simplified *in vitro* biofilm models to the much more complex clinical reality. It is important to use *in vitro* biofilm assays that closely mimic the dynamically changing clinical environment. This review uses ventricular assistant device driveline infections as a model of disease to demonstrate the morphological diversity and dynamics of clinical biofilms that are important for disease pathogenesis. We also provide insights into how to develop *in vitro* assays to address the complexity of device-related infections, focusing on pathogen-device interactions, infectious microenvironment, and selection of representative microorganisms and biomaterials.

Keywords: biofilm formation, biofilm migration, drip-flow reactor, driveline infections, infectious microenvironment, tunnel-based biofilms.

Introduction

Biofilms serve as a sophisticated infectious entity for many difficult-to-treat chronic infections, in particular those related to implantable medical devices. Formation of clinical biofilms is underpinned by host–device–pathogen interactions occurring at the specific anatomic sites of infection.[1] Ventricular-assist device (VAD) driveline infections are a model of medical device-related infection with a known association with microbial biofilms.[2] The driveline is a percutaneous tube connecting the pump and the extracorporeal controller unit of the VAD, conducting energy, controller algorithms and telemetric data. A typical HeartWare VAD (HVAD) driveline consists of an inner segment of smooth tubing made of polyurethane and another segment of outer tubing made of polystyrene velour (Fig. 1*a*, *b*), which is meant to enhance tissue integration of the embedded driveline. Driveline infections associated with VADs have a unique pathogenesis compared to other device-related infections such as catheter-related bloodstream infections or prosthetic joint infections. The exact mechanisms and factors involved in driveline infections are not fully understood, but recent research has started to uncover some important factors and interactions between the host, device and pathogen that contribute to the development and persistence of these infection.[3–5] Unlike many other *in vitro* studies that focus on the genetic determinants of invading microorganisms,[6,7] we studied pathogen–device interactions occurring around the implanted driveline and revealed 'modifiable' host, device and microbial factors that might determine the destiny of infection.[3,4] It is now known that the diverse microenvironments around an implanted driveline, including the skin exit site, the driveline tissue tunnel and the velour–tissue interface, may facilitate the invading pathogens to form biofilms and migrate, with distinct characteristics along the driveline (Fig. 1*a*).[3,4] It is challenging to mimic these biofilm infections *in vitro*. Here, we aim to illustrate the polymorphism of clinical biofilms in a single infection, using VAD drivelines as a model, and to shed light on how *in vitro* assays can be developed to closely mimic the dynamic *in vivo* environment.

Drivelines support the formation of clinical and *in vitro* microbial biofilms

Clinical evidence that supports the importance of microbial biofilms in driveline infections was recently published.[3,8] Using high-resolution scanning electron microscopy and quantitative colony-forming-unit enumeration assays, we examined explanted drivelines from patients with VADs who had driveline infections and control patients without infection.

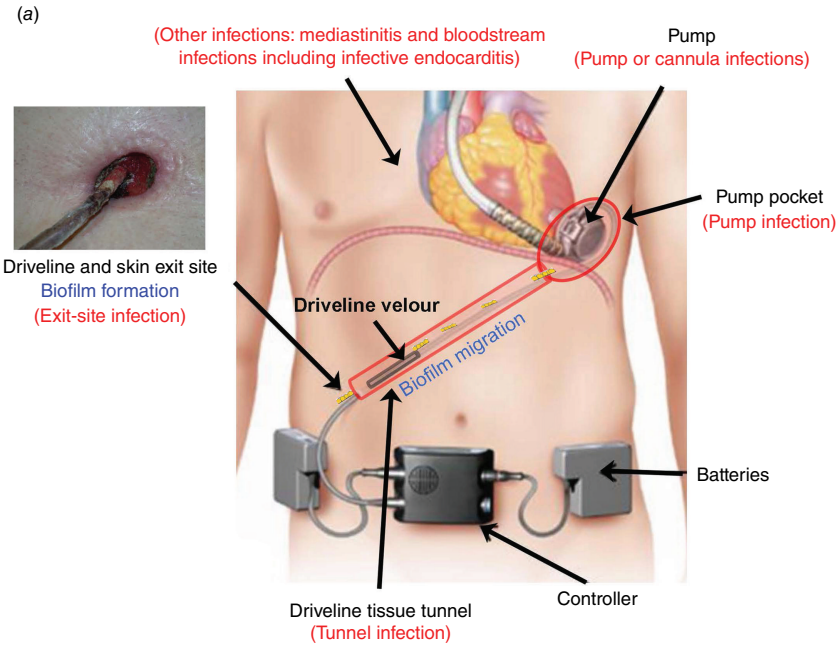

Fig. 1. (*a*) Ventricular assistant device (VAD), anatomic sites, biofilms and associated infections (reproduced with permission[5]). (*b*) An infected driveline explanted from a VAD patient.

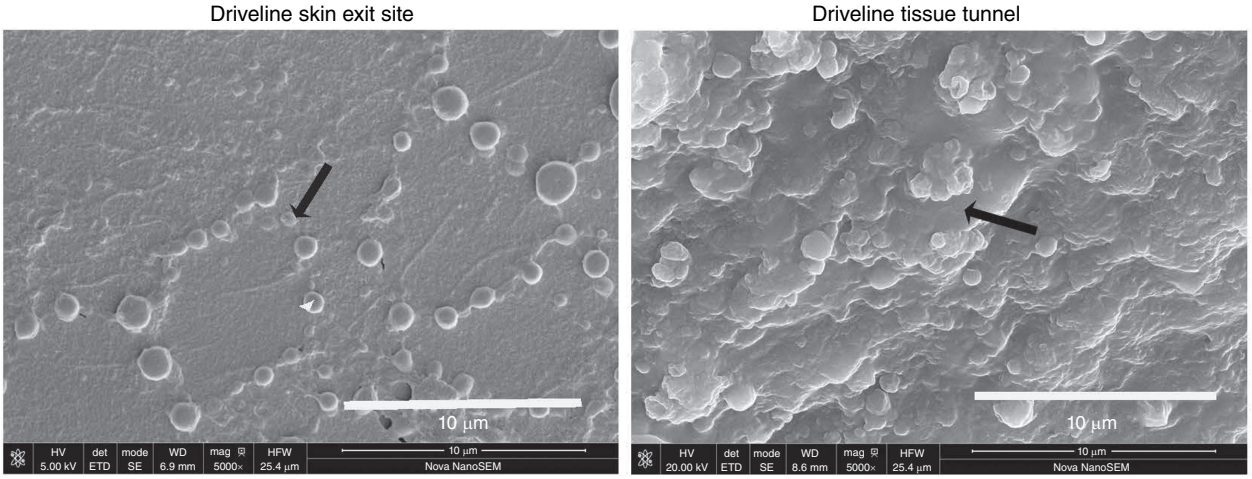

Fig. 2. Staphylococcal biofilm growths on an infected driveline at different anatomic sites, skin exit-site and driveline tissue tunnel (reproduced with permission[3]). Black arrows represent adherent monolayers and microcolony biofilms respectively.

We found monolayer biofilms on the smooth tube section of the driveline at the skin exit site and microcolony biofilms on the velour at the tissue tunnel–driveline interface (Fig. 2). We also analysed *in vitro* biofilm formation of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Candida albicans* on the driveline materials under clinically relevant environmental conditions mimicking those encountered at the driveline skin exit site and in the driveline tissue tunnel and found the predilection of different pathogens to different parts of the driveline.[4] The smooth tube of the driveline supported initial adherence of all four representative microbial species, with *P. aeruginosa* and *C. albicans* attaching to the tube surface at relatively higher densities. The scaffold provided by the three-dimensional structure of the driveline velour and the presence of the nutrient-rich driveline tunnel facilitated sporadically adhered *S. aureus* and *S. epidermidis* to form robust biofilms.[4]

Biofilm formation at the driveline exit site and *in vitro* modelling

Driveline exit-site infection is the most common infection associated with VAD implantation and often presents as a

chronic wound infection with a section of smooth tube or velour of driveline protruding from the skin exit site (Fig. 1*a*). Numerous *in vitro* and animal studies suggested that the formation of microbial biofilms at the skin exit site was the major cause of local driveline infections.[4,6,7] The unique clinical environment of the driveline exit site is characterised by an open space for the device–pathogen interaction, adequate nutrient and oxygen supplies, and low-level shear stress. We speculated that the microbial interaction with the implanted driveline at the skin exit site was made up of two phases.[4] Early adherence occurs soon after implantation, where microbial cells first encounter the surface of the driveline.[9] To mimic this first phase, we developed an *in vitro* early adherence assay whereby microbial cells, resuspended in a drop of liquid media, interact statically with the driveline surface for 90 min in the presence of air (Fig. 3*a*). The second phase is the formation of mature biofilms on the device surface in an environment that mimics a chronic wound. Device–pathogen interactions during this phase occur at a solid–liquid–air interface. As wound drainage may provide a low shear force and continuous nutrient supply, an *in vitro* drip-flow biofilm reactor assay was adopted and further modified to mimic the exit-site wound environment, by reducing the liquid flow to a very low rate of 5 mL min^{-1} $channel^{-1}$ (Fig. 3*b*).[4,10] This *in vitro* assay allowed the growth of monolayer biofilms of *S. aureus* and *S. epidermidis* on the driveline materials, morphologically similar to that observed at the skin exit site of infected, explanted drivelines from patients (Fig. 2).[3,4]

Formation of microcolony biofilms in the driveline tissue tunnel and *in vitro* modelling

Driveline tunnel infections are also frequently encountered.[3,5,11] These infections are difficult to manage and often require surgical interventions such as debridement and re-tunnelling of the infected driveline.[11,12] Examination of infected drivelines from patients with a VAD revealed microcolony biofilms of *S. aureus* and *P. aeruginosa* grown at the velour–tissue interface in the driveline tunnel.[3] The driveline tunnel differs from the exit site in its confined space for biofilm growth, limited oxygen supply and rich nutrients from the surrounding tissues.[4] Device–pathogen interactions in this environment occur at a solid–solid (tissue–device) interface with minimal liquid flow or shear force involved. We have developed a tunnel-based biofilm assay to closely mimic the tunnel environment (Fig. 3*c*). *S. aureus* and *S. epidermidis* that grow on drivelines as monolayer biofilms in the drip-flow biofilm reactor can form macro-colony biofilms in the enclosed agar tunnel, structurally resembling microbiology biofilms found on clinically infected drivelines explanted from the tissue tunnel.[3]

Biofilm migration and dynamics in driveline infections

The spread of driveline exit-site infection is known to cause more severe VAD-associated infections such as tunnel infection, bloodstream infection, pocket infection and pump infections.[4,6,13] Our recent clinical study dissected infected drivelines from patients undergoing VAD explantation and heart transplantation, and revealed a microbial burden on driveline sections extending from the exit site to the deeper tissues, implicating biofilm migration along the driveline surface in the tissue tunnel.[3] Two models of biofilm migration in the driveline tissue tunnel have been proposed, including the expansion of biofilms along the driveline surface and the releasing–seeding model. The latter allows the frontline of biofilms to release planktonic cells that further seed a remote area and regrow into a new biofilm. The expanding parental biofilms and newly grown biofilms eventually merge together and lead infections to the deeper tissues and parts of the driveline.

Host tissue integration of drivelines has been proposed as a defensive strategy against driveline infections.[14] Driveline velour was designed by VAD manufacturers such as Abbott Medical and Medtronic to facilitate host tissue in-growth and to stabilise the driveline in the subcutaneous tunnel. In our analysis of explanted drivelines, we found numerous micro-gaps at the velour–tissue interface (incomplete tissue integration) in the driveline tunnel.[3] It was hypothesised that these micro-gaps not only support the formation of a new biofilm at the driveline velour–tissue interface, but allow the biofilm to intermittently migrate to a remote area. Our newly published *in vitro* study has demonstrated the important role of micro-gaps contributing to biofilm formation and migration.[15] Such micro-gaps may serve as a reservoir for oxygen and tissue fluid and support biofilm growth, and a transit station for biofilm migration. Our tunnel-based biofilm assay in combination with wide-field microscopy can be used to monitor real-time biofilm migration in a tissue tunnel[4] (Fig. 3*c*, *d*). Micro-gaps can be fabricated in the agar tunnel using a surgical scalpel.[15]

Other important factors for *in vitro* modelling of complex biofilm infections

Representative biomaterials and microorganisms should be cautiously selected when modifying or developing *in vitro* biofilm assays to study a complex device-related infection. Tissue-culture treated polystyrene (TCPS) 96-well microplates have been widely used to study microbial biofilms, due to their commercial availability and low cost.[16] However, they are likely far from ideal to study the complexity of a biofilm-related device infection. A standardised TCPS surface does not reflect the unique surface chemistry of biomaterials used to manufacture medical devices such as silicone- or polyurethane-based drivelines. The surface chemistry, in particular the elementary composition and the presence of specific functional groups, have been found to be a determinant for microbial biofilm formation on medical devices.[17] For example, our recently published study found that the HVAD driveline made of Carbothane material was more resistant to biofilm formation by *Staphylococcus* spp. than its Pellethane predecessor.[15] Furthermore, when designing *in vitro* experiments to mimic *in vivo* biofilm infections, the most common and representative microorganisms seen clinically should be selected. For *in vitro* modelling of

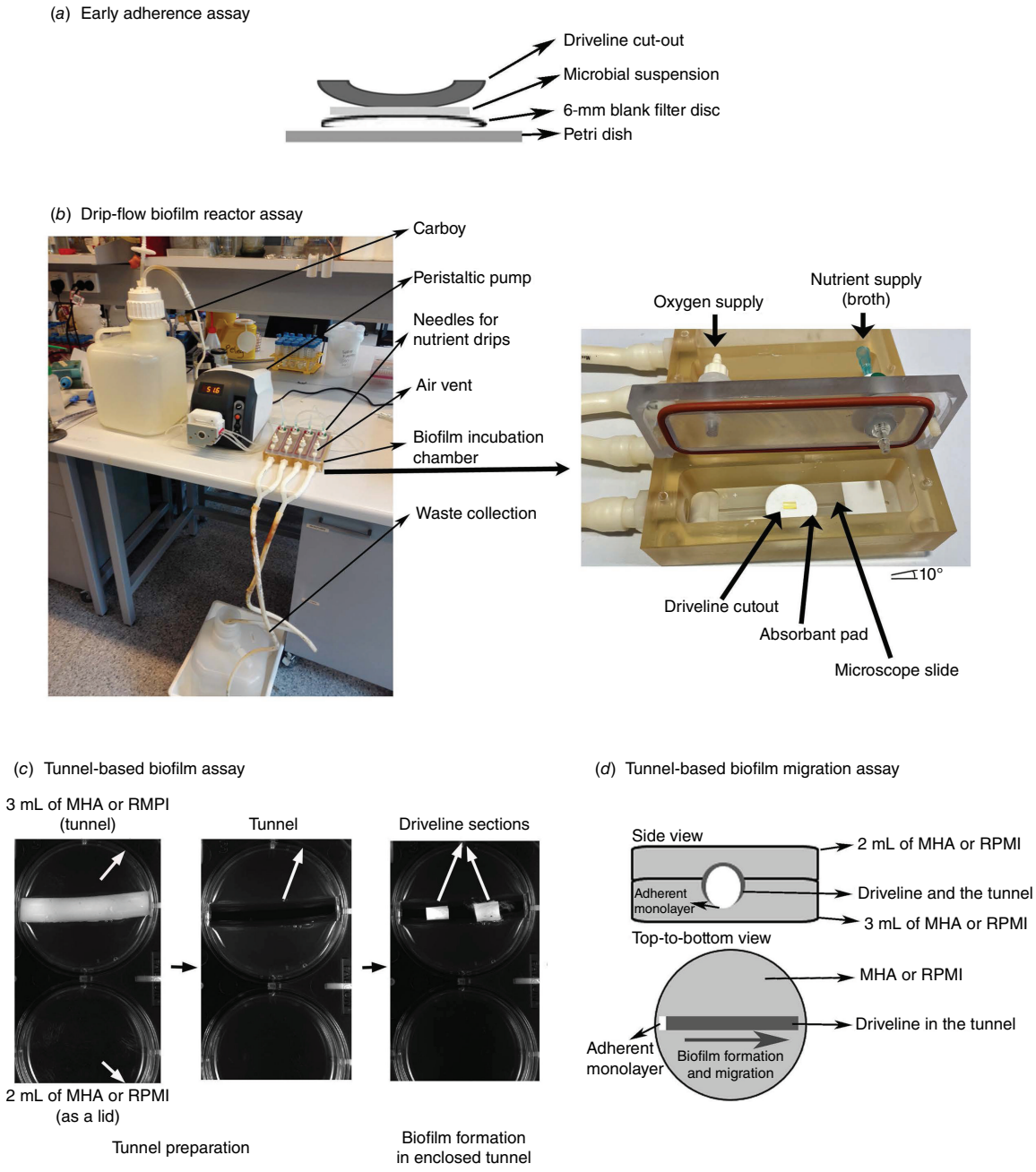

Fig. 3. Different biofilm assays mimicking diverse infectious microenvironment encountered in a driveline infection. (*a*) Early microbial adherence assay, (*b*) drip-flow biofilm reactor assay (reproduced with permission[4]), (*c*) tunnel-based biofilm growth assay and (*d*) tunnel-based biofilm migration assay. MHA, Mueller–Hinton agar; RPMI, Roswell Park Memorial Institute medium.

driveline infections, staphylococcal species should be included as they have been reported to account for over 50% of driveline infections[12,18] and other surgical site infections.[19] Other clinically important, biofilm-producing microorganisms that should be considered are *P. aeruginosa*, *C. albicans*, *Enterococcus faecalis*, *Klebsiella pneumonia* and *E. coli*.[16]

Conclusion

This review uses VAD driveline infections as an exemplar of how to study pathogen–device interactions and biofilm formation and migration on implantable devices using *in vitro* assays that closely mimic dynamic infectious microenvironments in patients. Methodologies recommended by this review can be extended to other biofilm-related medical device infections, such as peritoneal dialysis catheter infections or pacemaker infections.

References

1. Bjarnsholt T *et al.* (2013) The *in vivo* biofilm. *Trends Microbiol* **21**, 466–474. doi:10.1016/j.tim.2013.06.002
2. Salwiczek M *et al.* (2014) Emerging rules for effective antimicrobial coatings. *Trends Biotechnol* **32**, 82–90. doi:10.1016/j.tibtech.2013.09.008
3. Qu Y *et al.* (2020) Characterization of infected, explanted ventricular assist device drivelines: the role of biofilms and microgaps in

the driveline tunnel. *J Heart Lung Transplant* **39**, 1289–1299. doi:10.1016/j.healun.2020.07.015
4. Qu Y *et al.* (2020) Biofilm formation and migration on ventricular assist device drivelines. *J Thorac Cardiovasc Surg* **159**, 491–502.e2. doi:10.1016/j.jtcvs.2019.02.088
5. Qu Y *et al.* (2021) Ventricular assist device-specific infections. *J Clin Med* **10**, 453. doi:10.3390/jcm10030453
6. Toba FA *et al.* (2011) Role of biofilm in *Staphylococcus aureus* and *Staphylococcus epidermidis* ventricular assist device driveline infections. *J Thorac Cardiovasc Surg* **141**, 1259–1264. doi:10.1016/j.jtcvs.2010.07.016
7. Arrecubieta C *et al.* (2009) SdrF, a *Staphylococcus epidermidis* surface protein, contributes to the initiation of ventricular assist device driveline-related infections. *PLoS Pathog* **5**, e1000411. doi:10.1371/journal.ppat.1000411
8. Schoenrath F *et al.* (2021) Fluorescence *in situ* hybridization and polymerase chain reaction to detect infections in patients with left ventricular assist devices. *ASAIO J* **67**, 536–545. doi:10.1097/MAT.0000000000001260
9. Qu Y *et al.* (2010) Antibiotic susceptibility of coagulase-negative staphylococci isolated from very low birth weight babies: comprehensive comparisons of bacteria at different stages of biofilm formation. *Ann Clin Microbiol Antimicrob* **9**, 16. doi:10.1186/1476-0711-9-16
10. Goeres DM *et al.* (2009) A method for growing a biofilm under low shear at the air–liquid interface using the drip flow biofilm reactor. *Nat Protoc* **4**, 783–788. doi:10.1038/nprot.2009.59
11. Nienaber JJC *et al.* (2013) Clinical manifestations and management of left ventricular assist device-associated infections. *Clin Infect Dis* **57**, 1438–1448. doi:10.1093/cid/cit536
12. Trachtenberg BH *et al.* (2015) A review of infections in patients with left ventricular assist devices: prevention, diagnosis and management. *Methodist Debakey Cardiovasc J* **11**, 28–32. doi:10.14797/mdcj-11-1-28
13. Pinninti M *et al.* (2014) Driveline insulation as a conduit for left ventricular assist device pocket infection. *J Thorac Cardiovasc Surg* **148**, e135–e136. doi:10.1016/j.jtcvs.2014.03.035
14. Pereda D, Conte JV (2011) Left ventricular assist device driveline infections. *Cardiol Clin* **29**, 515–527. doi:10.1016/j.ccl.2011.08.004
15. Qu Y *et al.* (2023) Anti-infective characteristics of a new Carbothane ventricular assist device driveline. *Biofilm* **5**, 100124. doi:10.1016/j.bioflm.2023.100124
16. Qu Y *et al.* (2017) Optimizing microplate biofilm assays to screen anti-infective surfaces. *Trends Biotechnol* **35**, 3–5. doi:10.1016/j.tibtech.2016.09.001
17. Qu Y *et al.* (2020) Hyperosmotic infusion and oxidized surfaces are essential for biofilm formation of *Staphylococcus capitis* from the neonatal intensive care unit. *Front Microbiol* **11**, 920. doi:10.3389/fmicb.2020.00920
18. Bomholt T *et al.* (2011) Driveline infections in patients supported with a HeartMate II: incidence, aetiology and outcome. *Scand Cardiovasc J* **45**, 273–278. doi:10.3109/14017431.2011.577236
19. Seidelman JL *et al.* (2023) Surgical site infection prevention: a review. *JAMA* **329**, 244–252. doi:10.1001/jama.2022.24075

Data availability. Data sharing is not applicable as no new data were generated or analysed during this study.

Conflicts of interest. The authors declare that they have no conflicts of interest.

Declaration of funding. This work was supported by the Alfred Research Trust Major Grant to Yue Qu.

Author affiliations
[A]Infection Program, Monash Biomedicine Discovery Institute, Department of Microbiology, Monash University, Clayton, Vic. 3800, Australia.
[B]Department of Infectious Diseases, The Alfred Hospital and Central Clinical School, Monash University, Melbourne, Vic. 3004, Australia.
[C]Department of Cardiothoracic Surgery, The Alfred and Monash University, Melbourne, Vic. 3004, Australia.
[D]Centre to Impact AMR, Monash University, Clayton, Vic. 3800, Australia.

Biographies

Dr Yue Qu is a senior research fellow at the Department of Infectious Diseases, the Alfred Hospital and Monash University. His expertise is in translational research and contributed significantly to the field of medical device-related biofilm infections, from disease pathogenesis to prevention and treatment. His research has a broad coverage of bacterial and fungal pathogens, and different disease models, including bloodstream infections, vaginal candidiasis, VAD driveline infections, and many other medical device-related infections.

Prof. David McGiffin spent most of his career at the University of Alabama at Birmingham, where his major focus was thoracic transplantation, mechanical circulatory support and pulmonary endarterectomy. He returned to Australia in 2013 as Head of the Department of Cardiothoracic Surgery and Transplantation at the Alfred and Professor of Cardiothoracic Surgery at Monash University. He established a pulmonary endarterectomy program for chronic thromboembolic pulmonary hypertension at the Alfred, now the major referral program for Australia and New Zealand.

Prof. Anton Peleg FAAHMS is Director of the Department of Infectious Diseases at The Alfred Hospital and Monash University, Leader in the Centre to Impact Antimicrobial Resistance (AMR), Monash University, and Theme Leader for Infection and Immunity at the Monash Academic Health Research and Translational Centre. He completed his infectious diseases clinical training in Australia and then went to the USA and worked at the Harvard-affiliated hospitals, Beth Israel Deaconess Medical Center and Massachusetts General Hospital. He is a clinician–scientist with a research program that spans fundamental, translational and clinical research.