UNIVERSITY OF CAMBRIDGE
Department of Veterinary Medicine

PhD Project: Evaluating the antibiofilm activity of human urinary extracellular vesicles
Supervisor: Professor Tim Williams

Project Description

Extracellular vesicles (EVs) are small (30-1000nm) membrane-bound particles released by all cells into extracellular space and EVs can be isolated from all body fluids, including urine. Urinary EVs (UEVs) are predominantly derived from cells in the kidney, although some UEVs can originate from cells lining the lower urinary tract. Our laboratory has determined that UEVs are enriched in antimicrobial proteins and have in vitro bactericidal activity.

UTIs affect 400 million people annually worldwide resulting in over 150 million deaths. Up to 80% of UTIs are caused by *Escherichia coli* (*E. coli*) and various other bacterial species such as *Proteus mirabilis* (*P. mirabilis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Enterococcus faecalis* (*E. faecalis*), and *Staphylococcus aureus* (*S. aureus*) are responsible for the remainder of infections. A significant cause of UTIs is the formation of biofilms, which are surface-attached communities of bacteria embedded within a self-produce matrix, protecting bacteria from both the immune system and antibiotic treatment. Biofilms in UTIs often contain multiple species of bacteria and regularly lead to treatment failure and recurrent UTIs (rUTIs). Data from our laboratory shows that UEVs from most healthy individuals prevent the formation of biofilms formed by *E.coli, Pseudomonas* and *Klebsiella* spp., although the degree of inhibition varies between individuals independent of the UEV concentration. In addition, UEV activity against biofilms formed by species other than *E. coli*, and on (more pathophysiologically relevant) multi-species biofilms is yet to be established. Furthermore, the mechanism of this antibiofilm activity remains unclear; human airway cell derived EVs contain the miRNA let-7b-5p which targets and represses genes within *Pseudomonas aeruginosa* that promote the formation of bacterial biofilms, and previous data from our laboratory has demonstrated that let-7b-5p is also abundant within UEVs of healthy volunteers. Hence, the presence of let-7b-5p within UEVs may account for their antibiofilm activity, however further studies are needed to correlate the presence of let-7b-5p and perhaps other miRNAs and/or proteins within UEVs with their antibiofilm activity. We hypothesise that UEVs will be effective at inhibiting multispecies biofilms and that the antibiofilm activity of UEVs derived from human patients with rUTIs will be impaired. We also hypothesise that UEV antibiofilm activity is correlated with abundance of let-7b-5p.

The aims of this project are:

1. To study the antibiofilm activity of human UEVs against multispecies biofilms
2. To study the antibiofilm activity of human UEVs from patients with rUTIs
3. To study the abundance of let-7b-5p within UEVs and correlate the abundance of this miRNA with the degree of antibiofilm activity observed against E.coli
4. To study the miRNAome and proteome of UEVs that do and do not demonstrate antibiofilm activity

This project will involve:

- Isolation of uEVs from urine of healthy individuals using ultracentrifugation.
- Characterisation of uEVs: by nanoparticle tracking analysis, transmission electron microscopy and Western blotting for EV associated proteins (TSG101, CD9, Alix).
- Confirmation of uEVs antibiofilm activity against *E. coli* biofilms in collaboration with Dr Ash Zarkan (Department of Genetics): by use of 96-well plate assays in artificial urine media using endpoint (safranin dye) biofilm assay to measure rates of biofilm formation in wells containing uEVs vs. PBS (control).
- Determine if differences in the abundance of let-7b-5p (based on droplet digital PCR) correlate with differences in degree of antibiofilm activity observed between individuals
- Investigation of uEVs antibiofilm activity against a multi-species biofilm comprised of the six most common aetiological agents mentioned earlier: using an artificial bladder model. The effects of uEVs on the rate of catheter blockage (as a marker of biofilm formation) will be evaluated.
- Investigation of the antibiofilm activity of urinary EVs obtained from patients with recurrent UTIs (rUTIs) against *Escherichia coli*, *Proteus mirabilis* and the multispecies biofilms will also be investigated to ascertain if differences in antibiofilm activity exist between rUTI patients and healthy volunteers, since such differences might predispose rUTI patients to the formation of biofilms within the bladder leading to recurrent UTIs.
- Small RNA sequencing and/or proteomic analysis of UEVs that do and do not exhibit antibiofilm activity (the latter perhaps derived from rUTI patients) to ascertain differentially expressed miRNAs and proteins that might be important for antibiofilm activity.

How to apply: Contact the Supervisor to discuss the project before submitting an official application. More info on applying here: https://www.postgraduate.study.cam.ac.uk/courses/directory/cvvtmpvet