STUDY ON THE SURVIVAL MECHANISMS OF *PSEUDOMONAS AERUGINOSA* ON NANOPILLAR TOPOGRAPHY OF DRAGONFLY (*PANTALA FLAVESCENS*) WING

ABSTRACT

Biomedical implants are indispensable for those with tissue damage caused by trauma, tumor resection or wear and tear in the ageing population. Biomaterials provide temporary or permanent support at the site of tissue damage. Infection in the biomedical implants is a menace and could be life-threatening. The rate of bacterial infection in implants range from 0.5-17.0%. The infected implant needs to be removed along with the tissues at vicinity by revision surgery. The revision surgery inevitably makes the patient morbid and is cost ineffective.

Conventional techniques to prevent biomaterial associated infections employ coating the implant with antibiotics, release of antibiotic at a controlled rate, coating the implant with DNase I and glycoside hydrolase. Antibiotic coatings would prevent bacterial infection for relatively short period of time, as the biological molecules lose their potency over prolonged storage. Other setbacks of the antibiotic coated implants are cold-chain requirement and majorly the threat of bacteria developing drug resistance.

Development of physical structures that targets the shape and size of the bacterium rather than their chemical moieties, would likely serve as better antibacterial surfaces, as the bacteria developing resistance against physical means is unlikely. Recently, nanopillar topography identified on the wings of insects such as dragonfly, cicada and damselfly were reported to be bactericidal. Though the mechanism of bactericidal activity is ambiguous, nanopillars mimicked on the artificial surfaces also

rendered a bactericidal effect. Studies with *Staphylococcus aureus* and *Klebsiella pneumoniae* showed that these bacteria were reported to be killed in lower numbers. Spherical shape of *S. aureus* was claimed to be exposed to a lower number of nanopillars than rod-shaped bacteria and, non-motile nature of *K. pnemoniae* provided advantage over the motile bacteria, whose chance of getting damaged by the nanopillars is lesser. *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* was observed to show species dependent variation in the attachment pattern to the nanopillars (Hasan *et al.* 2013 & Cao *et al.* 2020). This leads to the question whether the different strains of *Pseudomonas aeruginosa* would employ mechanisms to overcome the bactericidal property of nanopillar wings. *P. aeruginosa* strains PAO1 and ATCC 9027 were tested for their viabilities on the nanopillar topography identified on the wings of dragonfly, *Pantala flavescens*. The *P. aeruginosa* strain PAO1 showed 43.47% viability when incubated with the wing of dragonfly for 48 h under static condition, while the strain ATCC 9027 showed only 0.2% viability under same experimental conditions. Nanopillars could be cidal to only those bacteria that are attached to it. Bacterial attachment studies indicated that the number of PAO1 attached to the wing surface was three times lower than that of ATCC 9027.

To validate this observation, clinical isolates of *P. aeruginosa* were tested for their viability and attachment on the wings of *Pantala flavescens*. All the three clinical isolates of *P. aeruginosa* tested showed 55-62 % viability till 48 h in PBS, and showed lower propensity to attach to the wing surface, similar to PAO1. These results suggest that *P. aeruginosa* strain PAO1 and clinical isolates likely evaded attachment to the wings of dragonfly and consequently exhibited higher viability, compared to ATCC 9027.

Bacterial attachment to substrate is governed by several factors such as adhesion force, surface appendages (flagella and pili), genes that aided reversible to irreversible attachment (gcbA and rsmZ), EPS secretion genes (pel and psl), phenazine gene and quorum sensing molecules. These molecules were analyzed during interaction of *P. aeruginosa* strains with coverslip and wing. Adhesion force analyses using scanning probe microscopy indicated that ATCC 9027 showed adhesion to 100% regions on coverslip and 88% regions tested on wing surface, whilst PAO1 showed adhesion to 92% regions on coverslip and 48% regions tested on wing surface.

Transcriptional level analysis suggested that both the strains of *P. aeruginosa* used the flagellar system for mechanosensing and surface attachment through upregulation of c-di-GMP and synthesis of pel polysaccharide. Genes that aid bacterial attachment were expressed by PAO1 in a delayed fashion compared to ATCC 9027. Analysis of quorum sensing molecules indicated that the AHL, 3-oxo-C12-HSL that activated LasR was highest in ATCC 9027 on coverslip (100%) > ATCC 9027 on wing (94%) > PAO1 on coverslip (69.63%) >PAO1 on wing (10.24%). Bacteria produce QS post-attachment to communicate with the neighboring cells. The pattern of QS molecules released by *P. aeruginosa* strains showed a similar trend observed in bacterial attachment as ATCC 9027 on coverslip > ATCC 9027 on wing > PAO1 on coverslip > PAO1 on wing. Since the initial cell count was the same in all the samples, the variation in the QS would be due to the variation in the number of cells attached to the coverslip or wing surface. These results further suggest that *P. aeruginosa* strains showed relatively lower adhesion force to the wing surface.

Cell wall tension experienced by the *P. aeruginosa* on the nanopillars was predicted using a previously reported mathematical model. The modeling results suggested that *P. aeruginosa* cells when attached to the nanopillars of *Pantala flavescens* experienced 0.15 mN/m to 64 mN/m tension, which was higher than the tension required for rupturing the bacteria (30-75 mN/m).

After implantation, biomaterials would inevitably be exposed to body fluids such as blood, tears or saliva. Behaviour of *P. aeruginosa* strains on the nanopillar topography was studied under flow conditions using flow cell. Coverslip and wing were stuck to the flow cell and the strains were analyzed for viability and attachment under three different shear rates of 1.0 s^{-1}, 1.8 s^{-1} and 2.4 s^{-1}. The viability and attachment results suggested that *P. aeruginosa* PAO1 was attached in higher numbers than ATCC 9027 to the wing surface, but were killed in relatively lower numbers than ATCC 9027.

P. aeruginosa under flow conditions was observed to secrete EPS in excess that probably masked the nanopillars and prevented from its bactericidal activity.

This study concludes that strains of *P. aeruginosa* exhibit survival mechanisms to overcome bactericidal effect of nanopillars on the wings of dragonfly.