Progress in resolving bio-nano interactions microscopically to understand the bactericidal activity of dragonfly wing nanopillars

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Bactericidal mechanism of nanototography is a highly debated, yet unresolved phenomenon. Investigation of bacteria-nanotopography interactions therefore has become a significant research interest for the development of advanced bactericidal nano textured surfaces for the control of bacterial adhesion to prevent their survival on biomaterials. The unique nanototography of dragonfly wings with nanopillars surface are of great interest among researchers to develop antibacterial surfaces with mechanical approach. The mechanical bactericidal activity of dragonfly wing is a property of this unique nanopillars which are made of hydrocarbons and usually known as cuticle layer. Due to their size, composition and beam sensitive nature of these nanopillars, it is difficult to characterise the bacterial-nanopillars interactions using traditional electron microscopic methods. However, to understand mechanistic bactericidal activity, characterisation is needed at a further later step where bacteria-nanopillar interaction takes place. This natural bio-nano interface needs to be resolved in order to understand the bactericidal activity and develop efficient nano structures on to biomaterials. Several attempts have been taken to characterise this bio-nano interface on synthetic surfaces although characterisation of such interface on natural surface has shown very little success . As the fabricated surfaces are different in chemistry, architecture and bactericidal activity, it is vital to characterise the interface on a natural surface.

Here, we reveal the bacteria-nanotopography interaction of dragonfly wing and gram-positive *Staphylococcus aureus* bacterium and discuss empirical approach on characterising the natural interface and their limitations. We have used TEM and Helium ion microscopy (HIM) to resolve the interfaces of Escherichia coli and *Staphylococcus aureus on* dragonfly wing nanopillars. Bacterial cells were incubated for 30 minutes on dragonfly wing was used to reveal the bio-nano interaction of bacteria and the nanotopography of dragonfly wing. Then samples were fixed with 2% glutaraldehyde and subjected to membrane staining and ethanol dehydration prior to imaging. In TEM samples were resin embedded, and for HIM samples were used without coating, so that we present uninterrupted natural interactions between *S.aureus* and nanopillars with clarity and detail. Ne milling followed by He imaging shows the interface between the nanopillars of dragonfly wing and Escherichia coli (Figure 1) Staphylococcus aureus (Figure 2). Using HIM we have identified that bacteria anchor to nanopillars only at certain positions and membrane in between is intact. We also see that Escherichia coli extra cellular polymeric substances (EPS) has been secreted on the nanopillars during this interaction. This causes bacteria to adhere strongly and makes a stress response. In order to understand the interaction clearly, bacterium was milled at various positions and microgrraphed at various angles. We have observed that Staphylococcus aureus bacteria contact the nanopillars only at certain points anchoring bacterium to the surface. Even after bacterium is flattened out, we could not observe membrane has collapsed. In between these anchor points membrane remains intact. It can be clearly noted from the images that bacterial membrane is blended with the nanopillars and their borders cannot be distinguished. This remarks integration of nanopillars with lipid bilayer. As nanopillars and membrane are organic and hydrophobic in nature this kind of dissolving interaction would be chemically expected. The results further confirms the findings in our previous paper.[1] However further biochemical assessments are necessary for a comprehensive explanation. These observations are different to some previous computational modelling predictions done without the use of microscopic evidence.[2,3].

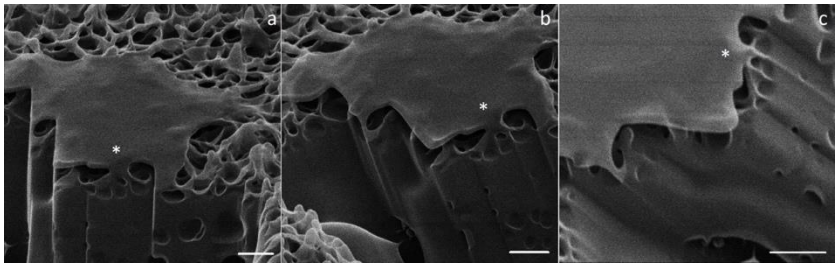
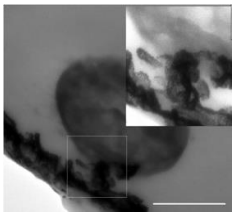


Figure 1: Bacteria-nanopillar interface between Staphylococcus aureus and dragonfly wing nanopillars. Several positions of flattened bacterium showed lumps and bacterium was milled using Neon beam and imaged underHelium beam without conductive coating to reveal bio-nano interactions. Three images show different angles of the same position. Those lumps were identified to be anchor points (*) where bacterial membrane made contacts with nanopillars at multiple positions. In between these anchor points membrane is flat and show no indication of any damage or leakage of internal material were observed. Scale bar 200 nm

Figure 2: Cross-sectioned Escherichia coli on dragonfly wing. Filopodia like extensions *and EPS connects bacterium to the nanopillars. Two unaffected nanopillars are marked for comparison.*



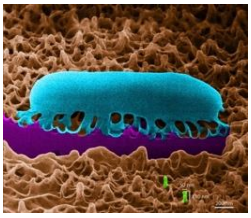


Figure 3: TEM micrograph showing interface between S.aureus and dragonfly wing. Scale bar 500 nm.

TEM image shows the resolved bio-nano interface with higher resolution for a 100 nm thick slice is shown in Figure 3. However, using TEM it is difficult to make a continuous spectrum across the bacterium and therefore we could only gain local information. Bacterium is about 1 um in length and we could not image the interaction along the entire bacterium. HIM is a versatile in this study and can be used to section a bacterium to expose the interface and image. As the milling and imaging can be done continuously, this can be used to construct a continued image over long period of the interface. This can be done without introducing conductive metal coating, therefore images are less vulnerable to have associated artefacts during the imaging process. However HIM could not resolve the bacterial membrane upto the details shown in TEM. Though they resolved the bio-nano interactions successively, these techniques are not capable of characterising biochemical interactions. Therefore further studies using super-resolution confocal microscopic techniques are necessary at this stage for concise understanding of the bactericidal process and the biochemistry involved during the process. The results from this research will contribute to an understanding of the mechanical and biochemical synergies that are necessary for the design of antibacterial nanotopographies.

References

1. Bandara et al., *ACS Appl. Mater. Interfaces*, **2017**, *9* (8), pp 6746 - 6760

- 2. Ivanova et al, *Nature Communications* **volume4**, Article number: 2838 (2013)

3. Ivanova et al., Small. 2012 Aug 20;8(16):2489-94