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The Insect (cicada) Wing Membrane Micro/Nano Structure – Nature's Templates for Control of Optics, Wetting, Adhesion, Contamination, Bacteria and Eukaryotic Cells

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Abstract

In order to survive, many insect species must utilise surfaces which are energy effective, material conserving and typically multifunctional. Insects are some of the oldest animals on the planet and survive in a diverse range of environmental conditions. It stands to reason therefore that they would possess features which are highly tuned and refined through the process of evolution.

The insect cuticle, and in particular the wings, have in recent times demonstrated a range of remarkable multifunctional properties which are of interest to biomimetic, medical, dental, material science, surface science, engineering, marine, biofouling, industrial and a host of multidisciplined researchers. Examination of the functional attributes and functional efficiency of insect cuticle wing membranes may provide valuable lessons for how to incorporate multifunctional properties into man-made materials, especially at surfaces and interfaces.

While there is an extensive diversity in insect wing structuring, the superfamily Cicadoidea (cicadas) highlight and demonstrate how simplistic insect architectures can exhibit varied and multifunctional properties. These include superhydrophobicity, self–cleaning, transparency, antireflection, high strength, ultra low particle adhesion/friction and control of bacterial and eukaryotic cell growth/adherence. This review, primarily focused on some of our recent studies, highlights the functions and functional efficiency of the insect cuticle by focusing on the cicada wing, as an exemplary example, illustrating features that are of particular relevance for biomimetic purposes.

Keywords: Biomimetic, Insect, Wing, Superhydrophobic, Self-cleaning, Contamination, Antibacterial, Cell growth, Antireflection, Replication.

Introduction

The pharmaceutical industry has long recognized the value of natural compounds from a variety of different flora and fauna. In addition to interesting chemistry, such plants and animals may also exhibit micro/nanostructures which are potentially rich blueprints for new technologies and surface architectures. The emerging industries however incorporating such aspects have so far only scraped the surface in relation to these free and abundant resources that have been refined by the imperatives of species survival.

Organisms produce a diverse range of materials considering they are constrained by synthesis conditions near ambient temperatures, in addition to significant energy constraints for manufacture and access to a limited range of starting materials. Through this process Nature has still managed to produce durable, 'cheap' and sometimes self-repairing materials that are easy to maintain and re-cycle. One of the more intriguing aspects of these materials is the incredible diversity in which structural shaping at the nano and microscale has taken place. Quite often this diverse structuring (often with similar chemistries) is utilised by different species for similar functions (e.g., anti-wetting and self-cleaning). Thus, nature can provide us with a range of alternatives to achieve a given functionality; each contribution with its own limitations and advantages.

One of the most striking nano-composite materials is the insect cuticle [1-5]. Recently micro- and nano-structures found on insect cuticle have been shown to exhibit a range of properties such as antireflection, specialised reflectance, superhydrophobicity, increased wetting, directed wetting, ultra-low adhesion and dew facilitated self cleaning [6-13]. The cuticle on insect wings in particular demonstrates a remarkably diverse range of micro and nano architectures [e.g., 8] some of which have been replicated [e.g., 14, 15]. Interestingly a number of these surfaces posses a form of structuring that achieves multiple functions for the organism [12]. These functions and properties often enhance the ability of the organism to survive in its hostile environment and are typically associated with maintaining and optimising the functional efficiency of the insect wing cuticle. Thus examination of the functional behaviour and functional efficiency of insect cuticle wing membranes may also provide valuable lessons of how to incorporate multifunctional properties into man-made materials; particularly at surfaces and interfaces.

As insects represent over 75% of all described species of animals, with many species still to be discovered, detailed descriptions of the tens of thousands of winged insect species will continue for many years in the foreseeable future. However, certain groups of insects such as those belonging to Hemiptera (e.g., cicadas, bugs, aphids and scale insects) have already shown promising aspects of wings which through reverse engineering have potential for solving a number of technological challenges [8,12-16]. In this review we describe the multitude of properties/functions of the thin wing membranes of cicada species with the view of replication for specific and multifunctional applications. In particular we describe the superhydrophobic, antireflective, self-cleaning properties/mechanisms, anti-bacterial action and also the potential for control of animal cell adherence/attachment. The aspects covered in this article may be of interest to biomimetic researchers, medical, dental and biotechnology researchers, engineers, biologists, surface scientists, entomologists and a range of other researchers including multidisciplinary scientists.

Cicada Species Diversity

The cicada belongs to a group of insects which have a characteristic feature of mouth parts which are highly efficient in extracting the liquid contents of plants (and some animals) [17]. The superfamily CICADOIDEA has more than 2000 described species worldwide and they are abundant in the tropics and subtropics. They exhibit a diverse range in size, shape and colouring (see figure 1).

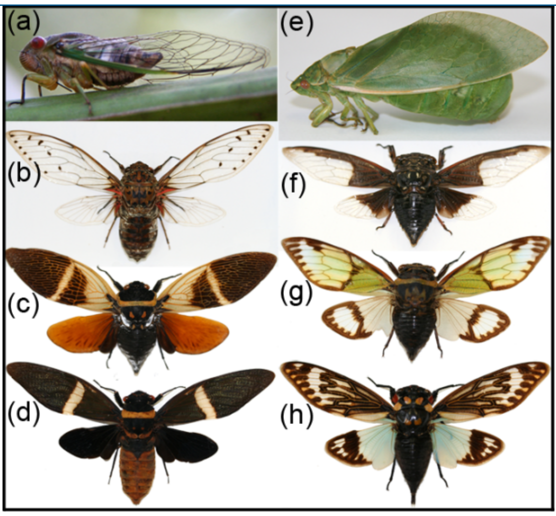

Figure 1: Various cicada species demonstrating diversity within this group of insects. (a) *Psaltoda claripennis*, (b) *Megapomponia intermedia*, (c) *Angamiana floridula*, (d) Black-winged cicada – *Tosena melanoptera*, (e) Bladder cicada - *Cystosoma saundersii* (f) *Cryptotympana aquila* (Walker), (g) Amazing cicada - *Salvazana mirabilis*, (h) Butterfly cicada - *Talainga binghami* (Photographs courtesy of Jolanta Watson).

Most people will hear the cicada (sound produced by the male) before making sight of the insect as they are quite vocal, producing sounds which may aid in pairing, promote aggregations and possibly repel some predators. The life cycle of the cicada comprises a cycle where eggs are inserted into foliage (branches and stems) and the hatched nymph (pronymph) after moving beyond the egg chamber moults to a free-limbed form. These drop to the ground and burrow into the soil and generally remain there for several years (some species remain for only a year and others for considerably longer time periods). Mature nymphs leave the soil under favourable conditions and moult (commonly on a nearby tree) sometimes during night-time hours. When cicada nymphs emerge from the ground and moult they live for varying periods of time in a form with fore and hind wings (up to several months). This period of time, although short, is a critical phase for the adult cicada. During this time the insect will use flight for finding suitable locations for food, location of mates and evading predators. Thus the functioning and maintenance of the wing, and particularly the thin wing membrane, is a critical aspect for survival of the insect.

Characterisation of cicada wing and cuticle micro/nanostructuring

Cicada species typically have a wing which is predominantly transparent as shown in figure 1 (a) & (b) however many species have translucent, coloured and opaque regions on the wings (figure 1 (c) – (h)). The cicada wings vary considerably in size with adult cicadas having wingspans as small as 4.5 cm (*Pauropsalta annulata*) and as large as 17.5 cm (figure 1 (b) - *Megapomponia intermedia*). The wings of most cicada species are characterized by a series of longitudinal veins, cross veins and the areas enclosed by these regions, known as cells (figure 2). The chemistry of the wing membrane of several species of cicada has been characterised showing protein, chitin and wax components [18, 19]. The strong presence of the hydrocarbon wax constituent in the epicuticle layer contributes to the general hydrophobicity of the membrane in most species [18].

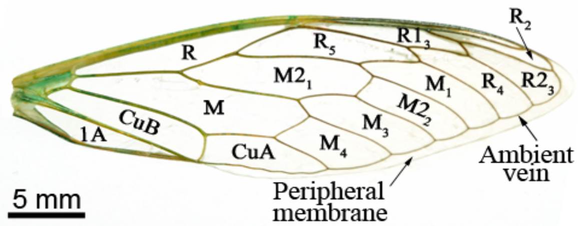

Figure 2: Structural morphology of the forewing of a cicada (example shown - *Psaltoda claripennis*) showing the vein arrangement and enclosed regions ('cells').

To date the wing micro/nanotopography of numerous species of cicada has been investigated [e.g, 8, 9, 12, 18, 20]. The cicada wing membrane has been characterised in relation to morphology by a variety of techniques including optical and Electron Microscopy (EM) as well as Atomic Force Microscopy (AFM) [e.g., 8, 9, 12, 20]. As the structuring of the wing membrane on many species have a spacing and size below the wavelength of visible light, instrumentation other than optical microscopy is required for complete characterisation. The structure size covers a significant range from less than 100 nanometres to several microns in height and spacing [18, 20-23]. Examples of the differing topography on cicada wing membranes are shown in figure 3. Generally, individual structures are spherically capped conical protuberances which often form arrays and cover both the dorsal and ventral areas of the fore and hind wings. The arrays are typically highly ordered although some species exhibit a more random assembly of protuberances (figure 3 (d) & (h)). The shape of the micro/nano protuberances characteristically consist of a gradual tapering from the base to the apex of the structure with a height and spacing of several hundred nm. Some species exhibit a significant broadening at the base forming a bulbous type construction (figure 3 (c)) while others show this feature at the top of the structures. In other cases the broadening may appear towards the middle of the structure as shown in figure 3 (g). Individual structures can be of micron sized dimensions (figure 3 (d) – (g)) and it is also evident that topographies are typically non-hierarchical in structure. The bladder cicada (*Cystosoma* sp) (figure 1 (e) & 3 (h)) demonstrates a disordered topography of relatively large sized curved projections (bumps) which are low in height and spaced many hundred of nanometres apart (centre-centre distance). Wings can also have hairs projecting from the membrane (figure 3 (i)) many hundreds of microns in length however this is not a common feature of this group of insects.

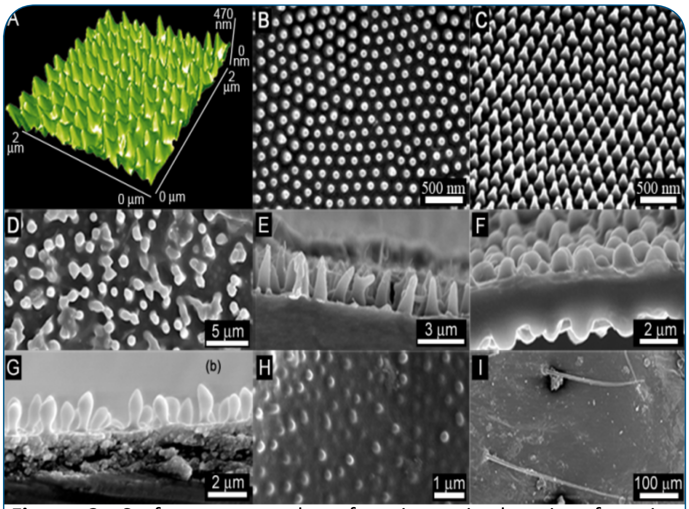

Figure 3: Surface topography of various cicada wing forewing membranes. (a) Atomic Force Microscope (AFM) image of Psaltoda sp. (b) Scanning Electron Microscope (SEM) images of *Meimuna* sp. (c) T*erpnosia* sp. titled 30°. (d) & (e) Brown coloured regions on the wing of *Tosena* sp. (top view and cross section). (f) Brown coloured region of the *Gaeana* sp. forewing (g). Cross-sectional view of Gudanga sp. (black cicada) topography of the dark region of the forewing. (h) Broad bump structuring of the bladder cicada *Cystosoma* sp. (i) Low magnification view of *Tettigarcta* sp. wing showing hairs. (parts (d) (e) and (f) have been reproduced with permission from [15 – copyright held by InderScience])

Transparency/Antireflection/Colour

The risk of predation as well as other behavioural influences have led to the evolution of various forms of colouration on insect cuticle. One of the more obvious functions that certain types of colouration can provide is camouflage, making the insect more difficult to detect in a canvas of undergrowth or in the tree canopy. The black cicada and similar species for example (figure 3 (g)) has a structuring where the dark pigmentation of the forewing ensures that the cicadas are superbly cryptic within the woodlands in which the cicada often inhabit [24], e.g., Mulga (*Acacia aneura*) and Creekline Mineritchie (A. *cyperophylla*). In contrast, the bladder cicada (*Cystosoma schemeltzi*) shown in figure 4 (a) blends in well with green coloured foliage.

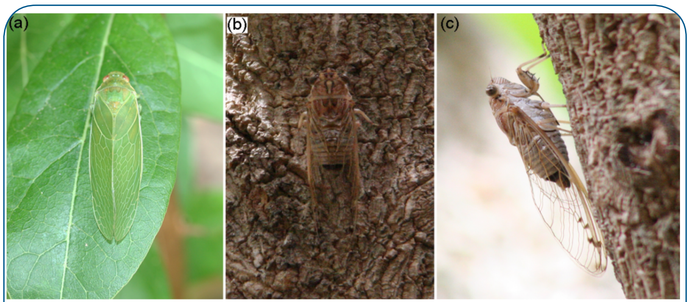

Figure 4: Cicadas photographed on vegetation illustrating optical properties enhancing camouflage for the insect. (a) Bladder cicada (*Cystosoma schemeltzi*) resting on a leaf. (b) The cicada *Tamasa tristigma* illustrating the transparency and the antireflective properties of the wing. The wing features are barely visible enhancing camouflage from the dark coloured body against the tree background. (c) Shows the cicada shown in (b) from a side view. At a distance the insect appears similar to a natural knot along the tree branch.

Pigmentation is not the only mechanism of cicada to be visually inconspicuous. Early studies by Bernhard and Miller in the mid 60's demonstrated that certain regions on the insect body can possess structuring which has an antireflective function [25]. For example, they showed that some moths have ordered hexagonal close-packed nm-size protuberances on their corneal surfaces (the cuticular lens or ommatidial surface). Numerous studies have shown various properties of these ommatidial structures including reflective and mechanical properties [26-28]. It has been suggested that these 'corneal nipple arrays' act as a survival mechanism whereby insects could evade predators through improved camouflage. Presumably the structuring may also enhance the efficiency of the eye. This form of structuring has also been reported on the wings of insects such as the hawkmoth where the reflectance of the wing was measured [29, 30].

A number of studies have also shown a similar sized structuring (e.g., figure 3 (a), (b), (c)) on the wings of numerous cicada species [e.g., 31] and the functional efficiency of the cicada antireflective structuring has also been measured [12]. The tapered protrusions on the wing constitute a change in optical impedance matching at the air-to-cuticle interface, enhancing photon collection and reducing reflectance [12, 26]. The effect occurs over a broad range of angles of incidence and a wide range of frequencies and can be described by the effective medium theory [32]. In order to understand the impedance matching one can view the wing arrays as a multilayered coating (gradient index coating) with each coating having a successively greater index of refraction. Thus the periodic features correspond to a pseudo gradient index coating consisting of structures with a small periodicity, height and repeat distance. Unlike some open-wing insects that perch on the tips of flora for quick take-off and rapid escape, cicada predator evasion is likely to rely heavily on crypsis from within a canopy. An antireflective coating which is transparent (minimising light reflection and also allowing the background to be seen) would thus aid the insect in being unnoticed by predators. This feature is illustrated in figure 4 (b) & (c) where the wing reflects very little light and enhances the natural camouflage of the insect body.

Yoshida et al have carried out a simple experiment by crushing wing nano-structuring on the hawkmoth which is very similar to many cicada species [30]. They demonstrated an increased reflectivity in the wavelength range of 200 to 800 nm after compressing the structuring confirming the likelyhood that it functions as an antireflective layer (figure 5 (a)-(c)). Other studies have also investigated the functional efficiency of the cicada nano-array as an anti-reflective coating by physical manipulation to alter the architecture. For example, AFM has been utilised to remove sections of the wing membrane and structuring [12]. Figure 5 (d) shows an AFM image of the outcome resulting from removal of a small section of wing cuticle to a depth of 300 nm. The optical image in figure 5 (e) clearly shows that the removal of the nano-structures produced a region on the membrane exhibiting higher reflectivity than the intact surrounding regions. Figure 5 (f) – (h) shows the effectiveness of the nanostructure in reducing reflections demonstrating reflectance of the membrane following removal to depths of 150, 60 and 200 nm (squares i, ii and iii, respectively) resulting in structure heights of 75, 165 and 25 nm. The structures with higher heights remaining after manipulation show greater effectiveness in reducing reflections. Taller structures provide a more gradual change in the refractive index (from air being unity to membrane with n ≈ 1.5) having the effect of reducing fresnel reflections.

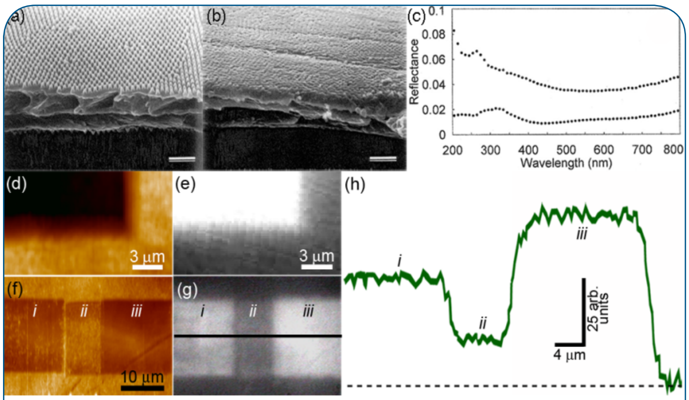

Figure 5: Reflective measurements after physical manipulation of nano-array wing structuring. SEM images of the antireflective structuring before (a) and after crushing (b). (c) Reflectance spectra of intact protuberances (lower trace) and the smoother wing after crushing the structuring (d) AFM image of a region on a cicada membrane following AFM-based nano-machining (e) Reflectance image of the manipulated section and surrounding intact region. (f) AFM image of regions (squares i, ii and iii, representing depths of removal of 150, 60 and 200 nm, respectively). (g) Optical images in the reflectance mode of the manipulated regions and surrounding intact surface, and (h) Reflectance intensity profile of the manipulated regions. (Parts (a) – (c) have been reproduced with permission from [30]. Parts (d)-(h) have been reprinted from [12] with permission from Elsevier.)

Interestingly cicada species like the black cicada which exhibits micro structuring on the coloured regions of the wing (figure 3 (g)) also have transparent wing regions, however nanometre structures similar to those in figure 3 (a) (antireflective structuring) are found on these areas. This provides strong evidence for specific dimensional structure size for specific functionality on selected regions of the wings. Regions of the wing where the antireflection property is required have the necessary structure dimensions less than the wavelength of light [8] while other coloured regions are not restricted by this wavelength condition.

Contaminant Adhesion/Friction

Insects will typically encounter a variety of air-borne contaminants which include plant matter and soil fragments [8]. Insects with relatively long or large wings such as some cicada species may be especially susceptible to fouling due to the high wing surface area and reduced ability of the insect to clean their extremities. As the cicada insect micro/nano structuring has multiple roles, wing contamination has the potential to impair a number of functional efficiencies such as the aerodynamic and anti-reflection effectiveness [12]. Small changes in wetting properties from contamination of the wing may also affect the ability of the insect to shed water and thus affect mobility and self-cleaning efficiency. As well, the growth of most microorganisms is provided by permanent or temporary water availability which may lead to the attachment of pathogens such as fungi and bacteria and the formation of biofilms. Changing the wetting properties of the cicada wing (by mild or heavy contamination) may reduce the ability of the insect cuticle to limit water coverage and thereby promote further contamination by secondary bodies.

The atmospheric environment surrounding insects contains a multitude of biological and anthropogenic particulate matter which can potentially contaminate the wing cuticle; for example bacteria, fungi, silica dust and plant material. Pollen grains are one of the most abundant components amongst the floating particles in the air (aeroplankton) surrounding most terrestrial organisms including human beings [33]. Other potential airborne contaminants can originate from soils. Naturally occurring silica particles composed principally of silicon dioxide (SiO_2) such as quartz can comprise as much as 90–95% of the sand and silt fraction of soil [34]. Exposure to, and inhalation of, a combination of various air-bourne particulates have been found to contribute to various diseases including lung cancer [35] and thus enhanced mechanisms for control such as the shedding of such particles is of great interest.

The adhesional properties of such contaminating particles of various dimensional scales (figure 6) have been investigated for a small number of cicada species with hydrophilic (silica), hydrophobic (C_{18}) particles and pollen grains [8, 9, 12, 15]. Superhydrophobic cicada wings exhibiting either micro or nanoarray structuring with sufficient roughness have demonstrated topographies for minimising these solid–solid contacts [8, 9, 12, 15]. Adhesional forces of contaminants when compared to other surfaces (hydrophilic insect cuticle, silicon hydrophilic wafer) were found to be extremely low (less than 20 nN) and comparable to other superhydrophobic insect cuticle (e.g., dragonfly) as shown in figure 6. It is evident that the topography of some cicada wing membranes results in minimal actual contact between the touching surfaces. The hydrophobic pattered surface decreases the contact area, number of menisci, van de Waals attraction and thus the total adhesive force. The bladder cicada cuticle (figure 3 (h)) showed much higher adhesion forces with contaminants (figure 6). As this cicada has a hydrophilic cuticle (figure 6) and has a low surface roughness, meniscus bridging is most likely a major contributor to the high adhesion.

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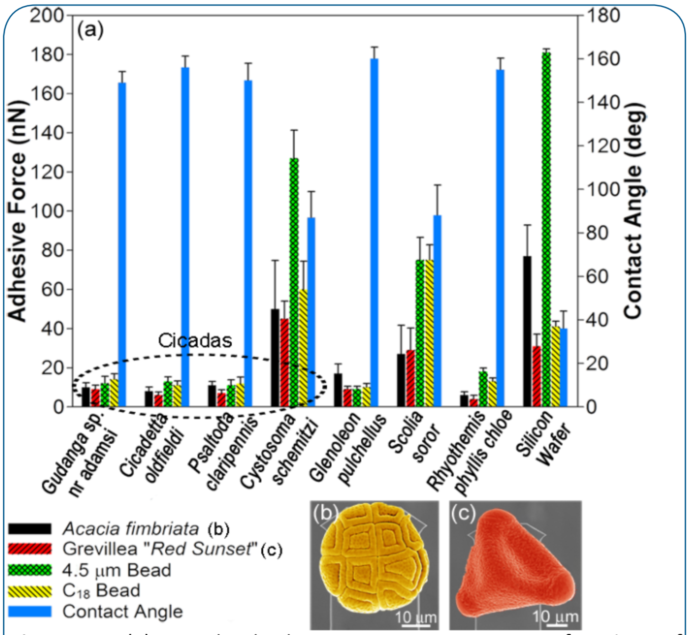

Figure 6: (a) Graph displaying insect type as a function of contact angle (right axis) and adhesion (left axis). The adhesion measurements were obtained using four different particles (two artificial- silica beads, and C_{18} particles and two natural pollens). The first three cicada species represent superhydrophobic surfaces and demonstrate low adhesion with particles. The other insects are a dragonfly and lacewing (also superhydrophobic) and 2 hydrophilic insect wing surfaces (a bladder cicada and a flower wasp *Scolia soror*). A flat unpatterned hydrophilic silicon surface is also shown to demonstrate the adhesional differences of the hydrophobic structured and structured/non-structured hydrophilic surfaces. (b) & (c) Illustrative examples of contaminating pollen particulates and their relative size in comparison to AFM levers ((b) Grevillea Red Sunset (*Grevillea olivacea* × *preissii*) and (c) Wattle *Acacia fimbriata*).

Frictional force measurements of particles on insect cuticle has shown that a hydrophobic cuticle exhibits lower friction. For example friction was a factor of 10 times less on a superhydrophobic cicada (*Cicadetta* sp.) than a hydrophilic species (bladder cicada) [15]. The low adhesion and friction of the hydrophobic cicada wings demonstrate the very small forces required to remove particles which are less strongly attached to the membrane. These factors will also facilitate a variety of removal mechanisms to contend with particle contact, such as wind and self-cleaning via droplet interactions.

Wetting Properties

Most insects must contend with interactions with water at various stages throughout their life cycle. In some cases they must also be able to maintain mobility during such contacts, for example in or on water bodies such as ponds and flight through heavy rain or fog. Interestingly the cicada wing membrane exhibits a wide range of wetting properties as indicated by static, advancing and receding contact angles and droplet adhesion measurements [8, 18, 20, 23]. Contact angles as low as 50 degrees, as well as superhydrophobic interactions with contact angles over 150° have been reported. The highly hydrophobic or superhydrophobic wetting states on cicada can be described by the Wenzel and Cassie-Baxter approximations [36, 37]. The theory by Wenzel makes the assumption that, when a liquid drop is placed on a surface consisting of protrusions, the liquid will fill the open spaces, as shown in figure 7 (a). This model predicts that roughness of the surface reinforces both hydrophobicity and hydrophilicity. Cassie and Baxter, on the other hand, consider the microstructures to be a heterogeneous surface composed of solid and air (figure 7 (a)).

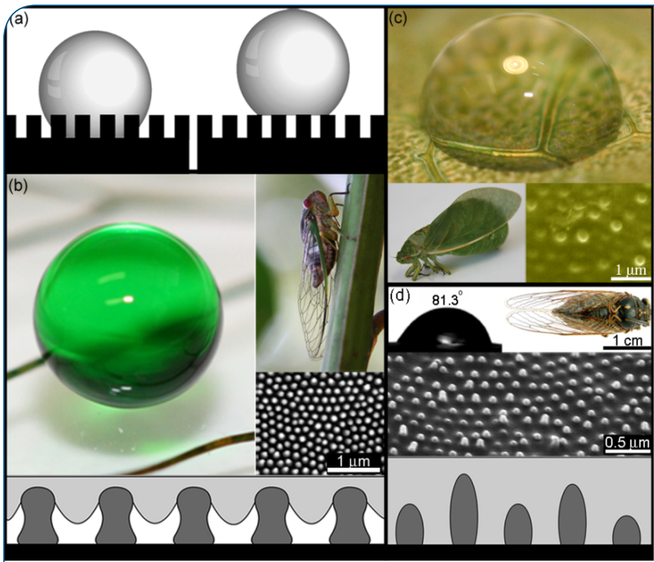

Figure 7: (a) Two wetting states of a liquid on the rough solid surface- Wenzel model and the Cassie-Baxter model where in the later the droplet rests on the top regions of the structuring. (b) – (d) Examples of wetting behaviour on the wing membrane of three different cicada species (b) *Psaltoda claripennis*, (c) Bladder cicada *Cystosoma schemeltzi*, and (d) *Leptopsalta bifuscata*. The highly ordered structuring shown in (b) exhibits a superhydrophobic interaction while the lower and more random forms (with 'defects') of structuring demonstrate hydrophilic interactions (c) & (d). (part (b) (bottom diagram) and part (d) reproduced by permission of IOP Publishing [31]).

The crucial assumption is that the space between the asperities will remain filled with air and the drop will sit on top of the surface as shown in figure 7 (a). Importantly, droplets sitting on these structures are not held up by the air layer but by contact with the structures (analogous to a body resting on a bed of nails) [38]. For an array of hemispherical top protrusions (like those shown in figure 3 (a) & (b)) the corresponding equations for the contact angle are

$$\cos\theta_{wenzel} = [\,1+4\phi_s({}^{h}/_{d}-0.25)]\cos\theta_y \quad (1)$$

$$\cos\theta_{cassie} = -1+\phi_B(\cos\theta_y+1)^2 \quad (2)$$

where ϕ_B is the ratio of the basal area of the protrusion over the total area, ϕ_s is the solid fraction of protrusions with $\phi_s = \pi \mathbf{d}^2/4l^2$, d is the diameter of the base of the protrusions, h is the structure height, and l is the center-to-center pitch (nearest-neighbor spacing for an ordered array). θ_y is the ideal contact angle of water on a smooth surface of identical chemistry. Equations (1) & (2) have been shown to be good approximations for well ordered structuring on cicada.

Figures 6 & 7 demonstrate extremes of wetting showing water droplet interactions on different cicada (2 of which are shown in figure 1 (a) & (e)). It is clear from figure 7 that ordered nanostructures on the cicada wing membrane such as those found on *Psaltoda claripennis* can result in superhydrophobic interactions. However it should be noted that cicada membranes with only microstructure and no nanostructure (e.g., micron sized structure height, spacing and diameter) such as shown in figure

3 (e.g., 3 (g)) may also result in superhydrophobic interactions. This has also been shown on other organisms including some plant surfaces.

The hydrophilic interaction on the bladder cicada (*Cystosoma* sp) and *Leptopsalta* sp highlight that structure with insufficient roughness (e.g., low height etc) can promote more hydrophilic wetting properties. Interestingly, their camouflaging green colour is lost to a significant degree when dehydrated [39]. It has been postulated that the hydrophilic wetting properties on the bladder cicada wing may potentially be required to maintain the intense colour of the membranes and aid in camouflage while resting on foliage [8]. Thus there may be advantages to having structuring which promotes a certain amount of wetting on some membranes. Varying wetting properties demonstrated on numerous cicada may be associated with specific behaviour, habits, environments and form of the insects.

Self-cleaning

While the insect cuticle can demonstrate very low adhesive forces with contaminating particles some local or external mechanism or environmental factor is required for complete removal of solid bodies. As many of the cicada wings demonstrate a very hydrophobic or superhydrophobic wetting behaviour, water droplets from rain can facilitate removal via impacting or rolling drops. This is often termed the 'lotus effect' where droplets can absorb or adsorb particles and easily remove them from the surface where contacting forces may be very low (see figure 6) as shown on some cicada membranes. Rolling and impacting droplets can easily collect contaminants from the cicada (figure 8 and supplementary video 1) on such surfaces and remove them completely from the wing.

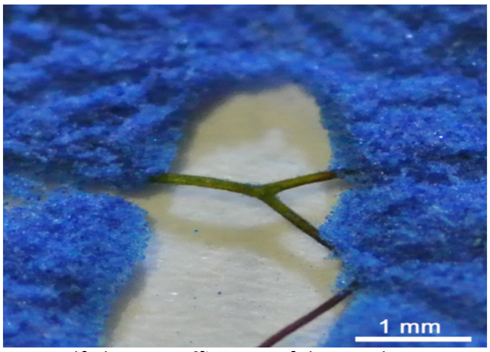

Figure 8: Self-cleaning efficiency of the cicada wing membrane of Psaltoda claripennis after one small water droplet was allowed to roll along the surface collecting silica particles (contaminants coloured with a dye to highlight the efficiency).

Although self-cleaning can be accomplished on the cicada wings with impacting or rolling droplets, rain may not be available for prolonged periods of time and, in extreme circumstances, may be absent during the short life span of the insect [40]. On the other hand, some cicada live in a humid environment in which condensation of atmospheric vapour takes place on a daily basis. Thus it seems plausible that some mechanism involving dew formation or fog conditions on the wing surface may also be involved in cleaning of the surface. A previous study of a superhydrophobic insect wing (a lacewing) showed that small water droplets could be removed from the membrane via a number of distinct mechanisms [41]. Surprisingly one of the removal processes did not entail droplets rolling off but took place based on a jumping droplet mechanism [42]. This jumping droplet phenomenon was first demonstrated to take place on artificial superhydrophobic surfaces [42]. This occurs when two or more droplets merge (from growth of the droplets on the surface) and changes in surface energy from the coalescence process (excess energy transformed into kinetic energy) propel the merged droplet from the surface. Observations from the study on the lacewing suggested that other superhydrophobic insect membranes may exhibit a similar process and potentially use this for self-cleaning in the absence of rain. Indeed when a cicada wing was examined it displayed the same jumping process and it was shown that condensation could actually clean the surface of a contaminated wing [13]. Droplets which housed contaminants could propel away from the wing surface. The process has been

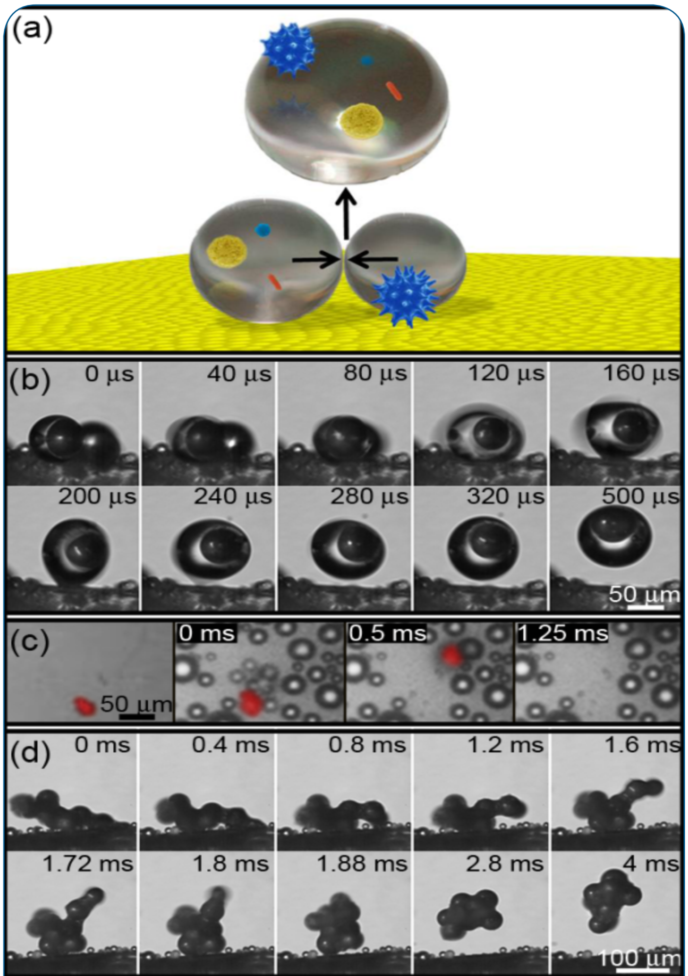

Figure 9: (a) Diagrammatic representation of the 'Cicada Effect' displaying two small droplets in close proximity, each containing various contaminants (e.g., bacteria, soil and plant particles). As the droplets grow in size they merge and the change in surface energy results in self-propulsion off the surface. (b) Self-cleaning showing a 50-µm-diameter silica particle initially captured inside a condensate drop. When this drop coalesced with a neighbouring drop, the capillary-inertial oscillation of the merged drop interacted with the superhydrophobic wing surface, resulting in an out-of plane jumping drop that carried away the particle. (c) Removal of a single pollen particle after coalescence with neighbouring drops (coloured in image to highlight particle). (d) Aggregating removal process: When water vapour condensed on a clump of 50-µm-diameter silica particles, the growing liquid bridge among the neighbouring drops led to particle aggregation. The aggregation process caused the particle clump to eventually jump off the surface. (Parts (b)-(d) from [13]).

termed the 'Cicada Effect' [43] as this was the first surface to demonstrate such an effect where various particles, including pollens, could be removed from the wing membrane [13]. The process is shown schematically in figure 9 (a) where two (or more) individual water droplets, housing various contaminants (or contaminants adhered to the droplet surface) such as pollen grains, silica and bacterium, combine and self-propel off the surface.

The propulsion process can be viewed in terms of changes in droplet surface energies [43]. Consider the case where two very small water droplets on a superhydrophobic surface merge to form a combined droplet. The maximum height, H_m, that can be reached by a droplet can be determined by integrating the velocity of a droplet over its time-of-flight resulting in Equation (3).

$$H_m = {}^{1}/_{2a} \ln[1+({}^{a}/_{b})V_0^{\,2}] \quad (3)$$

where $a= -3\rho_a C_D/2\rho_w R_m$, $b= -g = 9.8$ m/s^2, $\rho_{a,w}$ density of air and water, respectively, C_D =drag coefficient, R_m = droplet radius and V_o= initial launch velocity at surface. Equation (3) sets the upper limit for the maximum possible height that a merged droplet can reach based on all the released surface energy being converted to kinetic energy and ignoring adhesional forces to the surface.

A series of images captured in time show the 'Cicada Effect' where a silica particle 50 microns in diameter (figure 9 (b)) is propelled off the surface and an individual pollen grain (figure 9 (c)) is also forced off the cicada cuticle. Changes in surface energy can also propel larger clumps of particles from the surface where a thin layer of water (capillary forces) covers particle clumps and rearrangement of the aggregation releases excess energy (figure 9 (d)).

Interactions with living cells

Bacterial growth

When collecting insects such as cicada species for numerous topography, adhesion and wetting studies [e.g., 8, 12, 15, 44-46] observation of dead specimens showed significant decomposition from environmental action/microbial attack of the body with minimal decay of wings (see for example figure 10).

Figure 10: Example of cicada remnants showing intact wing membranes while other regions are degraded from environmental factors (e.g., microbial attack).

As well, as highlighted above in a number of previous studies, some cicada structuring demonstrates a nanostructured surface which can control the interaction of solids (e.g., natural organic contaminants such as pollens as well as hydrocarbons and silica particles, e.g., [8, 12]). A natural extension as suggested in such studies is investigating solid contacts of insect cuticle in aqueous conditions [8]. Collectively these observations/factors have lead to investigation of various bacteria with a range of insect surfaces including cicada membranes [47].

We have shown (along with various colleagues) that *Pseudomonas aeruginosa* and *Porphyromonas gingivalis* cells which contacted the surface of the wings of the cicada (*Psaltoda claripennis*) surface were killed with extreme efficiency by the wing surface (see CLSM images in figure 11 (a) [47] & (b) [48], and the accompanying SEM images in (c) & (e), respectively). While the wings were effective against other Gram-negative bacteria, Gram-positive bacteria were less susceptible. Interestingly, when we coated the cicada nanostructuring with a thin layer of Au, the effect was maintained which demonstrates that the effect is primarily from structure as opposed to chemistry (figure 11 (d)) [47]. When the bacterial cells adsorb onto the cicada nano structures on the wing surfaces, the cell membrane stretches in the regions between the pillars. If the degree of stretching is sufficient, this will lead to cell rupture. As Gram-positive cells exhibit greater rigidity, these cells have a greater natural resistance to this effect than Gram-negative cells for this form of structuring.

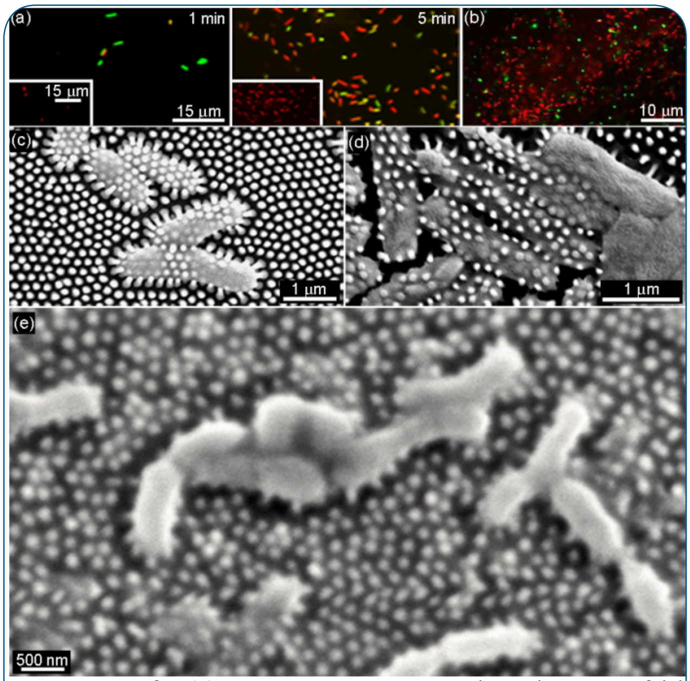

Figure 11: Confocal laser scanning microscopy (CLSM) images of (a) *Pseudomonas aeruginosa* and (b) P*orphyromonas gingivalis* cells adhering to the surface of cicada wing. Live cells in the CLSM images were stained with; (a) SYTO 9 indicated in green, while dead cells are stained with propidium iodide, indicated in red, and (b) LIVE/DEAD® BacLightTM Bacterial viability kit (ThermoFisher Scientific). Yellow cells are an indication of binding of both fluorescent dyes, which is also indicative of dead cells as propidium iodide is unable to stain healthy cells. SEM images revealing the nanostructure penetration of (c) *Pseudomonas aeruginosa* on the cicada wing, (d) *Pseudomonas aeruginosa* on the chemically altered (via Au coating) wing, and (e) *Porphyromonas gingivalis* cells on the cicada wing. (d) clearly demonstrates that topography rather than chemistry is the dominant factor in the bactericidal effect of cicada wings. (Parts (a) (modified), (c) & (d) have been reproduced by permission of John Wiley & Sons Inc. [47]).

Growth of Eukaryotic cells

Cicada wings typically possess arrays of hydrophobic nanostructures with a wide range of dimensions as described above. Importantly, these features in the context of a platform architecture for cell growth represent extensive scope for examining varied contact conditions (volume/area) [49]. Green et al were the first study to examine such cell interactions with insect wing cuticles including cicada wing membranes (see figure 12) [49]. The wide range of shape, spacing, height and size of structures allows one to potentially examine the conditions for stimulating desired specific cellular responses while inhibiting other cellular processes. Thus the cicada wing (and indeed insect wings in general) provide us with an excellent number of blueprints for directed control of cell behaviour for biological and clinical applications. This entails directional cell guidance (as structuring can be anisotropic and of micron dimensions) to varied cell adhesion/adherence (as nanostructure contact control is also possible). While the structuring on the cicada wing can kill some species of bacteria, the interaction/adhesion/adherence of eukaryotic (animal) cells can take various forms. This entails at least 3 distinct adhesional cell responses comprising 1) well adhered cell interactions; 2) cell sheets and 3) loosely adhered cells. The examples in figure 12 show these responses with a variety of cell lines on cicada wings (human retinal pigment epithelium, human umbilical endothelial, stem and cancer cells). It should be noted as some cicada surfaces can also represent a superhydrophobic surface (and thus structuring may hold air) the interaction of living cells may incorporate solid, liquid and air contacts (particularly at early stages). The temporal evolution of this 3 phase contact may also affect cell responses. Varied mechanical properties of the cicada structuring add yet another parameter at hand for cell response studies.

Recent studies on fabricated structuring not too dissimilar to those exhibited on some cicada species (e.g., figure 12) have demonstrated that co-cultured cell lines exhibit different responses [50-51]. This cell selectivity has shown nano structuring of particular dimensions can provide favourable conditions for some cells (e.g., endothelial cells) while inhibiting others (e.g., fibroblast cell growth) [50-51]. The tops of the nanostructures apparently can, in some circumstances provide insufficient ligand density, spacing, and clustering for the cells to form mature focal adhesions. Density of integrin binding sites may also explain different cell responses. As well, nano-topography may potentially hinder integrin clustering and/or alter orientation of cell binding sites. The varied cell responses shown in figure 12 demonstrate some of these aspects and the potential to use such natural templates for man-made designs.

Further studies of insect cuticle (including cicada membranes) with various cell types may provide insights into design of numerous biomedical surfaces where it would be advantageous to enhance the growth of a desired cell type, while inhibiting that of another ("cell-selectivity").

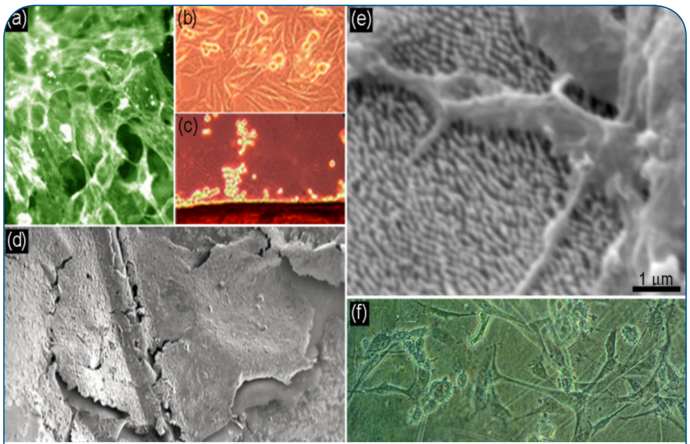

Figure 12: (a) Human retinal pigment epithelium (ARPE-19) grown on cicada wing. (b) C32 melanoma cells on control culture dish showing good adhesion. (c) Same cells as (b) grown on the cicada wing demonstrating low adhesion. (d) Stem cells grown a cicada membrane demonstrating a cell sheet response. (e) Periodontal ligament stem cells (high resolution image where wing nanostructuring is visible) (f) Human umbilical endothelial cells on cicada wing. (Part (a) has been reproduced by permission of John Wiley & Sons Inc. [49].

Replication

From the studies described the cicada wing membrane represents a multifunctional surface which is non hierarchical and in most cases is simplistic from 'evolutionary design'. The extensive properties (many of which are common to one form of structure and shape) are an intriguing feature of the membrane surface (see figure 13). The simplicity of the cicada structuring is an advantageous attribute when considering the structures for replication for man-made applications. High fidelity replication of insect wing surfaces can be undertaken using a variety of techniques [e.g., 14, 16]. These successful methods may provide advanced biomaterials for numerous applications including selective antibacterial, self-cleaning and cell growth architectural surfaces. While a comprehensive review of all possible replication techniques is beyond the scope of this review, a number of biotemplating methods are presented where the cicada wing itself is used in the replication process.

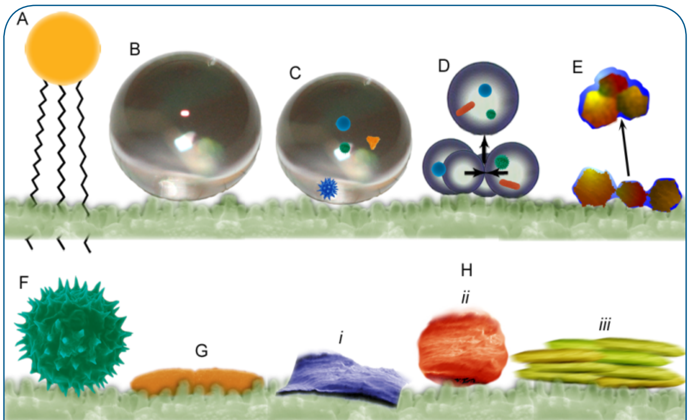

Figure 13: The multifunctionality of the cicada wing. (a) – Transparency, Antireflection, Colour, (b) – Superhydrophobicity, (c) – Self-Cleaning - Large rolling/impacting droplets, (d) – Self-Cleaning - self-propelling of smaller droplets, (e) – Self-Cleaning – aggregating particles, (f) - Low adhesive and frictional forces of contaminants, (g) – Selective anti-bacterial, (h) – Control of Eukaryotic cell adhesion with i – high adhesion, ii – low adhesion, iii - cell sheets.

It has been suggested that cicada micro/nanostructures on the wings could act as natural templates to transfer properties onto materials such as polymers and metals [45]. A polymer was previously tailored using this templating procedure on the wing membrane of several species of cicada [8, 12]. In those studies negative replicas were produced by laying whole wings on liquid Epon araldite resin held in a silicone rubber mould. The resin was polymerised at 60°C for 3 days. After cooling, the wing tissue was pulled away from the resin leaving an impression that was used to produce a positive cast. The casts were then formed by the application of PolyDiMethylSiloxane (PDMS). The resulting replicates of the surface closely matched the original topography of the cicada (compare figure 14 (a) with 3 (d), (e)). The wetting properties of the membrane can also be transferred with this technique. This is demonstrated in figure 14 where part (b) shows a flat PDMS surface and the contrasting replicated PDMS surface shown in (c), presenting a change in contact angle. Replication of cicada wing membranes using this process can produce self-cleaning surfaces as shown in figure 14 (d).

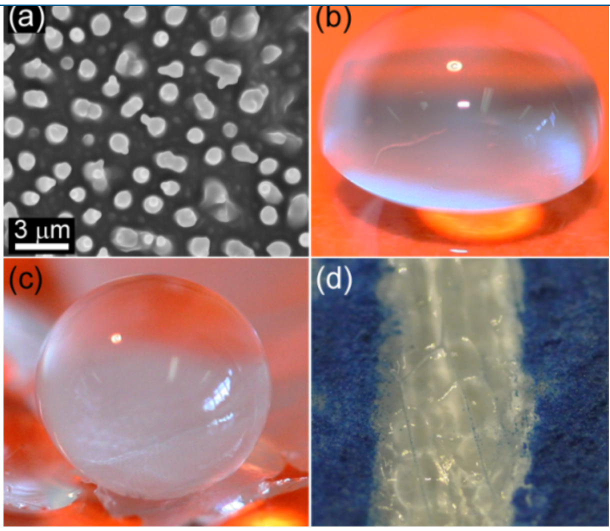

Figure 14: (a) Shows the PDMS replica of the cicada membrane from *Tosena* sp. (b) Photographs showing a 10 µl droplet deposited on an unpatterned hydrophobic PDMS surface. (c) Water droplet interaction on a polymer (PDMS) replica of the cicada *Gaeana* sp. (d) Self-cleaning efficiency of the polymer replica after one small water droplet was allowed to roll along the surface collecting silica particles (contaminants coloured with a dye to highlight the efficiency).

There are numerous examples of more elaborate methods for replication of these simple structures. An example of a more elaborate replica molding technique to fabricate a large area of nanostructures on polymethylmethacrylate (PMMA) polymer films has been used utilising a cicada wing bio-template [14]. In that study the cicada wing was attached to a supporting plate with an organic glue. A thick film of gold was deposited on the wing surface by thermal evaporation and another supporting plate was adhered onto the film with the organic glue and the Au film released from the cicada wing. The next part of the fabrication process involved transfer of the structure of the Au film to a PMMA film. A PMMA solution in anisole was cast on the Au mold heated and then mechanically peeled off from the Au mold. The resulting replicas have been shown to closely resemble the original cicada wing surface, including the anti-reflective property (see figure 15). Figure 15 (e) shows the measured reflectivity as a function of wavelength for the replicated PMMA film and a flat PMMA film without the nano-structured array. The reflectivity of the PMMA surface with nano arrays is on average less than 30% of that of the unpatterned flat PMMA surface at wavelengths in the UV and visible regions [14].

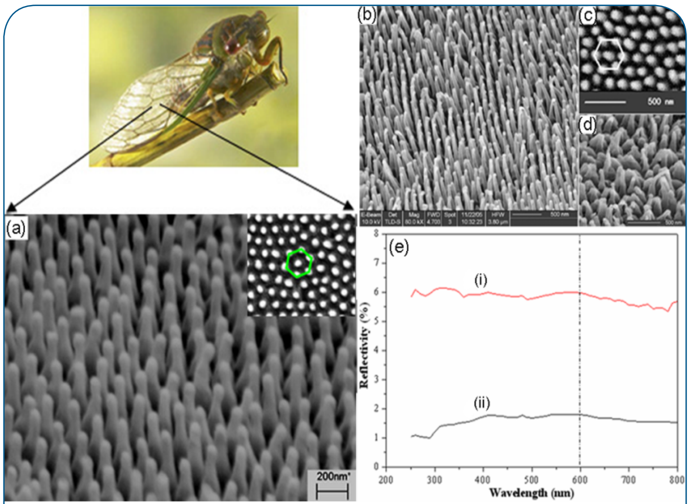

Figure 15: SEM images of a cicada wing (*Cryptympana atrata Fabricius*). (a) Large-scale perspective view. The inset is a top view. (b) – (d) SEM images of the replicated PMMA films with nano-arrays on the surface from the negative Au mold. (b) Large-scale perspective view and (c) higher-magnification top view showing a hexagonal pattern. (b) and (c) were obtained after the PMMA film was heated at 90°C for 30 min. (d) Perspective view after the film was heated at 60°C for 30 min. (e) Wavelength dependence of the measured reflectivity of an unpatterned flat PMMA film (i) and replicated PMMA film with nano-nipple arrays on the surface (ii) (Reproduced by permission of IOP Publishing [14]).

Conclusions

The multifunctional structuring of the insect wing membrane provides a rich and free blueprint for potential man-made materials. In this paper we have illustrated some of the varied properties and functions of the insect cuticle using the cicada as a representative example. The extensive properties in relation to interactions with liquids and solids (in terrestrial and liquid environments), lends the wing structuring to a diverse range of applications. In particular, superhydrophobic, antireflective, low adhesion/friction, excellent self cleaning properties, antibacterial and control of cell growth responses are features which are amenable for diverse purposes. These include multifunctional surfaces in specific environments such as anti-reflective self-cleaning materials exposed to humid environments. Possible applications may also include medical devices/materials such as orthopaedic implants, surgical tools, theatre surfaces, wound bandaging, contact lenses, artificial capillaries (e.g., catheters and inlet ports), dental implants and uses in numerous other areas (e.g., marine platforms, various membranes used in industrial applications (e.g., potable water filters) and a range of optical devices).

The varied structuring presented in this paper are relatively easy to replicate (especially when compared to some elaborate hierarchical structuring) and have other interesting attributes as they are incorporated into relatively thin membranes which can potentially be deployed in constrained geometric environments.

Acknowledgements

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References

1. Gorb S. Attachment devices of insect cuticle. The Netherlands, Dordrecht: Kluwer Academic Publishers; 2001.
2. Vincent JFV. Arthropod cuticle: a natural composite shell system. Composites Pt A. 2002; 33(10):1311–1315. doi:10.1016/S1359-835X(02)00167-7.
3. Vincent JFV, Wegst UGK. Design and mechanical properties of insect cuticle. Arthropod Struct Dev. 2004; 33(3):187–199.
4. Neville AC. Biology of the Arthropod Cuticle. , Berlin: Springer; 1975.
5. Chapman RF. The Insects: Structure and function. Cambridge: Cambridge University Press; 1998.
6. Watson GS, Cribb BW, Hu HM, Watson JA. Contrasting micro/nano architecture on termite wings: Two divergent strategies for optimising success of colonisation flights. PlosOne. 2011; 6(9):1-10. doi: 10.1371/journal.pone.0024368.
7. Vukusic P, Sambles JR. Photonic structures in biology. Nature. 2003; 424:852–855. doi:10.1038/nature01941.
8. Hu H-M, Watson JA, Cribb BW, Watson GS. Fouling of nanostructured insect cuticle: Adhesion of natural and artificial contaminants. Biofouling. 2011; 27(10):1125–1137. doi: 10.1080/08927014.2011.637187.
9. Watson GS, Cribb BW, Watson JA. Particle adhesion measurements on insect wing membranes using Atomic Force Microscopy. ISRN Biophysics. 2012; 947872. doi: http://dx.doi.org/10.5402/2012/947872.
10. Gorb SN, Kesel A, Berger J. Microsculpture of the wing surface in Odonata: evidence for cuticular wax covering. Arthropod Structure & Development. 2000; 29(2):129-35.
11. Parker AR, Lawrence CR. Water capture by a desert beetle. Nature. 2001; 414:33-34. doi:10.1038/35102108.
12. Watson GS, Myhra S, Cribb BW, Watson JA. Putative function(s) and functional efficiency of ordered cuticular nano-arrays on insect wings. Biophys J. 2008; 94(8):3352-3360. doi: 10.1529/biophysj.107.109348.
13. Wisdom KM, Watson JA, Qu X, Liu F, Watson GS, Chen C-H. Self-cleaning of superhydrophobic surfaces by self-propelled jumping condensate. Proc Nat Acad Sci (PNAS). 2013; 110(20):7992-7997. doi: 10.1073/pnas.1210770110.
14. Xie G, Zhang G, Lin F, Zhang J, Liu Z, Mu S. The fabrication of subwavelength anti-reflective nanostructures using a bio-template. Nanotechnology. 2008; 19(9):095605. doi: 10.1088/0957-4484/19/9/095605.
15. Watson GS, Hu S, Cribb BW, Myhra S, Brown CL, Watson JA. Micro and nano-structures found on insect wings - designs for minimising adhesion and friction. Int J Nanomanufacturing. 2010; 5(1/2):112-128. doi: 10.1504/IJNM.2010.029929.
16. Zhang G, Zhang J, Xie G, Liu Z, Shao H. Cicada wings: a stamp from Nature for nanoimprint lithography. Small. 2006; 2(12):1440–1443.
17. The Insects of Australia: A Textbook for students and research workers. Carlton, Victoria: Melbourne University Press; 1991.
18. Sun MX, Watson GS, Zheng YM, Watson JA, Liang A. Wetting properties on nanostructured surfaces of cicada wings. J Exp Biol. 2009; 212(19):3148-3155. doi: 10.1242/jeb.033373.
19. Tobin M, Puskar L, Hasan J, Webb H, Hirschmugl CJ, Nasse MJ, et al. High spatial resolution mapping of superhydrophobic cicada wing surface chemistry using synchrotron radiation infrared microspectroscopy. J Synchrotron Radiation. 2013; 20(Pt 3):482-489. doi: 10.1107/S0909049513004056.
20. Sun M, Liang A, Watson GS, Watson JA, Zheng Y, Ju J, et al. Influence of cuticle nanostructuring on the wetting behaviour/states on cicada wings. PLoS One. 2012; 7(4):1-7. doi: 10.1371/journal.pone.0035056.
21. Hu H-MS, Watson JA, Cribb BW, Watson GS. Multi-functional insect cuticles: Informative designs for man-made surfaces. World Academy of Science, Engineering and Technology. 2011; 59:1370-1374.
22. Watson JA, Hu HM, Cribb BW, Watson GS. Anti-wetting on insect cuticle – structuring to minimise adhesion and weight On Biomimetics. In: LD Pramatarova, editor. Croatia: InTech Publishing; 2011.
23. Sun M, Liang A, Watson GS, Watson JA, Zheng Y, Jiang L, et al. Wettability and adhesional differences on a natural template: The cicada wing. Sci Adv Mat. 2014; 6(7):1493-1500. doi: http://dx.doi.org/10.1166/sam.2014.1805.
24. Ewart A, Popple LW. Three new cicada species of the genus Gudanga (Distant Insecta: Cicadidae: Cicadettinae: Cicadettini) from Queensland; comparative morphology, songs, behaviour and distributions, Memoirs of the Queensland Museum. Nature. 2013; 56(2):355-406.
25. Bernhard CG, Miller WH. Function of corneal nipples in compound eyes of insects. Acta Physiol Scand. 1963; 58:381–382.
26. Bernhard CG, Miller WH, Moller AR. The insect corneal nipple array. A biological, broad-band impedance transformer that acts as a antireflection coating. Acta Physiol Scand Suppl. 1965; 243:1–79.
27. Stavenga DG, Foletti S, Palasantzas G, Arikawa K. Light on the moth-eye corneal nipple array of butterflies. Proc R Soc B. 2006; 273(1587):661–667.
28. Gibson CT, Watson GS, Myhra S. Determination of the spring constant of probes for Force Microscopy/Spectroscopy. Nanotechnology. 1996; 7(3):259-263. doi:10.1088/0957-4484/7/3/014.
29. Yoshida A, Motoyama M, Kosaku A, Miyamoto K. Nanoprotruberance array in the transparent wing of a hawkmoth, Cephonodes hylas. Zool Sci. 1996; 13(4):525–526. doi: http://dx.doi.org/10.2108/zsj.13.525.
30. Yoshida A, Motoyama M, Kosaku A, Miyamoto K. Antireflection nanoprotuberance array in the transparent wing of a hawkmoth Cephonodes hylas. Zool Sci. 1997; 14:737–741.
31. Sun MX, Liang A, Zheng Y, Watson GS, Watson JA. A study of the antireflection efficiency of natural nano-arrays of varying sizes. Bioinspiration & Biomimetics. 2011; 6(2):026003. doi:10.1088/1748-3182/6/2/026003.
32. Grann EB, Moharam MG, Pommet DA. Optimal design for antireflective tapered two-dimensional subwavelength grating structures. J Opt Soc Am A. 1995; 12(2):333–339. doi: 10.1364/JOSAA.12.000333.
33. Linskens HE, Cresti M. Pollen-allergy as an ecological phenomenon: a review. Plant Biosystems. 2000; 134(3):341-352. doi: 10.1080/11263500012331350495.

34. Smith JL, Lee K. Soil as a source of dust and implications for human health. Adv Agronomy. 2003; 80:1-32. doi: 10.1016/S0065-2113(03)80001-9.

35. Knaapen AM, Borm PJA, Albrecht C, Schins PF. Inhaled particles and lung cancer Part A: Mechanisms. Int J Cancer. 2004; 109(6):799-809.

36. Wenzel RN. Resistance of solid surfaces to wetting by water. Ind Eng Chem. 1936; 28(8):988–94. doi: 10.1021/ie50320a024.

37. Cassie ABD, Baxter S. Wettability of porous surfaces. Trans Faraday Soc. 1944; 40:546–551. doi: 10.1039/TF9444000546.

38. Shirtcliffe NJ, McHale G, Atherton S, Newton MI. An introduction to superhydrophobicity. Adv Coll Int Sci. 2010; 161(1-2):124–138. doi: 10.1016/j.cis.2009.11.001.

39. Moulds MS. Australian cicadas. Australia: New South Wales University Press; 1990. 193–195 p.

40. Azuma A. The biokinetics of flying and swimming. Tokyo: Springer; 1992.

41. Watson JA, Cribb BW, Hu H-M, Watson GS. A dual layer hair array of the brown lacewing: Repelling water at different length scales. Biophysical J. 2011; 100(4):1149-1155. doi: 10.1016/j.bpj.2010.12.3736.

42. Boreyko JB, Chen CH. Self-propelled dropwise condensate on superhydrophobic surfaces. Phys Rev Lett. 2009; 103(18):184501.

43. Watson GS, Gellender M, Watson JA. Self-propulsion of dew drops on Lotus leaves: A potential mechanism for self-cleaning. Biofouling. 2014; 30(4):427-34. doi: 10.1080/08927014.2014.880885.

44. Watson GS, Blach JA. Characterisation of cuticular nanostructures on surfaces of insects by Atomic Force Microscopy-'mining' evolution for smart structures. Proc SPIE: Smart materials II. 2002; 4934:378-385. doi: 10.1117/12.469733.

45. Watson GS, Watson JA. Natural nano-structures on insects – possible functions of ordered arrays characterized by Atomic Force Microscopy. Appl Surf Sci. 2004; 235(1-2):139-144. doi:10.1016/j.apsusc.2004.05.129.

46. Watson GS, Watson JA. The genius of the cicada wing. Australasian Science Magazine. 2013; 34(9):24.

47. Ivanova EP, Hasan J, Webb HK, Truong VK, Watson GS, Watson JA, et al. Natural bactericidal surfaces: cicada wings kill Pseudomonas aeruginosa cells. Small. 2012; 8(16):2489-2494. doi: 10.1002/smll.201200528.

48. Watson GS, Green DW, Schwarzkopf L, Li X, Cribb BW, Myhra S, et al. A gecko skin micro/nano structure–A low adhesion, superhydrophobic, anti-wetting, self-cleaning, biocompatible, antibacterial surface. Acta Biomaterialia. 2015; 21:109-122. doi: 10.1016/j.actbio.2015.03.007.

49. Green DW, Watson GS, Watson JA, Abraham SJK. New biomimetic directions in regenerative ophthalmology. Adv Healthc Mater. 2012; 1(2):140-148. doi: 10.1002/adhm.201100039.

50. Reynolds PM, Pedersen RH, Stormonth-Darling J, Dalby MJ, Riehle MO, Gadegaard N. Label-free segmentation of co-cultured cells on a nanotopographical gradient. Nano Lett. 2013; 13(2):570−576. doi: 10.1021/nl304097p.

51. Csaderova L, Martines E, Seunarine K, Gadegaard N, Wilkinson CDW, Riehle MO. A biodegradable and biocompatible regular nanopattern for large-scale selective cell growth. Small. 2010; 6(23):2755–2761. doi: 10.1002/smll.201000193.