

No safe landing for bacteria

SOME KINDS OF SKIN ARE THEIR OWN ANTISEPTICS, SAYS ANANTHANARAYAN

Bacterial infection is the reason for a great proportion of disease and is a risk that exists in all surgery. The need for antiseptics to eliminate or limit bacterial population adds to the complexity and cost of assuring public health and even to contain some forms of corrosion in industry.

The success of scientists in institutes in Australia, with co-workers in Spain, in discovering that natural materials are able to combat bacteria, not by chemical barriers but by physical structure, and then to mimic the structure with its antibacterial features in an artificial material is, hence, an event of importance. Professor Elena P Ivanova and colleagues report in *Nature Communications* that *black silicon*, a synthetic semiconductor, solar cell material with sharply rising nano-protrusions on its surface, so fashioned to absorb light more efficiently, shows bactericidal properties just like similar surfaces found in the natural world — the wings of the dragonfly, for instance.

Ivanova's group had confirmed only a few months earlier, in a paper in the journal *Small*, that it was the physical surface structure of the wings of the cicada, and not any chemical coating, which made for its antibacterial quality.

Many surfaces in the natural world need to stay free of wet and dirt to be able to function. The legendary lotus leaf, like the leaves of other plants that live in water, need to efficiently repel water so that they do not get weighed down. Similarly, the wings of insects need to be free of traces of wet for them to get about and survive. While water-repelling leaves and insect skin are found to be coated with water-repelling waxes, it has been found that the wings of the dragonfly, and others, also have a physical pattern that breaks a drop of water that falls on them into smaller drops, to have greater rebound and be lighter.

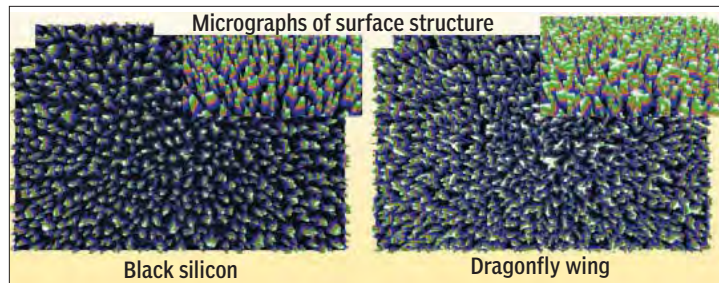
Many insects have evolved to have such *strongly water-repelling* or *superhydrophobic* surfaces. While water does not wet, and runs off these surfaces, it does wet specks of dirt and other contaminants and carries them



Elena P Ivanova



Bacterium disintegrating



Black silicon

Dragonfly wing

away, to leave the surface clean in addition to being dry. The observation that many superhydrophobic surfaces are also free of bacterial contamination was considered to be a case of the self-cleaning quality leading to clearing of biological material, an action that is called *antibiofouling*.

The first study of Professor Ivanova and group looked into the antibiofouling action of the wings of the cicada, which are highly hydrophobic and self-cleaning. The initial findings were that the wings were in fact not par-

ticularly effective in keeping away adhesion by a strain of common bacteria as many of the cells were able to attach themselves to the wings. But then it was found that once the bacterium landed in the wing, nano-pillars on the wing surface pierced the bacterium cell and caused cell components to spill out beneath the cell and between the nano-pillars.

Further study showed that the cells that had landed on the surface were, in fact, dead! It was found that most cells were killed off within five minutes of attaching to the surface and there was a cycle of 20 minutes of cells landing and getting killed, and getting cleared for more cells to land. Studies with Atomic Force Microscopy showed that the cells sank slowly into the wing surface for some 200 nanometres, in about three minutes, before there was a sudden downward movement that indicated rupture of the cell.

To eliminate the possibility of the cell death being due to some chemical component of the cicada wing surface, the researchers coated the surface with a 10 nanometre thick gold film. The fine gold coating, which left the surface topography unchanged, was found to affect the hydrophobicity of the surface. But, despite the new surface chemistry of the surface, the bactericidal effect was found to stay

unchanged. This demonstrated that it was the physical features, of sharp protuberances, that brought about the bactericidal effect, a conclusion of importance in the context of many bacteria developing resistance to biochemical agents, like antibiotics.

In the current study, reported in *Nature Communications*, Professor Ivanova and colleagues note that many surfaces with sharp protuberances at the microscopic scale display superhydrophobicity or biological activity at the molecular level. Taking off from what was seen with the cicada, the group looked at other surfaces that had similar structural characteristics. One such was a form of silicon, called *black silicon*, a form where the surface structure is of needle-shaped single crystals of silicon. The effect is that the normal reflectivity of metallic silicon is greatly reduced and most light that falls on the surface is absorbed — hence the name *black silicon*. Silicon being a semiconductor that is used in photocells, this form, with the property of absorbing light, was developed for increasing the efficiency of photocells.

Microscopic studies showed that both black silicon as well as natural surfaces like the cicada or dragonfly wing, had disordered nanopillar systems, forming clusters and groups of clusters. Such a grouping has also been shown to be a result of the forces that act when the structure is forming. While natural surfaces, in this grouping of spikes, consisted of fats and waxes, which are water-repellent, the surface of silicon is only moderately water-repellent. But the remarkable result was that the silicon structure was also equally bactericidal as the natural surfaces.

The bactericidal quality was assessed by culturing colonies of three different kinds of bacteria on the surfaces of dragonfly wing, black silicon and plain glass and silicon, for comparison for 30 hours. While the mix of bacteria that attached to the structured surfaces and the plain surfaces were different, the two kinds of structured surfaces were found to be lethal to the cells that attached to them. It can be seen in the table that despite differences of composition and hydrophobicity of natural materials and silicon, black silicon, which shares shape and construction, is as bactericidal as both cicada and dragonfly wings. The nature of the cicada wing structure limits its effectiveness to Gram-negative cells, but black silicon is like the dragonfly wing and is effective against all kinds of cells. Gold coating the black silicon and dragonfly wings again left the bactericidal property unchanged, like in the case of the cicada wings.

The results are significant — that nanomaterials that can be readily fabricated, in quantity, can be used to control bacteria growth. The structure of nanomaterials, in fact, can be optimised for exclusive antibacterial action, or for action along with rigidity, strength, etc, as required and could be better than as found naturally. This suggests that "novel antibacterial nanomaterials may open the way for new applications in the field of mechanobiology", say the authors.

THE WRITER CAN BE CONTACTED AT simplescience@gmail.com

Features comparison of insect wings and black silicon surfaces.			
Surface	Cicada wing (<i>R. claripennis</i>)	Dragonfly wing (<i>D. bipunctata</i>)	Black silicon
Surface characteristics			
Water contact angle	159°	153°	80°
Chemical composition	Lipids/waxes	Lipids/waxes	Mostly SiO ₂
Height of nanoprotuberances	200nm	240nm	500nm
Bactericidal activity			
Effectiveness	Gram-negative	Gram-negative Gram-positive Spores	Gram-negative Gram-positive Spores
Efficiency (*)			
Versus <i>P. aeruginosa</i> ATCC 9027	2.0 x 10 ⁵	3.0 x 10 ⁵	4.3 x 10 ⁵
Versus <i>S. aureus</i> CIP 65.8 ^T	N/A	4.6 x 10 ⁵	4.5 x 10 ⁵
Versus <i>B. subtilis</i> NCIMB 3610 ^T	N/A	1.4 x 10 ⁵	1.4 x 10 ⁵

(*) Average number killed over a square centimetre every minute, over three hours

IMPORTANT ROLE-PLAY An exciting enterprise

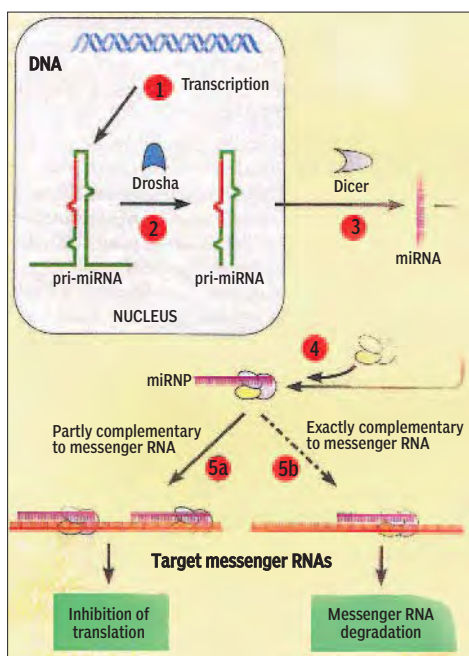
TAPAN KUMAR MAITRA EXPLAINS HOW MICRORNAS PRODUCED BY NORMAL CELLULAR GENES SILENCE THE TRANSLATION OF IMPORTANT MESSENGER RNAS

The finding that gene expression can be silenced by the introduction of double-stranded RNAs into cells raises the question of whether any normal genes produce RNAs that function in a comparable fashion. The search for such molecules has led to the discovery of microRNAs (miRNAs), a class of single-stranded RNAs about 21-22 nucleotides in length that are produced by genes found in all multicellular organisms studied so far. MicroRNAs bind to and regulate the expression of messenger RNAs produced by genes that are separate from genes that produce the microRNAs.

Genes that produce microRNAs are initially transcribed into longer RNA molecules called primary microRNAs, which fold into hairpin loops. The looped pri-miRNAs are then converted into mature miRNAs by sequential processing.

First, a nuclear enzyme called *Drosha* cleaves the pri-miRNAs into smaller hairpin RNAs, called precursor microRNAs, roughly 70 nucleotides long. The pre-miRNAs are then exported to the cytoplasm, where Dicer cleaves them into mature, single-stranded microRNAs 21-22 nucleotides long.

Each mature microRNA is assembled with a group of proteins to form a ribonucleoprotein complex (miRNP) that, in turn, silences the expression of messenger RNAs containing sequences complementary to that of the microRNA. In a few cases, the match is exactly complementary and the messenger RNA is destroyed by a mechanism similar to that observed with siRNAs. It is more common, however, for microRNAs to exhibit partial complementarity to messenger RNA sites. Such binding inhibits translation rather than triggering messenger RNA degradation, but this inhibitory effect usually requires the binding of multiple microRNAs to different, partially complementary sites within a given messenger RNA.



Genes coding for microRNAs represent about 0.5 to one per cent of the total number of genes present in the genomes of the multicellular organisms studied so far. For example, about 200-250 microRNA genes have been detected in the human genome and about 120 in worms. Many of the microRNAs produced by these genes control the activity of messenger RNAs coding for proteins that play important roles during development of the organism in which they occur.

The chicken is a production animal of major economic importance, crucial for food security throughout the world. More than 80 billion chickens are produced each year worldwide, making it the most numerous livestock species. Poultry production is also very efficient, imposing the least environmental impact of all our livestock in terms of gas emissions. However, climate change is likely to have an impact on future poultry production systems. We are likely to see the (re)emergence of diseases such as macroparasites (*coccidiosis*) and vector-borne pathogens within wild hosts (Avian Influenza from ducks and other poultry). Climate change will also have effects on heat stress, water shortage and salt tolerance, which will need new buildings and changes to other work practices in poultry production, particularly in Asia.

Our knowledge of the organisation and content of poultry genomes has increased rapidly over the past few years, culminating in the sequence of the chicken genome in 2004, a milestone in genetics and evolutionary biology. Recent advances in DNA sequencing technologies now make it possible to produce genome sequences of most living organisms, quickly and cheaply. Using these technologies we have also seen the completion of the genome sequences of the turkey, duck and many other avian species — with thousands to follow.

The "annotation" of the chicken genome — the decoding of the sequence of base-pairs (G, A, T, C) into the proteins and other functions it encodes has been under continuous improvement, as well as being a major challenge. The latest version has taken advantage of huge volumes of sequencing data derived from the RNA molecules themselves, the message read from the genome DNA sequence that is translated into proteins and other non-coding regulatory RNA species. The management and analysis of these sequence datasets has and continues to be a major challenge in the application of computer science — this field of "bioinformatics" is now a key area in the genetics and genomics of poultry and livestock species.

These new DNA sequencing technologies have revolutionised genetic studies within commercial and experimental poultry populations by allowing the rapid sequencing of many individuals for the detection of genetic variants, often unique to those populations sequenced. An international team of scientists, coor-



A MISSION TO TACKLE AN ENDEMIC DISEASE PROBLEM OF COMMON INTEREST TO INDIA AND THE UK WILL BE A GRAND CHALLENGE, WRITES DAVID W BURT

minated by The Roslin Institute, from the public and private sectors has undertaken a massive genome sequencing effort of over 24 chicken lines to develop a high-density genotyping array of 600,000 SNPs suitable for the characterisation of both layer and broiler chickens. This array is being used to identify and select DNA-based markers for the selection of genetic traits in poultry.

These new technologies were recently used to sequence the genomes of about 50 different avian species as part of an evolutionary study. These resources open up many new opportunities to explore the rich biology of birds and finally solve the phylogenetic history of this large and rapidly evolving avian reptile group. The availability of thousands of avian genomes will provide many new opportunities but also poses new challenges. How do we annotate so many species? The current model of one genome at a time will no longer be sustainable — we will need to annotate hundreds of genomes simultaneously, for example, within orders or families based on multiple genome alignments. How do we access the data on these genomes? We will still maintain high quality annotations of model species, such as the chicken and zebra finch, and probably ancestral species for each order (or family). For other species we will provide access to the genomes, gene annotations and new tools to visualise and search these resources. Whatever happens, it will be exciting and a grand challenge.

An area of collaboration between partners in the UK (The Roslin Institute and Royal School of Veterinary Studies, University of Edinburgh) and India (HSADL-IVRI, Bhopal) is to exploit these genome sequences to explore the reservoirs of Avian Influenza found endemic in domestic and wild bird populations in India. Avian Influenza, caused by the highly pathogenic virus H5N1, is taking a huge toll on the Indian poultry industry since the first outbreak in 2006. Despite eradication and confirmation of disease-free status, the re-emergence continues. Since each outbreak needs eradication of all poultry within a one-two mile radius, every episode of infection has a major economic and social impact on small and marginal poultry farmers of rural India. This is particularly acute in a country where backyard poultry is popular and thickly populated villages usually lie within the eradication zone. As a notifiable disease, Avian Influenza can have serious implications for international trade and further harm India's poultry industry.

This will also be an opportunity to share in resources and facilities and learn new skills and approaches from each other to tackle this endemic disease problem of common interest to India and the UK.

THE WRITER IS CHAIR OF COMPARATIVE GENOMICS, THE ROSLIN INSTITUTE, AND CAN BE CONTACTED AT dave.burt@roslin.ed.ac.uk

PLUS POINTS

Teen depression

A revolutionary way of identifying teenage boys most likely to develop clinical depression later in life has been discovered by researchers at the University of



Cambridge. Predicting those who may be at risk of depressive symptoms has been puzzling doctors for decades but now scientists have found the first biomarker — or biological signpost — for clinical

depression.

Teenage boys with a combination of depressive symptoms and raised levels of the stress hormone cortisol are up to 14 times more likely to develop clinical depression than those who show neither trait. Around one in six people suffer from clinical depression at some point in their lives and three-quarters of mental health diseases start before people are 24 years old. Researchers believe this latest discovery, published on Monday in the journal, *Proceedings of the National Academy of Sciences of the USA*, could help target treatment. Professor Ian Goodyer from the University of Cambridge, who led the study, said, "Depression is a terrible illness that will affect as many as 10 million people in the UK at some point in their lives. Through our research, we now have a very real way of identifying those teenage boys most likely to develop clinical depression. This will help us strategically target preventions and interventions at these individuals and hopefully help to reduce their risk of serious episodes of depression and their consequences in adult life."

Researchers analysed several early morning saliva samples taken within a week from more than 1,850 teenagers — and did the same again a year later. The samples showed cortisol levels were stable over the year and were then combined with self-reports about symptoms of depression. The teenagers were then divided into four groups, ranging from group one, who had normal levels of morning cortisol and low symptoms of depression over time, through to group four, who had elevated levels of morning cortisol and high symptoms of depression over time.

Teenage boys in group four were 14 times more likely to develop clinical depression than those in the first group. Teenage girls in this fourth group were only four times more likely than those in the first group to develop major depression — and were no more likely to develop the condition than those with either high morning cortisol or symptoms of depression alone. The findings suggest gender differences in how depression develops.

Paul Jenkins, chief executive of Rethink Mental Illness, said, "These findings represent a major development in our understanding of depression among teenage boys. When young people receive early intervention treatment, they have a much better chance of getting better and avoiding long-term mental health problems. The prospect of identifying boys at risk at an earlier stage, should enable us to make a big step forward in successfully treating serious mental illness."

EMILY DUGAN/THE INDEPENDENT

Psychopaths & sadists

Canadian researchers have confirmed what most people suspected all along — that Internet trolls are archetypal Machiavellian sadists. In a survey conducted by the group of psychologists, people who partake in so-called trolling online showed signs of sadism, psychopathy, and were Machiavellian in their manipulation of others and their disregard for morality. The researchers defined online trolling as "the practice of behaving in a deceptive, destructive or disruptive manner in a social setting on the Internet" for no purpose other than their pleasure.

To achieve the results, the team asked Internet users about subjects including how much time they spent online and whether they commented on websites such as YouTube. They were also given tests that measured their responses against psychology's "Dark Tetrad": narcissism, Machiavellianism, psychopathy and a sadistic personality. Questions also surrounded sadistic statements including, "I enjoy physically hurting people", "I enjoy making jokes at the expense of others" and "I enjoy playing the villain in games and torturing other characters".

The study — conducted by psychologists from the University of Manitoba, University of Winnipeg and University of British Columbia and published in the *Personality and Individual Differences* journal — said, "It was sadism, however, that had the most robust associations with trolling of any of the personality measures." It went on to claim that trolls were "agents of chaos" that exploited "hot-button issues" to inflame and exploit users' emotions.

"If an unfortunate person falls into their trap, trolling intensifies for further, merciless amusement. This is why novice Internet users are routinely admonished, 'Do not feed the trolls!'" the study warned. Most worryingly they based their conclusion on cyber-trolling being an "Internet manifestation of everyday sadism" rather than an on-line phenomenon.



THE INDEPENDENT