

Insects inspire new material

Insects' shells show the way to do better than plastics, says s ananthanarayanan

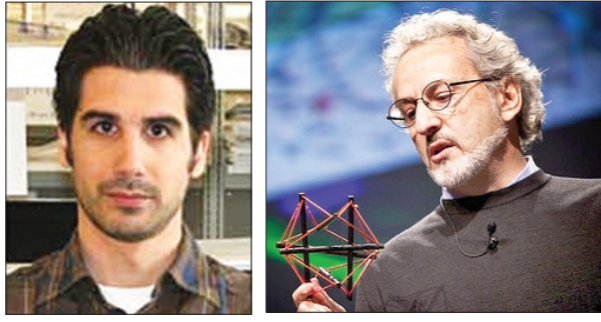
THE discovery of plastics was hailed as a miracle — a new, light, synthetic and easily workable material. But plastics are petroleum-based and have grown to be dangerous pollutants that last effectively for ever. Conversely, the material of shells and parts of the limbs of insects have evolved micro-structure that multiplies strength and the material does better than metallic sheets, at lower weight. They are versatile and, as they are biodegradable, they present no ecological challenge.

Researchers Javier G Fernandez, post-doctorate fellow, and Donald E Ingber, founding director of the Wyss Institute for Biologically Inspired Engineering at Harvard University report in the journal *Advanced Materials* that they have replicated the composition and structure of insect cuticle — to create what may be an alternative to consumer plastics and some medical applications.

The shells of insects consist of a material, *chitin* (pronounced *kytin*), formed by linking and chaining — a process called *polymerization* — molecules derived from the sugar glucose. The material is chemically similar to cellulose found in green plants. The chemical form allows links between adjacent chains, which creates a mesh and gives strength. In function, chitin is like the protein that makes up our skin. In its pure form, chitin is leathery but as the outer covering of insects it is modified, by combining with proteins, to form a toughened layer, or with chalk or limestone to form a hard shell. Chitin is said to be the second most abundant polymer on earth after cellulose, or *lignin*, which is the material found in wood and plants.

As instances of living material, insect exteriors show strength, they are lightweight, versatile and have the ability to interact with the biomolecular internals of the insect. The strength of the chitin-based material arises from its formation into a fibrous structure, embedded in a framework of elastic proteins. There are many proteins that can effectively fill the role and insect cuticle can take a great variety of forms of strength, resilience, appearance and texture, with variation of its components. But the structure of the interaction between chitin and proteins has not been understood and artificial materials of the same kind have been difficult to engineer.

Many efforts have been made to use chitin with synthetic components to create materials that can mimic these natural structures. But the efforts have not made progress because chitin is almost insoluble and difficult to work with in the laboratory. But a closely related form of chitin, called *chitosan*, has been found useful and has been approved as a material for wound dressings. Another useful material is *fibroin*, which is found in natural silk and is used in surgical sutures. Efforts were made to combine



Javier G Fernandez.

Donald E Ingber.



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chitosan and fibroin for medical applications where both strength and biocompatibility were needed, but without success, as the composites were found to be less hardy than the components.

Cuticle structure

Fernandez and Ingber explain in their paper that this seems to be because simply combining chitosan and fibroin ignores the organised, laminar arrangement of chitin and fibroin, laid close together but as separate components, in cuticle that is found in nature. "We therefore explored whether we could recapitulate the

times stronger than a simple blend of chitosan and fibroin without forming into layers. Fibroin does contribute to the strength and the ideal ratio was found to be one part of chitosan to two of fibroin. *Sbrilk* is also tougher, being able to absorb 1.5 times the amount of energy before breaking than chitosan and the deformation under forces is also significantly less than with chitosan. This property of

novel properties of these living materials by fabricating chitosan-fibroin laminates," they say.

They go on to describe how they dissolved chitosan in acetic acid and wet a glass sheet with the solution. When the acetic acid evaporated, it left behind a 12-micron layer of chitosan. This chitosan layer was cleaned of acid and then wet with a water solution of fibroin, from natural silk. When the water evaporated, it left behind a layer of fibroin, which was then treated with simple varnish, for protection.

The result was a clear, thin film with a structure of layers of the sugar-based polymer between layers of protein, with a distinct boundary. This is both in chemical form and construction, just like what is found in natural cuticle. The authors have named the material *sbrilk*, to acknowledge the *sbrimp*, which is a common source for chitin and *silk*, which is the source for fibroin. But more relevant to the study, the composite material was found to be twice as a

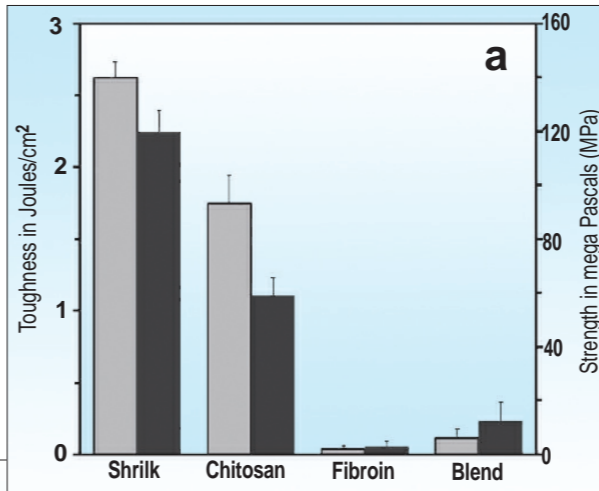
Sbrilk, of resisting external tension without deforming and damaging the contents, is like that of insect shells whose natural function is to protect. *Sbrilk* is also found to be twice as strong as nylon and equal in strength to aluminum alloys, but at half the weight!

Versatility

Natural cuticle comes in myriad forms — from the very elastic, in the joints of limbs, to the very hard, in the shells or protective covers and in wings. But the variations in physical qualities of cuticle do not seem to depend on the nature of the components of the materials — rather on the water content. It is found that *Sbrilk* also absorbs water, the chitosan and fibroin taking up water independently — chitosan twice as much as fibroin. Saturating *Sbrilk* with water reduces its strength by a factor 30 but, interestingly, the energy absorbed before breaking is reduced by a factor of only two. This comes about because, when the strength is reduced, the material becomes many times more elastic and can absorb a whole half of the energy before breaking than when it was 30 times stronger.

This property of varying elasticity by water content is like the ability of some insects to dynamically change the cuticle stiffness according to need. The *Rhodnius* bug, which spreads the Chagas disease, is able to hydrate and make its cuticle as much as 25 times harder in the course of feeding on the blood of its victim. *Sbrilk* also displays this ability to change its elasticity reversibly by changing water content. It is this property of insect cuticle that calls for the layer of proteins or wax that protects the deeper layers of the wings and shells of insects.

Forms of natural cuticle are also able to condense moisture from the atmosphere in arid climates, or repel water vapour in humid areas. The surface of some insect shells even act on the polarisation of reflected light, for different purposes. These features often depend on the micro-topography of the surface. The Harvard researchers report success in shaping the surface by casing the outer layers in a suitable mould, in a manner that has already been used with



chitosan. And again, because the elasticity of *Sbrilk* is changeable by moisture, it was possible to create *Sbrilk* objects in different shapes, like tubes or cylinders, before retracting the moisture to fix the new shape. *Sbrilk*, being biocompatible, could thus form a scaffold for tissue growth and repair.

"Based on its outstanding strength and versatility, as well as its low cost and density, *Sbrilk* is an excellent candidate as a biodegradable plastic that could have great value as a replacement for existing non-degradable plastics in a wide range of consumer product application areas, including disposable bottles, trash bags, packing materials and diapers that currently pile up in waste sites around our planet," say the authors.

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Another mince pie?

Jeremy Laurance reports on the discovery of a drug that may offer gluttons anti-ageing benefits of a low-calorie diet

IT is a challenging time of year to be told that eating less is good for you but evidence has, for a long time, suggested that cutting down on calories extends life. Now Italian researchers have identified a molecule produced when people diet that could lead to the development of a drug that mimics the effect of restraint, offering a longer life without the need for self-denial.

Experiments have shown that curbing the amount of food rats eat can extend their lives by 25 to 40 per cent. However, anti-ageing benefits are lost when the rats return to a normal diet.

Among humans, the population of the tropical Okinawan islands in Japan's extreme south-west are home to more than twice the national average of centenarians. As well as having a healthy diet, their longevity is attributed to their cultural habit of calorie control called *hara hachi bu* — or "eat until you are 80 per cent full".

Italian scientists from the Catholic University of Sacred Heart in Rome have now come a step closer to understanding how dieting works. It causes the body to activate a molecule called *Creb1* which, in turn, activates another set of molecules linked to longevity called the *sirtuins*.

While overeating ages the brain and can lead to Alzheimer's disease, calorie restriction increases the activity of *Creb1*, which is known to regulate memory and learning. Research in mice has shown that if they lack the molecule, the benefits in terms of improved memory from cutting down calorie intake are not seen.

Giovambattista Pani, who led the study published in *Proceedings of the National Academy of Sciences*, said, "Our findings identify, for the first time, an important mediator for the effects of diet on the brain. Our hope is to find a way to activate *Creb1*, for example through new drugs, to keep the brain young without the need for a strict diet." Biologists believe that restricting calories causes animals to go into a state normally reserved for near starvation. Instead of spending their energy reserves on reproduction, they shut down everything but their basic body maintenance, in preparation for a future time when breeding would stand a better chance of success.



Giovambattista Pani.

Self-preservation

- Jeanne Calment, the oldest person in history, who died in 1997 aged 122, attributed her longevity to a diet rich in olive oil, port and "regular smiling".
- Cleopatra, Queen of ancient Egypt, took baths of asses' milk to preserve the beauty and youth of her skin.
- In 18th-century France, noblemen were obsessed with the quest for longevity. Some believed that if you found a magic formula it should be stored inside a clock, literally to hold back time.
- Seaweed-eating nations often have higher-than-average life expectancies. In Iceland, dried seaweed known as *sof* is credited with helping nationals to live, on an average, to 83, thanks to its fat-absorbing qualities.
- Your parents make a difference if you want to live to a grand age — genetic inheritance plays the most important part.

The Independent, London

Cell coat and recognition

All the components undergo active turnover and this is particularly evident in cultured cells that leave a kind of 'footprint' on the glass to which they adhere, writes tapan kumar maitra

ANIMAL cells are frequently covered by a coat or *glycocalyx*, which generally appears as a layer 10-20 nm thick in direct contact with the outer surface of the plasma membrane. The glycocalyx contains the *oligosaccharide* side chains of *glycolipids* and *glycoproteins*, which are exposed to the outer surface of the membrane.

It can be considered to be a secretion product of the cell, which is an integral part of its surface and is renewed continuously. All the components of the cell undergo active turnover, and some of these are shed into the surrounding medium. We shall see that this is particularly evident in cultured cells, which leave a kind of "footprint" on the glass to which they adhere.

The cell coat has many other functions in addition to that of protecting the plasma membrane. For example, it may operate as a filter in certain blood capillaries (eg. the kidney) and in connective tissue. It may provide the cell with a special micro-environment, have a particular electrical charge, pH, and ion concentration. Certain enzymes may be concentrated in the glycocalyx — for example, the fuzzy coat that covers the intestinal *microvilli* contains all the enzymes used in the terminal digestion of the carbohydrates. Molecular recognition is one of the main functions of the cell coat.

The number and position of the various *oligosaccharides* it contains constitute a molecular

code or special fingerprint for each cell type. The antigenic properties of a given cell are related to its molecular recognition properties — for example, blood groups are antigens with specific terminal oligosaccharides. Other important antigens are those of histo-compatibility, which permit the recognition of cells from the same organism and rejection of alien cells (eg. a graft).

Molecular recognition is particularly significant in the nervous system, where a neuron can establish contact with several other neurons to form circuits of immense complexity.

The classic experiment of HV Wilson (1908) illustrates, in a dramatic way, the phenomenon of cell-to-cell recognition. If living sponges of different species and colours are forced through a fine silk mesh, they disintegrate into the motile cells. If these cells are all mixed together, after some time cells of the same colour will reaggregate among themselves. It has also been discovered that when similar cells attach to each other they form aggregates that are characteristic for a given cell population (eg. retinal cell, kidney or bone). Thus, the reaggregation is not necessarily species-specific: if cells of chick and mouse embryos are mixed, they reaggregate according to the type of cell population, rather than the species. There is considerable experimental evidence that these cases of cell-to-cell recognition

are dependent on the carbohydrate composition and distribution in the cell coat.

Contact inhibition and cancer cells

Normal cells growing in tissue culture show contact inhibition of movement and growth. When they make contact with neighbouring cells, junctions are formed and there is a slowing down movement. The rate of cell division also decreases and there is a gradual inhibition of cell growth. Contact inhibition is apparently dependent on a signal that is propagated only by cell contact and is not effective at a distance.

Cancer cells behave quite differently. They continue to move and the mitotic rate is not inhibited; the cells tend to pile up, giving rise to multilayers. These effects, referred to as loss of contact inhibition, can be studied in normal cultured cells that have been transformed into cancerous cells by certain *oncogenic* (cancer-causing) viruses.

Cancer cells also undergo many changes in their surface properties. For example, gap junctions tend

to disappear and the concentration of *mucopolysaccharides* in the cell coat increases. Cancer cells are less well-regulated than normal cells and enzymes may leak into the medium. They may also carry new antigens in the cell surface that occur in cancer cells.

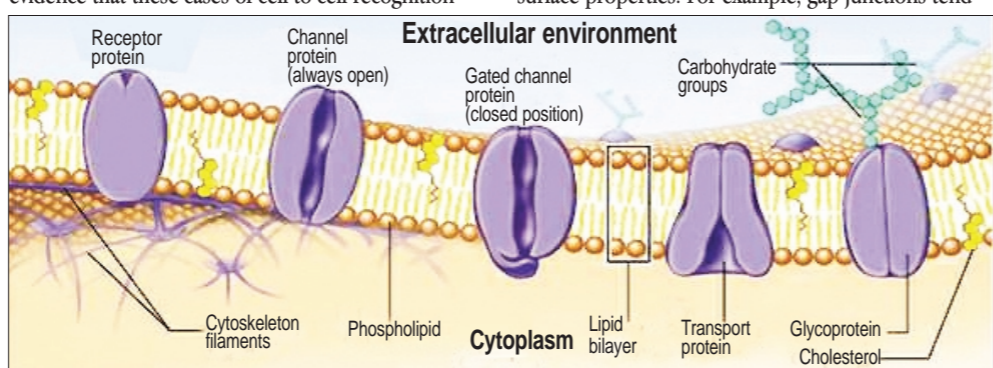
One of the major chemical components of the cell coat is *fibronectin*, a high molecular-weight glycoprotein that can be isolated from normal cultured fibroblasts by mild procedures, such as washing with a solution of urea. Together with other glycoproteins, fibronectin is found in the "footprints" that a moving cultured cell leaves on the substratum with which it comes into contact (a glass surface).

Fibronectin occurs widely in connective tissues, among others, and it thought to have a role in determining the distribution of cells within both embryonic and adult tissues. The presence of fibronectin increases cell adhesion to the substratum and to other cells and influences the morphology of the cell as well as induces locomotion and migration.

Fibronectin was originally called Large External Transformation-Sensitive) because it is absent or drastically reduced in cultured cells undergoing cancerous transformation. It is conceivable that the reduction of fibronectin, together with the uncoupling phenomenon mentioned above, could have adverse effects on the social behaviour of transformed cells. It has been observed that the addition of fibronectin to cultured transformed cells produces changes in their behavior, causing them to acquire a more normal appearance. For example, they attach more readily to the substrate, adopt a more flattened morphology and tend to align with one another in a monolayer.

The cytoskeleton of a transformed cell also becomes more organised under the influence of fibronectin.

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Courtesy: McGraw-Hill Companies, Inc.

Glyco-calyx— A coating or layer of molecules external to the cell wall. It serves protective, adhesive and receptor functions.

Bacterial chromosome or nucleoid— The site where the large DNA molecule is condensed into a packet. DNA is the code that directs all genetics and heredity of the cell.

Pilus — An elongate, hollow appendage used in transfers of DNA to other cells and in cell adhesion.

Mesosome — An extension of the cell membrane that folds into the cytoplasm and increases surface area.

Flagellum — Specialised appendage attached to the cell by a basal body that holds a long rotating filament. The movement pushes the cell forward and provides mobility.

