of these reactions was dose dependent, and increased after the booster immunization, so a second injection was not given to the highest-dose groups. In addition, lymphocytes — white blood cells of the immune system (which include T cells and B cells) — were reduced in number in most vaccinated individuals, but returned to normal 6–8 days after vaccination.

Vaccine-induced anti-RBD antibody levels were quantified at multiple time points. However, the latest time point assessed was at only two (Mulligan *et al.*) or three (Sahin *et al.*) weeks after the booster injection. All vaccinees developed low-level anti-RBD antibody responses after the first vaccination. As expected, the antibody levels depended on the vaccine dose, and they increased 10–15-fold after the booster. By three weeks after the booster, the antibody levels dropped. Antibody-mediated SARS-CoV-2 neutralization, as assessed by *in vitro* experiments, followed a similar pattern, and it also declined three weeks after the booster. This result stresses the importance of long-term follow-up to understand the durability of vaccine-induced immune responses. A decline in the response is expected over time, and such a follow-up is needed to determine the rapidity of this decline.

With the exception of the group who received the lowest vaccine dose, levels of neutralizing-antibodies against SARS-CoV-2 compared favourably with those in blood samples taken from people who had recovered from COVID-19 — commonly referred to as COVID-19 convalescent serum or plasma. Crucially, the magnitude and dynamics of the elicited antibody response indicate that a booster dose is essential for this vaccine.

Sahin and colleagues measured the responses of CD4 and CD8 T cells before the first vaccination and one week after the booster. Although most vaccinees showed convincing responses, the strength of the T-cell responses, as measured by the production of immune-system signalling molecules called cytokines, varied between participants, and there was no clear dose dependency in the responses.

In terms of what we have learnt from the results of these phase I/II clinical trials, the reactogenicity and early safety profile seem acceptable. However, it should be remembered that, as the authors acknowledge, this was a small group of individuals, and it was missing people from key age profiles and at-risk groups. The average age of the participants in the two trials was 35 and 37, respectively.

In another study[6], Pfizer and BioNTech reported a clinical trial that compared BNT162b1 with a different version of the vaccine, termed BNT162b2, that uses mRNA encoding the full-length spike protein. Among older adults, aged between 65 and 85, those vaccinated with BNT162b2 showed less systemic reactogenicity than did people vaccinated with BNT162b1. BNT162b2 was therefore selected to go forward to an ongoing phase II/III large-scale clinical trial[6].

So what do the data tell us about whether the vaccine generates immunity to COVID-19, and about the correlates of immune protection — the quality and quantity of vaccine-induced antibody and T-cell responses elicited? The results are encouraging but inconclusive. The presence of neutralizing antibodies is correlated with protection from SARS-CoV-2 infection in monkeys[7–9] (see pages 583, 572 and 578), and there are anecdotal reports for humans that are consistent with this[10]. However, a definitive interpretation of such data is complicated by the lack of standardized tests for assessing T-cell and neutralizing-antibody responses. Approaches to tackle this shortcoming are already being developed, for example by the SARS-CoV-2 Neutralization Assay Concordance Survey (go.nature.com/3iqh0jp), and the results should help to provide a way of comparing different vaccine candidates.

Taken together, the early clinical data for the Pfizer/BioNTech vaccine candidate hold promise, but many questions remain for this and other mRNA vaccines that target SARS-CoV-2. For example, what is the optimal dose, and what would be the best timing for a booster vaccination? How long does the vaccine-induced immune response last? Is the vaccine safe and effective in people with underlying health conditions, or those of minority-racial and -ethnic backgrounds, who are disproportionately affected by COVID-19? Whether the vaccine is safe in children should also be tested. In addition, there are logistical hurdles to consider when distributing and administering a vaccine that requires transport and storage at −80 °C. Above all, it needs to be established that the vaccine-elicited immune response prevents infection and disease.

Data to come from the ongoing large-scale phase II/III clinical trial — revealing efficacy and longer-term safety profiles — will be crucial for answering some of the remaining questions. This is especially important for pioneering RNA-based vaccines, such as BNT162b1 and BNT162b2, that lack the extensive safety record of vaccine candidates developed using a conventional approach.

The good news is that the final hurdle on the way to the finishing line — the completion of a properly controlled phase III clinical trial — is in sight. Ideally, this process will not be jeopardized by a premature rush, through an Emergency Use Authorization by the US Food and Drug Administration or other international regulators, to get a vaccine into use in the clinic before the trial has generated sufficient safety and efficacy information. As in any hurdle race, skill, speed and judgement are all needed to successfully and safely cross the finishing line.

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1. Krammer, F. *Nature* **586**, 516–527 (2020).
2. Jackson, L. A. *et al. N. Engl. J. Med.* https://doi.org/10.1056/NEJMoa2022483 (2020).
3. Mulligan, M. J. *et al. Nature* **586**, 589–593 (2020).
4. Sahin, U. *et al. Nature* **586**, 594–599 (2020).
5. Plotkin, S. A. *Clin. Vaccine Immunol.* **17**, 1055–1065 (2010).
6. Walsh, E. E. *et al. New Engl. J. Med* https://doi.org/10.1056/NEJMoa2027906 (2020).
7. Mercado, N. B. *et al. Nature* **586**, 583–588 (2020).
8. Yang, J. *et al. Nature* **586**, 572–577 (2020).
9. van Doremalen, N.*et al. Nature* **586**, 578–582 (2020).
10. Addetia, A. *et al. J. Clin. Microbiol.* https://doi.org/10.1128/JCM.02107-20 (2020).

Materials science

Tough lessons from diabolical beetles

Po-Yu Chen

Intriguing structures have been observed that link sections of the diabolical ironclad beetle's amazingly crush-resistant armour. These findings suggest fresh approaches for making tough, reliable joints for use in engineering. **See p.543**

The splendidly named diabolical ironclad beetle (*Phloeodes diabolicus*, Fig. 1) has an impressively tough exoskeleton — allowing it to survive attacks from predators, being stomped on by hikers and even being run over by cars. On page 543, Rivera *et al.*[1] reveal the secret of this beetle's crush resistance. Using a combination of advanced microscopy, mechanical testing and computer simulations, the authors find that layered, jigsaw-like joints and a variety of support structures connect the various parts of the exoskeleton, accounting for its toughness.

Natural materials, for example those found

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in bones, teeth and shells, often have exceptional mechanical performance, combining properties such as strength, toughness and self-healing capabilities in ways that cannot be achieved in conventional engineering materials[2,3]. These superior properties are partly due to the materials' hierarchical architectures: the constituents are assembled from repeating structures or patterns at several different size scales, ranging from the molecular to the macroscopic scale[4]. More importantly, the interfaces between building blocks at the various scales[5,6] lead to synergistic strengthening and toughening mechanisms[7]. Many efforts have therefore gone into developing hierarchically structured composite materials inspired by nature[8].

One such inspiration is the exoskeleton of the arthropod group of organisms, which includes insects and other jointed invertebrates. The arthropod exoskeleton is a multifunctional armour that consists of three main layers: an outermost, waterproof epicuticle; an underlying exocuticle; and an innermost endocuticle. The two inner cuticles provide protection and mechanical support for the organism.

In the cuticles, molecules of the polysaccharide α-chitin combine with proteins to form fibres, which assemble into a twisted 'helicoid' arrangement. The stacking of fibres in this twisted arrangement gives the cuticles a multilayered (laminated) microstructure. This structure is tough, energy-absorbent (that is, it can absorb the energy of impacts) and damage-tolerant, owing to its ability to deflect, twist and arrest crack propagation at the interfaces between layers[6,7]. But the intrinsic properties of chitinous cuticles are insufficient to explain the outstanding toughness of the exoskeleton of *P. diabolicus*.

Flying beetles have hardened forewings (elytra) to protect the underlying hindwings, which are used for flight. But *P. diabolicus* has lost its ability to fly, and its elytra are permanently locked together to provide protection from predators. Rivera *et al*. carried out macro-scale compression tests on entire exoskeletons of these beetles to investigate their toughness.

The authors found that these small insects (about 2 centimetres long) can withstand a maximum force of 149 newtons, which corresponds to a weight of approximately 15 kilograms. This is about 39,000 times the beetle's body weight, and roughly 10 times higher than the biting forces generated by potential predators. It is also substantially greater than the weight that could be withstood by the exoskeletons of three other terrestrial beetles tested in the researchers' experiments.

Rivera *et al*. then conducted a compositional analysis and microstructural characterization of the diabolical ironclad beetle's exoskeleton. This showed that the exoskeleton is protein-rich but does not contain inorganic minerals (as crustacean exoskeletons do), and that it has a considerably thicker endocuticle than do other insects. This thickness might aid the ability of the exoskeleton to absorb energy, but still cannot fully explain its toughness.

GETTY

Figure 1 | A crush-resistant insect. The diabolical ironclad beetle (*Phloeodes diabolicus*) has an exoskeleton that is so tough, the insect can survive being run over by a car.

Using an imaging technique called micro-computed tomography, Rivera and colleagues went on to observe some striking features at interfaces in the exoskeleton (Fig. 2): lateral supports at the interfaces between the elytra and the ventral cuticle (the shell on the underside of the beetle); and a rigid joint, known as a suture, that permanently fuses the two elytra together. There are three distinct types of lateral support, which are found in specific regions running from the front to the back of the exoskeleton. The authors describe the three types as interdigitated, latching and free-standing, on the basis of their interface geometries. Such variation in interfacial architectures is absent in other beetles, which have only interdigitated supports throughout their bodies.

Rivera *et al*. investigated the mechanical performance of the three distinct types of lateral support using compression tests and computational simulations. They observed that the interdigitated supports are the stiffest and strongest under compression. The latching supports are less so, allowing more deformation of the exoskeleton than do the interdigitated supports. Scanning electron microscopy revealed that the contacting surfaces in the latching supports are densely covered by micrometre-scale, rod-like protuberances, which might improve the frictional grip of the surfaces on each other. The free-standing supports lack a firm connection between the elytra and the ventral cuticle, thus allowing the two surfaces to slide easily past each other under compression.

The authors conclude that the strong and stiff interdigitated supports are used to protect the beetle's vital organs from being crushed, whereas the compliant latching and free-standing supports allow deformation of the exoskeleton, so that the beetle can squeeze into crevices in rocks or tree bark. Cockroaches have a similar shape-changing ability, which has inspired the design of a compressible robot that can squeeze into, and move within, tight spaces[9]. Such robots could be used to search for survivors in collapsed buildings after disasters. The functionally diverse support structures in *P. diabolicus* might now inspire new designs for compressible robots, or for armoured vehicles.

The hinges between the elytra of flying beetles typically have a 'tongue-and-groove' structure that facilitates repetitive opening and closing of the elytra during flight and landing[10]. By contrast, the suture between the elytra of *P. diabolicus* contains interlocked, jigsaw-shaped structures called blades (Fig. 2). Rivera and colleagues studied how the geometry, number and microstructural features of the blades affected the mechanical performance of the sutures, using a combination of computational simulations and mechanical testing of 3D-printed models of the sutures.

The authors report that sutures containing blades that have the ellipsoidal geometry found in *P. diabolicus* are tougher than are those that contain the hemispherical and triangular blades commonly observed in other terrestrial beetles. 3D-printed sutures consisting of two blades were the toughest, whereas those with five blades were stiffest and endured the highest forces before fracturing. Optimization of suture structures therefore involves a trade-off of toughness with stiffness and fracture resistance.

Finally, Rivera *et al*. fabricated jigsaw-shaped blades that had a laminated microstructure, mimicking that of the beetle's exoskeleton, and compared them with two types of blade that lack this microstructure. The beetle-inspired

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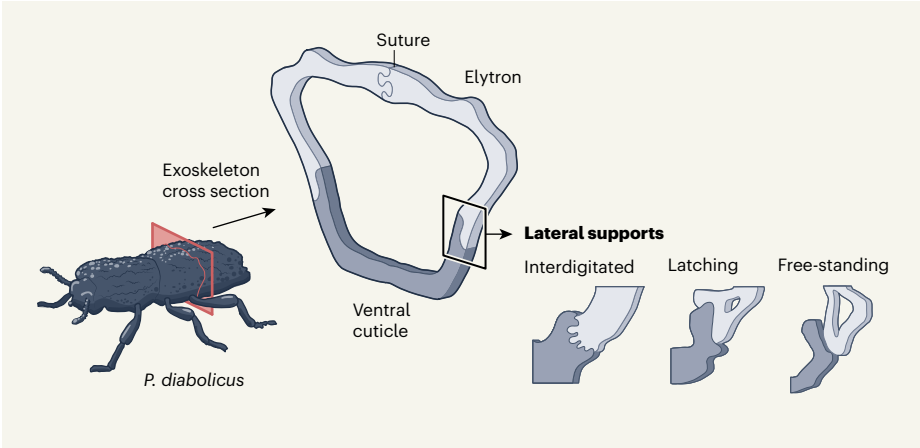

Figure 2 | Tough joints and interfaces. Rivera *et al.*[1] report that joints and supports in the exoskeleton of *Phloeodes diabolicus* help to account for the insect's remarkable crush resistance. A joint known as a suture locks together the beetle's hardened forewings (the elytra). The suture consists of interlocked, ellipsoidal structures called blades. This interlocked structure, combined with the layered microstructure of the blades (not shown), enhances the suture's toughness. Three types of lateral support connect the ventral cuticle to the elytra: interdigitated, latching and free-standing. The interdigitated joints are the stiffest and strongest under compression, whereas the latching and free-standing supports allow the exoskeleton to undergo some deformation when compressed.

laminated blades were significantly tougher than were the other two types, and absorbed more energy.

In engineering applications, commonly used joints between materials often fracture at their thinnest regions, where tensile stress is concentrated, leading to unpredictable and catastrophic failure[11]. By contrast, in Rivera and colleagues' bio-inspired blades, tensile stress results in localized delamination (separation of the layers); this causes the neck region of the blade to expand laterally, so that the interlocked blades grip each other even more tightly.

The laminated blades also fracture in a more predictable and gradual manner than do joints used in engineering, initially producing non-propagating, circumferential cracks. If the bio-inspired blades were used as jointing materials in practical applications, these cracks could be regularly inspected to pre-empt their final fracture, and would therefore be safer and more reliable than currently used jointing materials. However, further evaluation of Rivera and colleagues' laminated blades will be needed — for example, to characterize their properties under compression, bending and torsion, and to discover how they become fatigued over time — before applications can be considered.

The authors' work mainly focuses on mechanical performance at the submillimetre and the macroscopic scale, taking into account the behaviour of the interfacial structures and laminated microstructures of *P. diabolicus*'s elytra. However, the effects of structural features at lower hierarchical levels (smaller scales) in the elytra still need to be explored, using multi-scale modelling and experiments. Emerging methods[12] that use artificial intelligence and machine learning might accelerate the search for hierarchically structured materials, based on these elytra, that have superior mechanical properties for engineering compared with currently available materials.

In the meantime, Rivera and co-workers' approach — integrating advanced characterization methods with mechanical testing, simulations and 3D printing — provides a template for investigations of other remarkable natural materials that have complex architectures. And the authors' work demonstrates that you should never underestimate the capabilities of insects.

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1. Rivera, J. *et al. Nature* **586**, 543–548 (2020).
2. Meyers, M. A., McKittrick, J. & Chen, P.-Y. *Science* **339**, 773–779 (2013).
3. Chen, P.-Y., McKittrick, J. & Meyers, M. A. *Prog. Mater. Sci.* **57**, 1492–1704 (2012).
4. Naleway, S. E., Porter, M. M., McKittrick, J. & Meyers, M. A. *Adv. Mater.* **27**, 5455–5476 (2015).
5. Dunlop, J. W. C., Weinkamer, R. & Fratzl, P. *Mater. Today* **14**, 70–78 (2011).
6. Barthelat, F., Yin, Z. & Buehler, M. J. *Nature Rev. Mater.* **1**, 16007 (2016).
7. Huang, W. *et al. Adv. Mater.* **31**, 1901561 (2019).
8. Yaraghi, N. A. & Kisailus, D. *Annu. Rev. Phys. Chem.* **69**, 23–57 (2018).
9. Jayaram, K. & Full, R. J. *Proc. Natl Acad. Sci. USA* **113**, E950–E957 (2016).
10. Sun, J. & Bhushan, B. *RSC Adv.* **2**, 12606–12623 (2012).
11. Malik, I. A., Mirkhalaf, M. & Barthelat, F. *J. Mech. Phys. Solids* **102**, 224–238 (2017).
12. Gu, G. X., Chen, C.-T., Richmond, D. J. & Buehler, M. J. *Mater. Horiz.* **5**, 939–945 (2018).

Tumour biology

Seeds of cancer in normal skin

Inigo Martincorena

Sequencing the genomes of individual skin cells called melanocytes has revealed a rich landscape of DNA changes. These insights shed light on the origins of melanoma, an aggressive type of cancer. **See p.600**

Mutations occur in our cells throughout life. Although most mutations are harmless, they accumulate in number in our tissues as we age, and if they arise in key genes, they can alter cellular behaviour and set cells on a path towards cancer. There is also speculation that somatic mutations (those in non-reproductive tissues) might contribute to ageing and to diseases unrelated to cancer. However, technical difficulties in detecting the mutations present in a small number of cells, or even in single cells, have hampered research and limited progress in understanding the first steps in cancer development and the impact of somatic mutation on ageing and disease. Tang *et al.*[1] report work on page 600 that overcame some of these experimental limitations to explore somatic mutations and selection in individual melanocytes — the type of skin cell that can give rise to the cancer melanoma.

The epidermis is the skin's outermost layer. Just 0.1 millimetres thick, the epidermis is battered by mutation-promoting ultraviolet rays over a person's lifetime, and is the origin of the vast majority of skin cancers.

To understand the extent of somatic mutation in a human tissue, and the origin of skin cancers, a previous study[2] used DNA sequencing of small biopsies of normal epidermis. This revealed not only that mutations are common in normal cells, but also that mutations in cancer-promoting genes favour the growth

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