If an insect is injured, can it repair its skeleton in a manner which is mechanically strong and viable? Previous work has described the biological processes that occur during repair of insect cuticle, but until now, there has been no biomechanical assessment of the repaired area. We analysed the biomechanics of the injury repair process in the desert locust (*Schistocerca gregaria*). We show that after an incision, a healing process occurred which almost doubled the mechanical strength of locust tibial cuticle, restoring it to 66% of the original, intact strength. This repair process occurred by targeted cuticle deposition, stimulated by the presence of the injury. The cut surfaces remained unrepaired, but a patch of endocuticle was deposited, reinforcing the area and thus increasing the effective fracture toughness. The deposition rate of endocuticle inside the tibia increased fourfold compared with uninjured controls, but only on the dorsal side, where the incision was placed. The limb is highly loaded during jumping, so this partial restoration of strength will have a profound effect on the fitness of the insect. A finite-element model provided insights into the mechanics of the repair, predicting that the patch material reaches its ultimate strength before the fracture toughness of the existing cuticle is exceeded.

1. Introduction

The exoskeletal body parts of arthropods are made from cuticle: an acellular chitin/protein composite material, mainly comprising two histological layers: an outer layer of hard, stiff exocuticle and an inner layer of softer endocuticle. Cuticle is secreted by a cellular epidermis which is attached to a basal membrane. After molting, during the normal growth process, the new, soft exoskeleton quickly sclerotizes and hardens. From this point on, the overall thickness of the cuticle gradually increases for several weeks by deposition of further layers on the inner surface, orchestrated by the epidermal cells.

Insects and other arthropods respond to injury by a process not unlike that found in mammals. First, coagulation occurs and melanin is formed, giving rise to a clot that seals the wound and provides a scaffold for cell migration. Then, cells in the underlying epidermis migrate to restore epidermal continuity across the damaged area. Subsequently, these cells secrete new endocuticle on the inner surface [1–7]. Many mammalian tissue types, such as skin and bone, can be fully restored by tissue secretion and remodelling [8,9]. In adult insects, however, damage in sclerotized parts of the cuticle exoskeleton is not completely healed: the new endocuticle creates a patch and the clot remains, creating a kind of scar tissue at the wounded area [1,2]. No previous work has been done to investigate the mechanical strength of this construct. Our aims for the present work were

(a) To introduce damage in a form of an incision in the tibiae of adult locusts and measure the reduction in bending strength thus caused.
(b) To characterize the effectiveness of the repair process by measuring bending strength and toughness as a function of time after incision.
To measure cuticle deposition as a function of time, near the injury site and remote from it.

To investigate the mechanics of the repair process using a finite-element modelling approach.

2. Material and methods

Female adult desert locusts (Schistocerca gregaria) were kept in a controlled 12 h (35.8°C)/12 h (20°C) day/night cycle and fed with fresh plants and dried cereals ad libitum. Thirty-two insects were randomly selected to receive injuries; a further 64 were used as controls. Injuries were applied 30 days after the final moult. Using a scalpel a transverse incision was made, perpendicular to the tibial axis on the dorsal side of the hind tibia (approx. 5 mm distal to the tibiofemoral joint) such that the epidermis was breached (figure 1a). The depth of the cut was controlled by using a purpose-built slotted guide. All subjects received the same size injury: average length $L = 780 \pm 60 \text{ mm}$ (s.d. $n = 32$; figure 1a). Trial and error was used to find an ‘ideal’ cut length—that is, an incision deep enough to breach the epidermis but shallow enough, so that it would not extend further during normal locomotion.

After incision, the locusts were separated into individual small tubs (approx. $20 \times 1 \times 10 \text{ cm}$), maintaining the same environmental and feeding conditions. These tubs were large enough to comfortably allow the insect to be fully mobile, and while jumping was not eliminated entirely, separating the locusts from one another discouraged the excessive use of the hind tibia (for jumping, kicking, and fighting) often observed when they are housed together. After a recovery period of between 1 and 50 days, the injured legs were removed from the insects under sedation by cutting just below the tibiofemoral joint. Immediately after removal, legs were set in a well of polymer cement (Howmedica® Surgical Simplex P Bone Cement mixed at a 1:1 ratio for rapid hardening). Cantilever bending tests were performed by applying a position-controlled load to failure. Displacement was gradually increased at a rate of $5 \text{ mm min}^{-1}$ using a mechanical testing machine (Zwick/Roell Z005, Ulm, Germany), with the purpose-built testing rig shown in figure 1b. Failure was characterized by a sudden drop in load (figure 1c) and visual fracture at the incision point (figure 1b). After testing, the samples were stored in a 3.7% glutaraldehyde fixative for 24 h, after which they were preserved in 70% ethanol solution.

Images were taken of the fracture surfaces of each sample using a Zeiss Ultra Plus scanning electron microscope (5 kV; Oberkochen, Germany). Cuticle dimensions (radius and thickness at various locations around the circumference, cut length and cut depth) were measured using FIJI software (an open source image processing package based on IMAGEJ). Control subjects received no incision but were otherwise treated in the same manner; they were tested at times varying from 3 to 63 days post-moult.

Standard engineering formulae were used to calculate strength and toughness, as follows. The nominal stress to failure $\sigma_f$ was calculated using

$$\sigma_f = \frac{Fx}{I},$$

where $F$ is the applied force at failure, $x$ is the distance from the failure location to the loading point, $r$ is the average outer radius of the tibia and $I$ is the second moment of area of the cross section, which is given (assuming a circular section of radius $r$)
Fracture toughness $K_c$ was calculated using

$$K_c = Q \sigma_f \sqrt{m},$$

(2.3)

where $a$ is the half-length of the cut measured around the circumference and $Q$ is a factor which depends on $a$, $r$, and $t$ (given by Takahashi [10]). Individual measurements of radius, thickness and cut length were taken for each sample. These were used to calculate the failure strength for each test, a unique shape factor $Q$ for each injured leg, and hence the fracture toughness for each sample.

2.1. Finite-element modelling

We created computer models using ANSYS finite-element software. The aim was not to reproduce the exact geometry of the tibia, but rather to gain insights through a simplified model in the form of a circular tube (figure 2) having dimensions similar to the average values found experimentally: radius 440 μm, thickness 100 μm with a slot of length $L = 780$ μm located a distance 2 mm from the fixed end. The width of the slot was varied from 10 to 100 μm, and we also created a slot which more closely modelled the wedge-like shape of the scalpel used to make the cut. Repair was simulated by depositing a layer of material, varying from 20 to 100 μm, on the inside surface of the tube. All material was assumed to be linear-elastic and isotropic, with a Young’s modulus of 8 GPa and a Poisson’s ratio of 0.3.

Testing was simulated by applying a force to create cantilever bending. An appropriate size for the mesh elements was chosen using critical distance theory [11] that allows one to estimate the appropriate length scale on which to study material fracture processes. Given a material tensile strength $\sigma_o$ and fracture toughness $K_c$, the critical distance $G$ is found by

$$G = \frac{\pi}{\pi} \frac{(K_c)^2}{\sigma_o^2}$$

(2.4)

Using the experimental values for fracture toughness and nominal strength reported below gives $\Gamma = 0.101$ mm, so we used a mesh element size of 0.1 mm throughout the model. Stress intensity ($K$) values were calculated using a standard approach (described in [12]) in which the length of the crack is extended by a small amount $\delta a$ and the change in deformation of the sample under load is used to calculate the change in stored...
strain energy in the body, $\delta W$. $K$ can then be found by

$$K = \left(\frac{E}{7} \frac{\delta W}{\delta a}\right)^{1/2}. \quad (2.5)$$

3. Results and discussion

Our tests on control subjects that had not received any incisions allowed us to establish normal baseline rates for cuticle formation in the absence of injury. We found that, in adults following their final moult, cuticle was deposited at an average rate of 1.8 $\mu$m d$^{-1}$ (s.d. = 2.4, $n = 24$) in an initial modelling phase which lasted on average 21 days. This rate is in agreement with the results of previous work [13], which examined growth up to 14 days. After 21 days, we found that the deposition rate decreased considerably, to 0.4 $\mu$m d$^{-1}$ (s.d. = 0.4, $n = 40$). Therefore, for our injury experiments, we used subjects which were 30 days post-moult and thus in a relatively dormant state as regards cuticle deposition.

Figure 3a shows results of cuticle thickness as a function of time post-injury. Cuticle thickness was measured from the fracture surfaces, at the following locations (figure 1a): T1 (dorsal side, at the incision); T2 (ventral side, opposite the incision); and T3 (medial and lateral sides, for which results were averaged as no differences were found). Cuticle thickness increased considerably with time at the injury site (T1) but only slightly elsewhere. Regression analysis showed that the increase in thickness over time at T1 is statistically significant ($p = 0.0061$), whereas the changes in T2 and T3 are not significant ($p = 0.077$ and 0.216, respectively). As figure 3b shows, the rate of deposition near the injury (1.6 $\mu$m d$^{-1}$; s.d. = 1.3, $n = 13$) was similar to that found in uninjured control subjects immediately post-moult, but much greater than in controls of the same age, i.e. adults more than 20 days after the moult. However, the measured deposition rates remote from the injury site, on the other surfaces (T2 and T3) remained low after injury, comparable to uninjured controls of the same age (average 0.39 $\mu$m d$^{-1}$; s.d. = 0.41, $n = 40$). As a result of 20–50 days of repair, the cuticle thickness on the dorsal side increased to 146 $\mu$m (s.d. = 36 $\mu$m, $n = 9$), becoming significantly thicker than the ventral side (109 $\mu$m, s.d. = 11 $\mu$m, $n = 9$, $t$-test $p = 0.003$). This is in marked contrast to uninjured controls in which the dorsal and ventral sides developed the same thickness ($T1 = 90 \mu$m (s.d. = 10 $\mu$m, $n = 40$), $T2 = 97 \mu$m (s.d. = 10 $\mu$m, $n = 40$)). The lateral/medial thickness T3 was similar in all cases (injured insects T3 = 82 $\mu$m (s.d. = 16 $\mu$m, $n = 18$); controls T3 = 73 $\mu$m (s.d. = 9 $\mu$m, $n = 80$)).

The above results imply the existence of a repair process of cuticle deposition which is targeted to the damaged area. However, this repair process did not always occur. From a total of 32 subjects tested, repair (which we defined as visible deposition of cuticle at the incision site—figure 4) occurred in only 15 subjects. Of the others, there was no deposition present in 12 cases; in the remaining four cases, it was unclear from the scanning electron microscopy (SEM) observations whether deposition had occurred or not so these samples were discarded. A possible explanation for non-repair in these subjects is that our scalpel cut may sometimes have caused a relatively wide incision, and/or a relative displacement of the cut surfaces, preventing the recovery of a continuous epidermal layer (figure 4). As mentioned above, the recovery of the epidermis is an essential first step, without which the cascade of repair activities culminating in cuticle secretion cannot occur. The results shown in figure 3 refer only to the cases where repair occurred.

To compare mechanical behaviour, we followed procedures established in our previous work [14]. Bending strength was defined as the nominal stress to failure, which is the maximum tensile stress that would occur, at the failure location, in an undamaged circular tube having the same average diameter and thickness as measured for a given sample. We also calculated an apparent fracture toughness. Fracture toughness, $K_F$, is a measure of the ease with which a crack can propagate in a material. Here, we calculated $K_F$ from the bending strength, assuming the incision to be a sharp crack of the same size in a circular tube [10,14]. As figure 5 shows, both strength and toughness approximately doubled as a result of repair. Uninjured controls of the same age had a strength of 172.4 MPa (s.d. = 31.5 MPa, $n = 40$). In subjects where repair did not occur, the incision lowered the average strength to 54.3 MPa (s.d. = 19.1 MPa, $n = 12$) and this was unchanged over time. In subjects where repair occurred, however, strength rose over the first 10 days and then remained approximately constant at an average of 113.7 MPa (s.d. = 16.4 MPa, $n = 15$), which is 66% of the original, uninjured strength. This increase in the nominal stress to failure was reflected in a similar increase in the bending moment to failure, from 2.50 N mm (s.d. =

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Figure 3. (a) Cuticle thickness as a function of time post-injury for the 15 subjects in which repair occurred; (b) cuticle deposition rate post-injury at the injury location (T1) and elsewhere (T2, T3), compared with uninjured controls in the immediate post-moult modelling period (less than 20 days) and the later dormant period (more than 20 days). (Online version in colour.)
Figure 4. SEM photos of repaired and non-repaired cuticle. Panels (a) and (b) show the fracture surface of a tibia that has received an incision (dashed line) and subsequently repaired the area by depositing cuticle (enhanced on the photo by red shading). At high magnification (b), there is a clear difference between the scalpel-cut surface and the fracture surface of the new cuticle. Panels (c) and (d) show the fracture surface of a tibia in which repair did not occur. Panels (e) and (f) are longitudinal sections of tibiae 30 days after receiving incisions. In (e), the incision (dashed line, arrowed) caused a relative displacement of the two sides of the cuticle, which are indicated by white lines. No repair occurred. In (f), there was no such displacement and new cuticle formed (red area) to repair the injury. (Online version in colour.)
1.63 N mm, \( n = 12 \) to 4.71 N mm (s.d. = 0.52 mm, \( n = 15 \)). The tibia will experience high bending moments in vivo while walking, and is loaded almost exclusively in bending during activities such as kicking and jumping [15,16].

In previous work [14], we measured a fracture toughness for uninjured cuticle using the same protocol, finding a value of 4.12 MPa m\(^{-1}\) (s.d. = 0.4, \( n = 9 \)) for fresh, fully hydrated cuticle and 2.06 MPa m\(^{-1}\) (s.d. = 0.6, \( n = 9 \)) for dried cuticle. In this study, we found a value of 3.07 MPa m\(^{-1}\) (s.d. = 0.99, \( n = 12 \)) in cases where no repair occurred (figure 5), which falls between the above two values, probably because a certain amount of dehydration took place in the area around the incision. Repair increased the measured toughness more than twofold, to 7.01 MPa m\(^{-1}\) (s.d. = 1.32, \( n = 15 \)). This was not due to an increase in the toughness of the material itself: it indicates the effect of the newly deposited cuticle in hindering crack propagation by bridging the crack, thus acting as a patch that reduces the local concentration of stress along the edge of the incision. All of these strength and toughness values displayed statistically significant differences from each other (t-test, \( p < 0.05 \)).

The original cut surfaces were still clearly visible after failure (figure 4a–d), demonstrating that cuticle is not being deposited directly across the incision. This must inevitably limit the strength of the repaired area, because the original cut will still remain and act as a stress concentrator. However, it is clear that the deposition of the cuticle patch goes far beyond what would be needed to simply seal the wound and prevent ingress or egress of fluid. Continuing for more than 20 days post-injury and considerably increasing the local thickness, this cuticle clearly plays a mechanical role. This can be quantified by comparing the resulting strength of the limb to the bending stress that arises in it during jumping, which was estimated [15] to be 42 MPa. Defining a safety factor as the ratio of strength to applied stress, our incision reduced the safety factor to 1.3, making fracture during jumping highly probable. After repair, the safety factor rose to 2.7, so even though this is less than the original intact strength (safety factor 4.1), it is clear that it will greatly reduce the probability of failure in vivo.

Our finite-element model gave a result for the fracture toughness of unrepaired cuticle (using equation (2.5)) which was comparable to that found above using the formula from Takahashi (in equation (2.3)). The finite-element result was 26% lower: this difference may have been due to a different assumption about the crack front, which Takahashi assumed to be parallel to the tube radius rather than horizontal. This result was not affected by the choice of slot width. Using our model, we were able to estimate the increase in effective fracture toughness as a result of the newly deposited endocuticle. For a new layer thickness of 20 \( \mu \)m, which is comparable to the average measured value (19 \( \mu \)m; s.d. = 1.6 \( \mu \)m, \( n = 21 \)), we estimated that measured toughness would increase by a factor of \( \times 3.3 \), somewhat larger than the experimental increase which was \( \times 2.3 \). Using a thicker endocuticle layer of 100 \( \mu \)m, comparable to the maximum value measured experimentally (110 \( \mu \)m) gave a predicted toughness increase of \( \times 7.7 \). One reason why these predicted increases are higher than the experimental value is that the stress in the cuticle patch may become very large before the critical stress intensity is reached (figure 2c). In our model, the maximum stress in the patch was not affected by the thickness of the patch material but was found to be inversely proportional to the width of the slot. This is because the patch material that spans the slot is loaded by the opening displacement of the slot faces: if we make the slot wider this will not affect the amount by which the slot opens under load, but it will affect the amount of material spanning the slot, and thus the strain that arises in this material. In our most accurate model of the slot, based on measurements of the scalpel used, we found that for an applied stress intensity equal to 3.07 MPa m\(^{-1}\) (the material’s fracture toughness), the maximum stress in the patch material was 99 MPa, whereas at the higher stress intensity of 7.01 MPa m\(^{-1}\) (when failure occurred in the repaired samples), this stress was 226 MPa. These values are comparable to the measured strength of cuticle (172.4 MPa), implying that failure in the repaired samples is occurring not because a critical stress intensity has been reached but, rather, because we have reached the strength of the material in the patch, causing the patch to break and thus no longer protect the cut area.

This is the first ever biomechanical study of injury repair in an arthropod. By contrast, the literature on this subject for mammalian tissues is extensive, demonstrating the importance to mammals of the ability to create repairs which are mechanically strong. For example, cracked or broken bones can be completely repaired and remodelled to the point where they are morphologically and mechanically identical to uninjured bones [9]. However, in some mammalian tissues, the result of healing may be a material that is structurally different but functionally satisfactory [8], and in tissues such as tooth enamel and articular cartilage, repair is absent or limited, presenting major problems for the
continued functionality of these body parts. We have shown that an effective system exists to repair the locust leg in a targeted manner. An incision large enough to breach the epidermis can stimulate the resurrection of a dormant modelling process, which might be the same as that used for cuticle growth immediately post-moult. However, following injury, this process is now confined to the injured area, placing new cuticle where it can be most effective to improve strength, significantly increasing the ratio between strength and \( \text{in vivo} \) stress. However, we also found that this only occurred in about half of the injured subjects, and we suspect that this is related to the type and severity of the injury, providing a stimulus for further work.

Data accessibility. All original data can be made available on request: please contact David Taylor at dtaylor@tcd.ie.

Authors’ contributions. E.P. carried out the experimental work and analysis. J.-H.D. advised on experimental protocols and biological interpretations. D.T. supervised the overall project, assisted in data analysis and prepared the manuscript. All authors discussed the results and commented on the manuscript.

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