

Design Strategies of the Mantis Shrimp Spike: How the Crustacean Cuticle Became a Remarkable Biological Harpoon?

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Abstract

Spearing mantis shrimps are aggressive crustaceans using specialized appendages with sharp spikes to capture fishes with a fast movement. Each spike is a biological tool that have to combine high toughness, as required by the initial impact with the victim, with high stiffness and strength, to ensure sufficient penetration while avoid breaking. We performed a multimodal analysis to uncover the design strategies of this harpoon based on chitin. We found that the spike is a slightly hooked hollow beam with the outer surface decorated by serrations and grooves to enhance cutting and interlocking. The cuticle of the spike resembles a multilayer composite: an outer heavily mineralized, stiff and hard region (with average indentation modulus and hardness of 68 and 3 GPa), providing high resistance to contact stresses, is combined with a less mineralized region, which occupies a large fraction of the cuticle (up to 50%) and features parallel fibers oriented longitudinally, enhancing stiffness and strength. A central finding of our work is the presence of a tiny interphase (less than 10 μm in width) based on helical fibers and showing a spatial modulation in mechanical properties, which has the critical task to integrate the stiff but brittle outer layer with the more compliant highly anisotropic parallel fiber region. We highlighted the remarkable ability of this helicoidal region to stop nanoindentation-induced cracks. Using three-dimensional multimaterial printing to prototype spike-inspired composites, we showed how the observed construction principles can not only hamper damage propagation between highly dissimilar layers (resulting in composites with the helical interphase absorbing 50% more energy than without it) but can also enhance resistance to puncture (25% increase in the force required to penetrate the composites with a blunt tool). Such findings may provide guidelines to design lightweight harpoons relying on environmentally friendly and recyclable building blocks.

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ABSTRACT

Spearing mantis shrimps are aggressive crustaceans using specialized appendages with sharp spikes to capture fishes with a fast movement. Each spike is a biological tool that have to combine high toughness, as required by the initial impact with the victim, with high stiffness and strength, to ensure sufficient penetration while avoid breaking. We performed a multimodal analysis to uncover the design strategies of this harpoon based on chitin. We found that the spike is a slightly hooked hollow beam with the outer surface decorated by serrations and grooves to enhance cutting and interlocking. The cuticle of the spike resembles a multilayer composite: an outer heavily mineralized, stiff and hard region (with average indentation modulus and hardness of 68 and 3 GPa), providing high resistance to contact stresses, is combined with a less mineralized region, which occupies a large fraction of the cuticle (up to 50%) and features parallel fibers oriented longitudinally, enhancing stiffness and strength. A central finding of our work is the presence of a tiny interphase (less than 10 μm in width) based on helical fibers and showing a spatial modulation in mechanical properties, which has the critical task to integrate the stiff but brittle outer layer with the more compliant highly anisotropic parallel fiber region. We highlighted the remarkable ability of this helicoidal region to stop nanoindentation-induced cracks. Using three-dimensional multimaterial printing to prototype spike-inspired composites, we showed how the observed construction principles can not only hamper damage propagation between highly dissimilar layers (resulting in composites with the helical interphase absorbing 50% more energy than without it) but can also enhance resistance to puncture (25% increase in the force required to penetrate the composites with a blunt tool). Such findings may provide guidelines to design lightweight harpoons relying on environmentally friendly and recyclable building blocks.

Introduction

The arthropod cuticle is one of the most remarkable and versatile biological material ^[1]. Not only it provides protection to all body parts, but it also forms highly sophisticated appendices, dedicated to specific functions. The cuticle enables basic tasks such as physiological exchanges, sensing and actuation^[2–4], walking, climbing and adhesion on different surfaces ^[5], swimming and flying^[6]. Food treatment (e.g. through mandibles and stomach teeth) ^[7], mechanical^[8,9] and optical shielding^[10], as well as puncturing and capturing^[11] are additional ingenious functions performed with the cuticle. It is remarkable that such multifunctionality is obtained thanks to a tiny and lightweight biological structure, formed by the epidermis, renewed periodically by the moult process, and essentially comprising a thin superficial waterproof layer (the epicuticle) made of a lipid-protein matrix, and a thicker internal fibrous layer (the procuticle), generally subdivided into exo- and endocuticle depending on secretion time ^[12,13]. The basic building blocks of the procuticle are the chitin-protein microfibers, composed of a crystalline α -chitin core which is coated by a helical sheath of protein

units [14–17]. Matrix proteins are also found outside the microfibers when considering higher structural organization. The chitin-protein microfibers interact with a (partially) proteinaceous and hydrated matrix, which features a rich variety of proteins, enabling selective cross-linking [17–19]. The relative fraction of protein and matrix is not well known for the mantis shrimp and may depend on the considered region. Previous data on decalcified crab cuticle indicates that chitin content should range between 50 to 75% dry weight [20]. In areas of the cuticle where hardness and wear resistance are major requirements, the chitin-protein biocomposite is reinforced either by mineral incorporation in the matrix or by sclerotization that cross-link the matrix, sometimes with the help of transition metals (e.g. Mn, Zn, Fe) as seen in insect mandibles [21] and spider fangs [22]. In the procuticle, the chitin-protein microfibers assemble in larger bundles to form fibers and, in turn, the fibers form higher order structural motifs: arrays of locally aligned fibers are assembled into beds, and several beds are stacked into a three-dimensional helicoidal twisted plywood structure, resembling a man-made laminate, but at the nano/micro length scales [23,24]. The regular rotation of the fibers in horizontal beds gives rise to a periodic lamellate aspect, each lamella corresponding to a rotation of 180°. The composition of the matrix, the type of sclerotization, the relative fraction of reinforcing minerals, together with the tunable fiber organization and lamellar thickness are at the basis of the outstanding versatility of the arthropod cuticle and, consequently, they are key elements of the evolutionary success of arthropods in a variety of very dissimilar environments [24,25].

An instructive example of how the cuticle has evolved into highly specialized tools, even within the same organism, is found in the stomatopod crustaceans (or mantis shrimps). During their evolution, these marine animals modified their cuticle for feeding, hunting and defense purposes [9]. Stomatopods are traditionally subdivided into two branches based on the structure of their anterior appendages [26–28]: the well-known “smashers”, that use a hammer-like club to destroy hard-shell preys, and the less-famous “spearers” (**Figure 1 A**) that have a harpoon-like appendage to impale and grasp their (generally) soft-body preys. Thanks to an efficient “amplification system”, which includes a dedicated area (the saddle) to store and release elastic energy [29], together with an ingenious system of articulation [30], stomatopods can deploy their anterior appendages at impressive velocities (up to 6 m/s for the spearers and to 23 m/s for the smashers) [31]. Repetitive impacts at such velocities require excellent damage tolerance, and previous investigations have revealed that the hammer-club of the smashers shows numerous strategies to cope with damage [28,32–34]. Macroscopically, the size of the club is smaller than the critical radius at which, according to contact mechanics, the damage response switches from quasi-plastic to brittle, thus hampering catastrophic failure [33]. Microscopically, the club presents three different regions solving different mechanical functions: a heavily mineralized impact region characterized by mineral gradients [32,35] and by oriented fluorapatite (FAP) crystals perpendicular to the outer surface to enhance impact resistance; beneath the impact region there is a less mineralized periodic region showing the common helicoidal twisted plywood pattern, which may dissipate possible cracks generated during impact [32]; on the lateral sides, the periodic region is encircled by a striated region with chitin fibers well aligned along a preferential orientation [32]. This belt-like area is believed to provide later confinement to the periodic region, hence preventing high tensile stresses and the associated tensile cracking [28].

Although deployed at smaller velocities, the anterior appendage of the spearer is not less fascinating. It is a biological tool solving multiple functions: its base presents a flat surface used to hammer the opponents and sharing some of the construction strategies seen in the smasher [28,35]. On the top, it is decorated by several spikes (**Figure 1B**) which, in analogy to man-made harpoons, have to penetrate and grab preys within a fraction of second during a high-speed capturing event [31]. This task requires the combination of conflicting mechanical requirements: the spike must be tough to cope with the initial impact with the victim, it must also be stiff to avoid large deflections while penetrating a moving target and it must be strong to prevent rupture and prey lost. These constraints should come together with additional strategies to retain the prey. By comparing the spike cuticle of spearing limbs with different body parts (i.e., cephalic shield and abdominal tergites) we revealed some compositional and microstructural modifications of the cuticle in the spike. These include: (i) the replacement of the leathery inner epicuticle (commonly having an organic nature) by a highly mineralized exocuticle reinforced by FAP, and (ii) the development of a unidirectional fiber region

interrupting the classical helicoidal twisted plywood structure in the less mineralized endocuticle^[36]. Here, we investigate the spike in more details and at multiple length scales using micro-computed tomography (micro-CT), backscattered and secondary electron imaging based on scanning electron microscopy (BSE-SEM and SE-SEM), elemental analysis by energy dispersive spectroscopy (EDS), confocal Raman spectroscopy, depth sensing nanoindentation and multimaterial 3D printing. The central aim of our work is to elucidate the structure-function relationship and the mechanical behavior of the spike, highlighting the morphological, compositional, and microstructural adaptations allowing this biological tool, which is built very quickly, to be an effective natural harpoon.

Results and Discussion

Figure 1: Morphology and biomechanics of a spike from the spearing mantis shrimp *L. maculata*. (A) Photograph of a spec

Morphology and biomechanics of the entire spike

The raptorial appendage of the spearer zebra mantis shrimp *Lysiosquillina maculata* (Figure 1A) features 10 spikes of increasing size, regularly arranged along the dactyl (Figure 1B). The morphology and the external surface of distal spikes were characterized by high resolution micro-CT and SEM. Each distal spike is a tapered, thin and elongated offensive weapon (Figure 1C). In contrast to the straight needle-like shape common to many man-made harpoons^[11], it has a curved form and an elliptical cross-section (Figure 1D). The local curvature of the spike varies along its length such that 3 regions can be identified: a slightly curved zone close to the base, a fairly straight segment in the middle and a region with the highest curvature before reaching the tip (Figure 1D, the length of the arrows is proportional to the local curvature k). This allows the spike to orient its sharp end at an almost right angle with respect to the dactyl within a short distance, while avoiding a large angle at the base, which may cause stress localization upon bending. A curved rather than a straight profile may be linked to the curved trajectory of the appendage during a strike^[31] and could as well facilitate the parking of the spikes within dedicated holes in the propodus at resting position (**Figure S1**). In nature, the shape of puncturing systems is highly diverse and closely connected with their function^[11]. A straight offensive tool such as the harpoon of the cone snail^[37], the bee stinger^[38] or the mosquito proboscis^[39] may be used to maximize penetration depth^[11], whereas highly curved devices like crustaceans claws^[40] or spider fangs^[41] should enable puncture along different trajectories and facilitate prey retention^[35,37]. The unusual form of the spike, featuring both curved and straight zones, may be a trade-off between large penetration depth and prey grabbing. Micro-CT imaging of the spike cross-section reveals a thin highly mineralized cover bordering a less mineralized but much thicker layer with a central cavity (Figure 1D). This dual mineralization is a specific reinforcement feature of raptorial appendages used for impaling or smashing preys as well as for fighting with opponents^[28,32,34–36]. In general, the electron dense region is particularly pronounced on the impact surface and much less in other region of the cuticle^[9,42]. After a heavily mineralized and sharp tip, characterized by a radius of curvature of about 20 μm , which should facilitate puncture into the tough skin of the preys, the spike exhibits a spatially varying geometry. The cross-section has an ellipsoidal profile and the cross-sectional area increases gradually from tip to base, always having a pronounced eccentricity (**Figure S2**). An additional peculiarity is a small rotation (of about 15°) of the cross section around the longitudinal axis of the spike (insert in Figure 1D), resulting in a slightly twisted shape. From a biomechanical viewpoint, such a slender and eccentric hollow beam may suffer from low bending and torsional rigidity, thus questioning the ability to remain straight during a capturing event. We evaluate how cross sectional properties evolve along the longitudinal length of the spike from tip to base and we estimate the consequences for bending and torsional resistance using second area and polar moments as surrogate for mechanical properties (Figure 1F). Both maximum and minimum second moments of area (I_{max} and I_{min}), corresponding approximately to dorso-ventral and lateral loading, increase proximally and show a steeper slope starting at 90% of the spike length, where the spike begins to merge with the appendage. The polar moment J (a proxy for torsional rigidity) follows a similar trend, being bounded by I_{min} and I_{max} . Despite a fairly high eccentricity, the ratio $I_{\text{max}}/I_{\text{min}}$ suggests only a moderate mechanical anisotropy, with resistance to dorso-ventral loading (i.e., parallel to the prevalent hunting movement of the

raptorial appendage) being in average 3.3 times higher than lateral loading. Additionally, the ratio I_{\max}/I_{\min} is fairly constant for about 60% of the spike length. Although simplified, this analysis suggests that the spike is not only very stiff along the longitudinal direction but it can also face multiaxial loading, consistent with the assumed ability of the spearing mantis shrimps to control and direct the strike^[31].

The outer surface of the spike is decorated with two reinforcing serrated ridges, running all the way from tip to base (Figure 1E). Such “shark fin-shaped” serrations are part of the highly mineralized layer; with the exception of a zone close to the tip, they have fairly constant dimensions (i.e., $\sim 100\ \mu\text{m}$ in height and $\sim 55\ \mu\text{m}$ in length, **Figure S3**) in the first third of the spike, while their size decreases when approaching the base, suggesting that only a portion of the spike (closer to the tip) is engaged to impale preys. Serrations are prominent features in many diverse biological cutting tools, ranging from teeth to insect stingers^[43], and could have a dual function: they should help to cut through the tissue of the prey by acting as local stress concentrators and, owing to their specific shape, they may prevent the prey from slipping off the spikes by interlocking with the damaged tissue^[11]. In addition to the serrations, the surface of the spikes features a characteristic “grooved” pattern (Figure 1E and Figure S3). The grooves have a periodic spiral arrangement, starting and ending at two opposite serrated ridges, and have dimensions smaller than the serrations (i.e., $\sim 5\ \mu\text{m}$ in height and $\sim 35\ \mu\text{m}$ in width, Figure S3). The grooves should provide the spike surface with a controlled roughness which may increase frictional forces as an additional strategy at a smaller length scale to enhance prey retention. From a biological viewpoint, grooves should reflect the specific growth pattern of the spike outer layer, regulated by the epidermis cells which are arranged into parallel arrays.

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Figure 2: Microstructure of the spike. (A) Representative polished transversal section imaged in BSE-SEM accompanied on

Microstructure

The microstructure of the spike, observed with SEM on fractured and polished samples, features four regions with distinct fiber architecture (**Figures 2 A, B** and summary scheme in I). The first innermost layer, named inner helicoidal region (IHR, Figure 2C), belongs to the endocuticle^[36] and displays a twisted plywood organization consisting of numerous lamellae gradually increasing in thickness from about 1.5 to 10 μm when moving outwards. Within each lamella, layered beds of chitin-protein fibers embedded into a protein-mineral matrix are stacked with regular changes in fiber directions between each bed, resulting into stepwise and rough fracture surface (Figure 2F). Pore canals, pervading the cuticle up to the epicuticle, are visible at high magnification SEM and have a characteristic twisted-ribbon shape^[44] with unmineralized dark lumens (Figure 2C). They serve for the secretion of the cuticle and perhaps provide supplementary “waterproof” capability, as inferred by their lipid content^[36]. In the second layer, referred as the striated region of the endocuticle (STR), the helicoidal pattern is replaced by highly aligned fibers parallel to the long axis of the spike as highlighted by a longitudinally fractured sample showing fiber sheets (oriented vertically in Figure 2G). In this region, the pore canals lose their typical twisted-ribbon aspect and cross perpendicularly the horizontal fiber beds, giving a characteristic striated pattern typically observed in polished (transverse) sections, where the canal lumen appears dark and the mineralized fibrous sheath appears bright (Figure 2D). While the twisted plywood motif is universal in arthropods cuticle, the parallel-fiber organization is very unusual^[45]. In stomatopods, striated regions with parallel longitudinal fibers have been found only in specific locations including the dactyl club of the smasher^[32], the impact area of the dactyl in the appendage carrying the spikes of the spearer^[28] and the back defensive spikes located at the uropods (posterior structures found in crustaceans)^[42]. One common feature of those locations is a challenging biomechanical environment, solving critical offensive or defensive tasks. Most interesting, sandwiched between the striated and the external region, there is an additional layer of the endocuticle^[36] presenting a twisted plywood organization (Figure 2E), called outer helicoidal region (OHR). This location has two unusual features: it is composed of a very limited number of lamellae, spanning a width of only a few micrometers, and it appears darker under backscattered electron (BSE) imaging, indicating a lower mineral content. Even more, when going

from this outer helicoidal region to the exterior of the spike exocuticle, the helicoidal architecture is not lost immediately, but there is a transition region (TR) having high mineral content (arrows in Figure 2E) and characterized by one interface lamella. The last region of the spike cuticle identified here is the external highly mineralized region (HMR), where larger and more isolated fibers achieve a last half rotation within a heavily mineralized matrix before the twisted plywood organization is finally lost towards the spike outer surface [36]. The presence of a continuous twisted plywood at the interface between the highly mineralized region and the less mineralized outer helicoidal region is a remarkable feature of the spike that may enhance the anchoring between such dissimilar layers, as suggested by the rough fracture surface at that location (Figure 2H). A closer examination of fracture surfaces (**Figure S4**) highlights that in this highly mineralized region, crystals form nanometer sized crystallites, in analogy with those found in the dactyl club's outer layer [35]. The remaining of the highly mineralized region presents a very low fraction of chitin-protein fibers [36], but is still travelled by numerous pore canals, running perpendicular to the interface with the outer helicoidal region (Figure 2E). The spike cross-section is largely dominated by the striated and the inner helicoidal regions: for about two-third of the spike length, 90% of the wall thickness is only due to those two regions with the contribution of the outer helicoidal region and of the highly mineralized region being about 3% and 7%, respectively (**Figure S5**).

Figure 3: EDS elemental mappings and profiles (line scans) on (A) BSE-SEM image of a polished cross-section of the spike

Composition

The spike has a spatially tuned and graded chemical composition featuring 3 different minerals: fluorapatite (FAP), amorphous calcium phosphate (ACP) and amorphous calcium carbonate (ACC). The minerals are closely related to the underlying microstructural organization and fiber architecture. Elemental microanalysis by energy dispersive spectroscopy of X-rays (EDS) on representative locations highlights the presence of C, O, P and Ca as major chemical elements and of F, Na and Mg as minor components [36]. Those elements are unevenly distributed in the different regions as shown on a transverse cross-section (**Figure 3 A**) using 2D elemental mapping (Figures 3B-D) and line scans (Figures 3E, F and **Figure S6**). The highly mineralized region is rich in Ca and P together with F; a complementary Raman analysis, focusing on the outer part of the spike cuticle (**Figure 4 A**), shows a fairly sharp phosphate peak centered at 965 cm^{-1} (Figure 4B), indicating that FAP is most likely the predominant mineral phase of this region^[7,46]. The sharpness of the peak further suggests a high degree of crystallinity [35]. Confocal Raman imaging reveals interesting spatial heterogeneities within the highly mineralized region. Firstly, the phosphate band presents higher intensity at the outer surface as well as in the transition region close to the individual lamella anchoring the highly mineralized region to the less mineralized thin outer helicoidal region (arrows in Figure 4C). This agrees with variations in P concentration detected by EDS (Figure 3E). The same zone co-localizes with an increased crystallinity of FAP, estimated by the full width at half maximum (FWHM) of the phosphate peak (Figure 4D). One additional feature of the highly mineralized region is a central band having relatively low phosphate intensity and crystallinity, along with some traces of ACC (arrows in Figure 4F). Comparison of the phosphate intensity at the 0° and 90° polarization angle confirms spatial differences in the orientation of the mineral phase, with less oriented mineral found in regions with higher FWHM (Figures 4E and **Figure S7**). A similar tuning of the mineral phases has been reported at the impact region of the spearing dactyl, with small amounts of sulfate co-localized with the more crystalline FAP regions [35]. The presence of fluorapatite, rather than the more common amorphous calcium carbonate, is a remarkable compositional modification at the surface of spearing and smashing appendages of mantis shrimp [35]. In decapods crustaceans, a similar modification involving FAP occurs in the external layer covering the molar area of the crayfish mandible^[7]. This mineral is used not only to enhance the biomechanical performance of the cuticle, providing superior impact and wear resistance, but the presence of fluor decreases the solubility of apatite, thus improving its chemical stability. At the interface between the highly mineralized and the outer helicoidal regions there is an abrupt transition in the mineral from FAP to ACP as indicated by the sudden shift in phosphate position (Figure 4G). A central observation of the compositional analysis performed here is that the thin

outer helicoidal region has the lowest mineral content as inferred by Ca and P concentrations both showing a minimum there (Figure 3E). Moving further towards the interior of the spike, ACP still predominates within the striated region, whereas in the inner helicoidal region it is gradually replaced by Mg-rich ACC [36].

Figure 4: Raman spectroscopy of the outer regions of the spike cuticle. (A) BSE-SEM image highlighting the locations of the

Nanoscale biomechanical properties: indentation modulus, hardness and damage behavior The region-dependent chemical composition and microstructural organization of the spike cuticle are reflected into spatially varying mechanical properties, as measured by depth sensing nanoindentation (**Figure 5 A**). The outer FAP-dominated region is clearly the stiffest and hardest location (indentation modulus of 64.9 ± 3.5 GPa and hardness of 4.0 ± 1.0 GPa). Both mechanical parameters show a considerable drop (almost a factor of 2 for the modulus and of 5 for hardness) in the thin outer helicoidal region, which displays the lowest values of the entire spike, consistent with the low mineral content and compositional modifications. Moving to the striated region, indentation modulus and hardness increase and the former also shows slightly higher values with respect to the inner twisted plywood (Table 1), which is conceivable considering the highly ordered arrangement of the fibers. Furthermore, a small negative gradient of about 4 GPa is observed in the indentation modulus in this location, probably reflecting the corresponding decrease in Ca content. To better characterize the spatial tuning in local mechanical behavior from the heavily mineralized to the parallel fibers region, we performed high resolution nanoindentation with a sharp cube corner probe (Figures 5B-D and **Figure S8**). The high-resolution maps confirm the unusual drop in modulus within the outer twisted plywood region, with values slightly below 20 GPa and no differences between longitudinal and transverse sections, consistent with the isotropic nature of the twisted plywood organization. In particular, the transition region (featuring an individual lamella with fairly high mineral content) has a clear signature in the mechanical maps (Figures 5C and D) with a modulus of about 40-45 GPa, allowing a gradual switch from the highly mineralized to the outer helicoidal region. One additional feature of the highly mineralized region is a quite heterogeneous mechanical behavior (**Figure S9**), likely reflecting the spatial variations of phosphate intensity and crystallinity measured by Raman spectroscopy. A similar mechanical modulation featuring stiffer crystalline domains and less stiff amorphous islands has been reported for the heavily mineralized impact region of the spearer appendage [35]. Although a thin outer helicoidal region enclosing highly co-aligned fibers has been found in other biological penetrating tools such as the uropod back spike of the mantis shrimp [42] and the spider fang [45], a novel feature observed here is that this region, sandwiched between the hard outer cover and the stiff parallel-fiber region, presents substantially lower mechanical properties (modulus and hardness) than the neighborhood.

Figure 5: Nanomechanical properties of the spike cuticle measured by depth sensing nanoindentation. (A) Profile plots of in

Table 1: Local mechanical properties (indentation modulus E, and hardness H) of the different regions of the spike cuticle measured with nanoindentation (with a cube corner probe). HMR: highly mineralized region, TR: transition region, OHR: outer helical region, STR: striated region, IHR: inner helicoidal region. Values are reported as mean \pm standard deviation.

Region	E [GPa]	H [GPa]
HMR	68.33 ± 5.05	2.96 ± 0.39
TR	42.35 ± 4.88	1.51 ± 0.23
OHR	21.13 ± 3.49	0.62 ± 0.16
STR	32.46 ± 3.87	0.85 ± 0.08
IHR	31.00 ± 3.18	0.85 ± 0.09

To further elucidate the possible biomechanical role of the outer helicoidal region, we performed qualitative

fracture experiments: high load indentation was used to induce cracking and the sample surface was examined in SEM (Figure 6). Indents placed in the striated region (along the fiber axis, transverse section) require a fairly high load (~ 1000 mN) to induce widespread damage: cracks follow a rather straight path and spread radially in the matrix between fiber stacks (Figure 6A). A central remark is that all cracks going towards the highly mineralized region are stopped by the outer helicoidal arrangement (Figure 6B and **Figure S10**). This is consistent with the extensive delamination-based damage observed in transverse sections (Figure 2A) and confined within the striated region by the inner and outer twisted plywood. The damage observed in Figure 2A was not induced by indentation but, most likely, by sample preparation. Specifically, sample dehydration and related shrinkage are possible sources of local cracking. Since mineral is believed to replace water, the amount of dehydration (and shrinkage) should be higher in STR than in HMR (which is more mineralized and therefore should contain less water). At the same time, cracks were not observed in the OHR and IHR, which have a comparable degree of mineralization than STR but helicoidal arrangements preventing crack growth. A comparable damage behavior has been reported for the uropod back spike, which has a similar multilayered microstructure, but lacking the external highly mineralized region^[42]. Indents performed in the highly mineralized region require smaller loads (i.e., 50 - 500 mN) to generate extensive cracking. In this heterogeneous region, a relevant observation is that damage is more likely observed far from the transition region, which is also the most compliant zone of the highly mineralized region. Indeed, indents placed in the vicinity of this transition region either do not cause cracks (Figure 6C) or induce damage running parallel to the transition region and its lamella (Figure 6D). Remarkably, no cracks are observed crossing the interface between the highly mineralized and the outer helicoidal region. Typical high-load indentation curves for the highly mineralized region (HMR) and for the less mineralized but highly anisotropic region (STR) are shown in **Figure S11**. HMR featured a characteristic pop-in event (highlighted by the arrow) associated with sudden damage beneath the contact surface. Conversely, the indentation curve of STR is free from pop-in events, indicating a more progressive damaging behavior. The tiny dimension of the OHR (less than 10 μm in width) precluded a quantitative assessment of the fracture toughness of this region as previously done for the impact surface of both spearer and smasher stomatopods^[33,35]. Nevertheless, our indentation-based fracture study proves the critical role of the OHR for decoupling damage mechanisms between HMR and STR regions. A closer examination of the surface of a fractured spike highlights the damage behavior of the regions from a different perspective (Figures 6E and F). There is a clear transition between the rather straight fractured surface of the highly mineralized region and the much rougher surface at its interface with the outer helicoidal region. Inside the latter, individual lamellae can be seen, suggesting a plausible role in crack deflection and a corresponding switching from a more brittle to a more ductile damage behavior. Furthermore, between the fiber beds of the outer helicoidal region, unbroken pore canals are present. As in decapods crustaceans^[44], canals of the stomatopods also contain fibers^[36] that may act as bridges between the chitin-protein fibers beds, possibly enhancing fracture resistance^[47].

Figure 6: Indentation induced damage and fracture behavior in the spike cuticle. (A-B) SE-SEM images of cracks produced

The importance of the thin helicoidal region for the overall damage behavior of the spike is further demonstrated using multilayered 3D printing (**Figure 7**). We designed spike-inspired multilayered samples with and without a thin twisted plywood (mimicking the outer helicoidal region) sandwiched between a stiff monolithic but brittle outer region (mimicking the highly mineralized region) and a more compliant but anisotropic parallel-fiber region (mimicking the striated region). We used cuboid samples and performed macroscopic penetration tests at high strain rate using a blunt tip (Figure 7A). Notched samples were tested in 3-point-bending up to fracture (Figure 7B). The twisted plywood region connecting the stiff/brittle with the compliant/anisotropic region has a strong impact on the strength, energy absorption and failure characteristics of the spike-inspired system in both scenarios. Considering penetration tests, force-displacement curves show two peaks (Figure 7A): the first one happening just before the breaking of the stiff outer layer and the second due to damage propagating within the underlying fibrous and more compliant region (Figure 7C). Interestingly, the presence of the plywood not only increases the force needed to propagate the damage in the softer region (by 40%) but also the force necessary to break the stiff layer (by 25%). At the same time,

it has no influence on the contact stiffness, as indicated by the same initial slope of the curves. Concerning the 3-point-bending tests (Figure 7B), in the sample without twisted plywood, a main crack nucleates from the notch (Figure 7D-I) and propagates straight in the parallel fibers region with only minimal delamination (Figure 7D-II). As soon as the crack meets the stiff outer region, a catastrophic failure is observed and the broken sample “splashes away” from the supports (Figure 7D-III). In the second scenario, the crack also starts from the notch within the parallel fibers region (Figure 7D-pI) and propagates straight (Figure 7D-pII) up to the helicoidal region. There, the main crack branches symmetrically into two smaller cracks which propagate horizontally, causing delamination. A similar pattern is repeated one more time, while the crack advances in the helicoidal plywood region (Figure 7D-pIII). Such crack branching and delamination are important toughening mechanisms which delay catastrophic failure, increasing by almost 50 % the energy absorption of the synthetic sample. Interpreting this behavior in the context of the spike may suggest that, that although HMR and STR are well-suited to reinforce the spike under contact forces (HMR) and axial loading (STR), the combination of these two regions is not efficiently dealing with damage propagation. The thin plywood region is therefore required to fulfill this task.

Figure 7: Mechanical tests on 3D printed samples mimicking the spike cuticle. (A) Blunt penetration and (B) 3-point-bending.

Conclusions The invention of harpoons is a fundamental milestone not only in the evolution of natural life but also for humans^[48]. In Nature, evolution produced a large variety of harpoons and puncturing devices of increasing complexity, ranging from the passive and fairly simple cacti spines^[49], to the more sophisticated offensive tools such as spider^[41] and viper fangs^[50], scorpion stinger^[51], and even including drilling machines as in mosquito or parasitic wasps^[52,53]. Particularly fascinating are the chitinous penetrating tools of arthropods, which often allow piercing through several layers of a similar material, and possessing multiple modifications to fulfill such task^[54]. The spikes emerging from the spearing appendage of the mantis shrimps are biological harpoons deployed very quickly to impale and grab moving fishes, which have the bad luck to swim nearby. This is a challenging task requiring the ability not only to resist the initial impact with the scaly skin of the prey, but also to penetrate it for several millimeters to avoid that the fish swims away. Here, we have investigated the spike using imaging and material characterization techniques, to elucidate the many design strategies of this biological tool. From the material point of view, the spike cuticle of stomatopod is a chitin-based multilayered biocomposite, reinforced by different minerals together with compositional gradients and specific fiber arrangement^[36]. A hard heavily mineralized outer shell of crystalline FAP, essentially lacking fibers, is designed to resist to impact and wear. This exterior cover is combined with inner fiber-rich regions characterized by lower mineral content and by amorphous minerals (ACP and ACC). Two distinct fiber architectures are observed: a unidirectional arrangement bordered by helicoidal twisted plywood of dissimilar size. The latter is ubiquitous in arthropod^[12] and presents two main biomechanical advantages^[45]: starting from a strongly anisotropic elementary building block (the chitin-protein fiber), it provides the cuticle with in-plane isotropic mechanical behavior and high shear stiffness^[41,55]. Moreover, the helicoidal twisted plywood arrangement has remarkable fracture resistance obtained by interacting with cracking at different levels^[56], essentially enhancing crack driving force^[57] and reducing strain energy release rate^[58]. Conversely, the parallel-fiber organization is less usual^[45] and is used here, well oriented along the long axis of the spike from tip to base, to enhance stiffness and bending resistance. The combination of a parallel fiber with a twisted plywood region is therefore well-suited to support a complex mechanical environment with axial, bending and torsional loading. A central finding of our work is that, in the spike cuticle, the parallel fibers region is “joined” to the highly mineralized cover by a thin fiber-rich twisted plywood region, showing compositional modifications which make it the most compliant zone of the entire spike. In the cuticle, plywood (or Bouligand structures) are generally thick regions occupying a major part of the cuticle width and previous works have demonstrated the superior fracture toughness of Bouligand structure^[34,59,60]. The unique feature observed here is the presence of an extremely thin plywood structure (i.e., less than 10 μm in width) sandwiched between two very dissimilar regions and allowing the integration of a highly mineralized brittle outer layer with a less mineralized but highly anisotropic straight fibers region. Not only the plywood is very effective to stop crack propagation (both from the striated region to the hard shell

and vice-versa), as demonstrated by nanoindentation fracture experiment and by 3-point-bending tests on spike-inspired synthetic systems, but it also increases the force required to penetrate both the stiff layer and the underlying compliant fibrous matrix (as assessed on 3D printed replicas). Considering the attachment of different materials, introducing a more compliant region at the interface joining two dissimilar components is a construction principle common to other biological systems^[61]. Tendon, for example, attaches to bone through a transition zone which is not only more compliant than bone but also than tendon^[62]. This region, which co-localizes with the unraveling and splaying out of tendon fibers into smaller fibrils^[61] and which is made up of fibrocartilage^[63], is believed to protect the attachment region by reducing stress-concentration, effectively strengthening the interface^[64]. In analogy with the tendon-bone attachment, the thin and more compliant twisted plywood region may offer protection against stress localization at the transition between the hard and the parallel fibers region, hence increasing the damage tolerance of the spike. Helix reinforced composites are common in engineering applications and the construction principles of biological materials can improve the performance of the synthetic counterparts^[65]. Focusing on the mantis shrimp dactyl club, Bouligand and herringbone arrangements as well as nanoscale features of the impact surface, have inspired the design of impact resistance man-made composites^[66]. In light of the endless advancement in nano- and micro-scale manufacturing methods, the biological tool investigated here could inspire the design of new synthetic harpoons for example based on environmentally friendly and recyclable building units as seen in the spike cuticle, with improved wear resistance and puncture abilities for repeated piercing on different surfaces.

Experimental Section

Stomatopods and sample preparation : The specie of spearing mantis shrimps used in this study is *Lysiosquilla maculata* (Fabricius, 1793) also called the striped mantis shrimp (Figure 1A). Eight living stomatopods from Kendary (Indonesia), ranging from 20 to 40 cm in length, were delivered by Marine Life (Paris, France) and were kept in captivity in proper tanks at the Functional Morphology Lab (ULiege). Spikes were harvested from 3 different individuals after euthanasia, for a total of 9 spikes analyzed with the different analyzed with the different methods. All specimens were dissected in the intermolt stage C4, corresponding to entire and mature cuticle^[67]. Distal spikes were then separated from each other (Figure 1B). Samples imaged with micro-CT ($n = 2$) were directly embedded in epoxy resin to minimize movement artefact. Samples used for SEM ($n = 4$), Raman ($n = 3$) and nanoindentation ($n=6$) were dehydrated in a graded series of ethanol bath (50%, 70%, 90%, and 100%) and embedded in epoxy resin (EpoFix resin Kit, Struers Inc., Germany) under vacuum. The resin blocks were then polished (Rotopol-2, Struers, Germany) with series of silicon carbide disks of decreasing grain size (P800, P1200, P2400, P4000, Matador, Germany). Final polishing steps were performed with a diamond spray (DP-Spray P 1 μm , Struers, Germany) and an aluminum suspension (Eposil F, 0.1 μm , ATM, Germany).

Micro-CT imaging and processing: Micro-CT scans of resin embedded spikes were done at a nominal isotropic voxel size of 2 μm . The micro-CT (Skyscan 1272, Bruker, Belgium) was operated at tube voltage of 60 kV and current of 166 μA , in combination with a 0.25 mm thick aluminum filter. The samples were rotated over 180° with a rotation step of 0.2° (corresponding to 940 projections), with an exposure time of 3600 ms and a frame averaging of 4, leading to a scan time of approximately 4 h. A bigger sample including also the dactyl was scan at a lower resolution of 15 μm . Scans were reconstructed using Nrecon (v.1.7.5.2, Skyscan), and further analyzed with ImageJ (v.1.52a), Matlab (R2018a; The Mathworks, USA) and CTAn (v.1.19.4.0, Skyscan). Each virtual spike was first aligned along its principal axes of inertia using BoneJ (v.1.4.3)^[68], a module of ImageJ. Images were binarized using a global threshold based on Otsu's method^[69] and filtered to extract the biggest connected component. Geometrical properties of the cross-sections were measured using the Matlab Image Processing Toolbox. Using the general formula $I = \int z^2 dA$ where z is the shortest distance of the area element dA from a given axis, the planar (I_x and I_y) and polar ($J = I_x + I_y$) second moments of area passing through the centroid were computed. Major and minor axis were deduced by fitting an ellipse having the same second moments of area as the current cross-section. Eccentricity was computed as the ratio of the distance between the foci of the ellipse and the length of its major axis. Principal moments of area about the minor (I_{\min}) and the major (I_{\max}) axis of each cross-section, corresponding approximately to dorso-ventral

and lateral loadings, were calculated based on Mohr's circle theory. To compute the curvature of the spike, firstly a centroid profile line was extracted smoothed and down-sampled, to reduce the noise. At each location along the profile line, the local radius of curvature was computed by fitting circles, always considering three neighboring points. The curvature vector was obtained by dividing the unit vector pointing from the middle point of the triple to the center of the circle by the radius of curvature^[70]. A similar procedure was followed to compute the radius of curvature at the tip.

Scanning electron microscopy and energy dispersive spectroscopy : Observations of polished sections were carried out with an environmental scanning microscope ESEM-FEG XL-30 (FEI, Nederland) in low vacuum mode (0.4 Torr) at 20 KeV accelerating voltage. Images were taken using a back-scattered electron detector. A silicon drift detector of X-rays (Bruker, USA) with a super-ultra-thin window was used to obtain energy dispersive spectra and to perform elemental mapping using a Quantax analyzer and the software Esprit 2.1 (Bruker, USA). To measure the different elements concentration, the following energy peaks were used: Carbon K α peak (0.277 keV), Phosphorus K α peak (2.013 keV), Calcium K α peak (3.690 keV), Magnesium K α peak (1.253 keV), Fluorine (K α peak at 0.677 keV) and Sodium (K α peak at 1.041 keV). Fractured samples were sputter-coated with silver (SCD030, Balzers Liechtenstein) and observed in high vacuum mode using the same microscope with an acceleration voltage of 15 kV and a secondary electron detector. Measurements of grooves and serrations were made using ImageJ software^[71] on SEM-BSE micrographs of the spike in longitudinal and transversal sections to quantify their length and depth.

Raman spectroscopy : Polished sections of the spike were analyzed by Raman spectroscopy with a green DPSS laser ($\lambda = 532\text{nm}$, LabRAM 300, HORIBA Jobin Yvon, Japan). Spectra were acquired along straight lines at several different positions within the highly mineralized region, the outer helicoidal layer and the striated region of the cuticle. Each spectrum was acquired for 5 seconds. High resolution Raman mapping was performed on the outer region of the spike cuticle with the same microscope by focusing the laser beam through an Olympus MLPlan objective (NA 0.75), using an integration time of 1 s and resulting into a nominal pixel size of 500 nm. The mapping was performed with two different polarization angles (0 and 90 deg). The obtained spectra were analyzed with Rstudio (Rstudio Team, 2015) to extract the phosphate intensity (peak intensity in the 952 to 968 cm^{-1} region), the carbonate intensity (peak intensity in the 1077 to 1083 cm^{-1} region), the phosphate position (wavelength at which the phosphate ν_1 vibration mode is the highest), the FWHM (width of the phosphate ν_1 vibration mode at its half height) and the polarization ratio (intensity of the 960 cm^{-1} band in 0 / 90 deg polarization).

Depth sensing nanoindentation : Nanoindentation tests were conducted with a TriboIndenter TI-950 (Bruker, USA) on re-polished surface (the same samples used for SEM were probed), considering both transversal and longitudinal sections. We first used a Berkovich diamond tip to perform nanoindentation grids spanning across the different regions of the spike with indents spaced 10 μm apart (grid size in Figure 5A: 32x5 indents). We employed a trapezoidal load-controlled function (10s-5s-10s for loading, holding, and unloading segments) with a peak load of 5000 μN . Higher resolution nanoindentation mapping covering $\sim 45 \times 45 \mu\text{m}$ areas was carried out on two locations at the interface between the highly mineralized region and the outer helicoidal region after scanning the surfaces with the tip of the indenter to check the roughness (average RMS roughness in the range $\sim 16\text{-}25 \text{ nm}$ depending on the location). We used a sharper cube corner probe and a displacement-controlled indentation (10s-5s-10s) with a maximum penetration depth of 200 nm, allowing a smaller spacing of 1.5 μm between indents^[72]. Both tips were calibrated in fused quartz. Force-depth curves were analyzed with the Oliver and Pharr method^[73] to extract the indentation modulus and hardness. High lateral resolution nanoindentation data were used to generate 2D maps of the indentation modulus (Figures 5C and D and S8) and raw data were interpolated with the MESHGRID function of Matlab. A qualitative high load fracture study was performed on polished samples with a high load 10 N transducer (Omniprobe, Bruker, USA) equipped with a Berkovich probe. Several indentation loads ranging from 50 mN to 1000 mN were used to generate surface damage in different locations of the spike.

3D printing and testing: Spike inspired samples were designed with IronCAD (2020, USA), fabricated using a 3D multimaterial polyjet printer (Objet 260, Stratasys, USA) and tested. Specifically, we performed

penetration tests with a blunt tip (5 mm tip radius) and 3-point-bending tests (MTS Criterion C43.304, USA). For the penetration tests we considered cuboid samples having the following dimensions: 4 cm x 3 cm x 3 cm (height, width and length). For 3-point-bending, we fabricated beam-shaped samples, with dimensions: height (H) 4 cm, width (W) 3 cm and length (L) 20 cm. Each sample was a multilayer composite, featuring an outer monolithic region (mimicking the heavily mineralized shell) and inner fiber-reinforced region. For the fiber reinforced part, two different architectures were considered: *i*) a thick region composed of unidirectional parallel fibers, all oriented perpendicular to the longitudinal axis of the sample (mimicking the striated region) and *ii*) a two-layer system with a thin helicoidal twisted plywood region (mimicking the outer helicoidal region) sandwiched between the monolithic part and the unidirectional fibers array. The monolithic layer as well as the fibers were printed with a rigid glassy polymer (commercial name Vero White Plus), having Young's modulus at room temperature in the range of 2-3 GPa [74]. For the matrix embedding the fibers we used a rubbery polymer (commercial name Tango Black Plus) with a tangent modulus at room temperature of about 0.5-1 MPa [75]. Based on the printer resolution and considering the finite size (i.e., up to 150 μm) and the properties of the interface between fiber and matrix [76], the 3D printed fibers had a diameter of 600 μm . For the region mimicking the helicoidal region structure, 10 sheets were stacked with a pitch angle of 18°. At the bottom surface of the samples, a rigid layer made up of Vero White Plus was added to prevent excessive sink-in at the contact point with the supports. A notch (0.15 cm deep) was directly printed in the samples to trigger crack initiation within the parallel fiber array. Penetration tests were done at 10 mm/min whereas three-point-bending tests (span length of 16 cm) were conducted at 60 mm/min, considering preload of 5 N and with a 30 kN load cell. During tests, samples were imaged with a HD camera at 15 fps and movies were used to analyze deformation mechanisms. Mechanical tests were performed 1 day after printing to have always the same post-curing time. Fabrication, storage, and testing were done in a room with controlled humidity and temperature to minimize experimental variability.

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References:

- [1] S. A. Wainwright, J. M. Gosline, W. D. Biggs, J. D. Currey, *Mechanical Design in Organisms*, Princeton University Press, Princeton, **1982**.
- [2] T. Hunger, R. A. Steinbrecht, *Tissue Cell* **1998**, *30*, 14.
- [3] G. a Boxshall, *Biol. Rev. Camb. Philos. Soc.* **2004**, *79*, 253.
- [4] P. Fratzl, F. G. Barth, *Nat.* **2009** *462*, 442.
- [5] S. Gorb, *Attachment Devices of Insect Cuticle*, Kluwer Academic Publishers, **2001**.
- [6] J. H. Dirks, E. Parle, D. Taylor, *J. Exp. Biol.* **2013**, *216*, 1924.
- [7] S. Bentov, P. Zaslansky, A. Al-Sawalmih, A. Masic, P. Fratzl, A. Sagi, A. Berman, B. Aichmayer, *Nat. Commun.* **2012**, *3*, 839.
- [8] J. R. A. Taylor, S. N. Patek, *J. Exp. Biol.* **2010**, *213*, 3496.
- [9] N. A. Yaraghi, A. A. Trikanad, D. Restrepo, W. Huang, J. Rivera, S. Herrera, M. Zhernenkov, D. Y. Parkinson, R. L. Caldwell, P. D. Zavattieri, D. Kisailus, *Adv. Funct. Mater.* **2019**, *29*, 1970232.
- [10] G. Agez, C. Bayon, M. Mitov, *Acta Biomater.* **2017**, *48*, 357.
- [11] P. S. L. Anderson, *J. Exp. Biol.* **2018**.
- [12] A. C. Neville, in *Biol. Integument*, Springer Berlin Heidelberg, **1984**, pp. 611–625.

- [13] P. Compère, C. Jeuniaux, G. Goffinet, in *Crustac. Revis. Updat. from Trait. Zool. Vol 1* (Eds.: J. Forest, J.C. von Vaupel Klein, F.R. Schram), Koninklijke Brill, **2004** , pp. 59–144.
- [14] H. O. Fabritius, C. Sachs, D. Raabe, S. Nikolov, M. Friák, J. Neugebauer, in *Chitin* , Topics In Geobiology, **2011** , pp. 36–58.
- [15] D. Raabe, P. Romano, C. Sachs, H. O. Fabritius, A. Al-Sawalmih, S.-B. Yi, G. Servos, H. G. Hartwig, *Mater. Sci. Eng.***2006** , *421* , 143.
- [16] E. Belamie, G. Mosser, F. Gobeaux, M. M. Giraud-Guille, *J. Phys. Condens. Matter* **2006** , *18* , S115.
- [17] Y. Politi, B. Bar-On, H. O. Fabritius, *Springer Ser. Mater. Sci.* **2019** , *282* , 287.
- [18] K. M. Rudall, W. Kenchington, *Biol. Rev. Camb. Philos. Soc.* **1973** , *48* , 597.
- [19] M. N. Horst, J. A. Freeman, *Crustacean Integument* , CRC Press, **1993** .
- [20] P. Compère, C. Jeuniaux, G. Goffinet, in *Crustac. Revis. Updat. from Trait. Zool.* (Eds.: J. Forest, J.C. von Vaupel Klein), Leiden, Boston, **2004** , pp. 44–118.
- [21] B. W. Cribb, A. Stewart, H. Huang, R. Truss, B. Noller, R. Rasch, M. P. Zalucki, *Naturwissenschaften* **2008** , *95* , 17.
- [22] Y. Politi, M. Priewasser, E. Pippel, P. Zaslansky, J. Hartmann, S. Siegel, C. Li, F. G. Barth, P. Fratzl, *Adv. Funct. Mater.***2012** , *22* , 2519.
- [23] M. M. Giraud-Guille, *Tissue Cell* **1984** , *16* , 75.
- [24] H. O. Fabritius, A. Ziegler, M. Friák, S. Nikolov, J. Huber, B. H. M. Seidl, S. Ruangchai, F. I. Alagboso, S. Karsten, J. Lu, A. M. Janus, M. Petrov, L. F. Zhu, P. Hemzalová, S. Hild, D. Raabe, J. Neugebauer, *Bioinspiration and Biomimetics* **2016** , *11* , DOI 10.1088/1748-3190/11/5/055006.
- [25] P. Chen, A. Lin, Y. Lin, Y. Seki, A. Stokes, J. Peyras, E. Olevsky, M. Meyers, J. Mckittrick, *J. Mech. Behav. Biomed. Mater.***2008** , *1* , 208.
- [26] C. Van Der Wal, S. T. Ahyong, S. Y. W. Ho, N. Lo, *PeerJ***2017** .
- [27] J. T. Haug, C. Haug, A. Maas, V. Kutschera, D. Waloszek, *BMC Evol. Biol.* **2010** , *10* , 290.
- [28] L. K. Grunenfelder, G. Milliron, S. Herrera, I. Gallana, N. Yaraghi, N. Hughes, K. Evans-Lutterodt, P. Zavattieri, D. Kisailus, *Adv. Mater.* **2018** , *30* , 1705295.
- [29] M. Tadayon, S. Amini, A. Masic, A. Miserez, *Adv. Funct. Mater.* **2015** , *25* , 6437.
- [30] P. S. L. Anderson, T. Claverie, S. N. Patek, *Evolution (N. Y.)*. **2014** , *68* , 1919.
- [31] M. S. deVries, E. A. K. Murphy, S. N. Patek, *J. Exp. Biol.* **2012** , *215* , 4374.
- [32] J. C. Weaver, G. W. Milliron, A. Miserez, K. Evans-Lutterodt, S. Herrera, I. Gallana, W. J. Mershon, B. Swanson, P. Zavattieri, E. DiMasi, D. Kisailus, *Science (80-.)*. **2012** , *336* , 1275.
- [33] S. Amini, M. Tadayon, S. Idapalapati, A. Miserez, *Nat. Mater.* **2015** , *14* , 943.
- [34] N. A. Yaraghi, N. Guarín-Zapata, L. K. Grunenfelder, E. Hintsala, S. Bhowmick, J. M. Hiller, M. Betts, E. L. Principe, J.-Y. Jung, L. Sheppard, R. Wührer, J. Mckittrick, P. Zavattieri, D. Kisailus, *Adv. Mater.* **2016** , *28* , 6835.
- [35] S. Amini, A. Masic, L. Bertinetti, J. S. Teguh, J. S. Herrin, X. Zhu, H. Su, A. Miserez, *Nat. Commun.* **2014** , *5* , 3187.
- [36] Y. Delaunois, S. Smeets, C. Malherbe, G. Eppe, D. Lecchini, D. Ruffoni, P. Compère, *J. Struct. Biol.* **2021** , *213* , 107810.

- [37] J. R. Schulz, A. G. Norton, W. F. Gilly, *Biol. Bull.***2004** , 207 , 77.
- [38] J. Wu, S. Yan, J. Zhao, Y. Ye, *PLoS One* **2014** ,9 , e103823.
- [39] M. K. Ramasubramanian, O. M. Barham, V. Swaminathan, *Bioinspiration and Biomimetics* **2008** , 3 , 046001.
- [40] M. Vittori, V. Srot, K. Žagar, B. Bussmann, P. A. van Aken, M. Čeh, J. Štrus, *J. Struct. Biol.* **2016** , 195 , 227.
- [41] B. Bar-On, F. G. Barth, P. Fratzl, Y. Politi, *Nat. Commun.* **2014** , 5 , 2041.
- [42] S. Li, P. Liu, W. Lin, J. Tian, C. Miao, X. Zhang, R. Zhang, J. Peng, H. Zhang, P. Gu, Z. Zhang, Z. Wang, T. Luo, *ACS Appl. Mater. Interfaces* **2021** , DOI 10.1021/acsami.1c02867.
- [43] M. A. Meyers, P.-Y. Chen, A. Yu-Min Lin, Y. Seki, *Prog. Mater. Sci.* **2008** , 53 , 1.
- [44] P. Compère, G. Goffinet, *Tissue Cell* **1987** ,19 , 839.
- [45] Y. Politi, M. Priewasser, E. Pippel, P. Zaslansky, J. Hartmann, S. Siegel, C. Li, F. G. Barth, P. Fratzl, *Adv. Funct. Mater.***2012** , 22 , 2519.
- [46] G. Leroy, N. Leroy, G. Penel, C. Rey, P. Lafforgue, E. Bres, *Appl. Spectrosc.* **2000** , 54 , 1521.
- [47] W. Huang, D. Restrepo, J. Y. Jung, F. Y. Su, Z. Liu, R. O. Ritchie, J. McKittrick, P. Zavattieri, D. Kisailus, *Adv. Mater.***2019** , 31 , 1901561.
- [48] S. S. Hughes, *J. Archaeol. Method Theory* 1998 54**1998** , 5 , 345.
- [49] S. B. Crofts, P. S. L. Anderson, *Pro. R. Soc. B***2018** , 285 .
- [50] S. B. Crofts, Y. Lai, Y. Hu, P. S. L. Anderson, *Biol. Lett.* **2019** , 15 , 20180905.
- [51] Z. L. Zhao, T. Shu, X. Q. Feng, *Mater. Sci. Eng. C***2016** , 58 , 1112.
- [52] U. Cerkvenik, B. van de Straat, S. W. S. Gussekloo, J. L. van Leeuwen, *Proc. Natl. Acad. Sci.* **2017** , 114 , E7822.
- [53] X. Q. Kong, C. W. Wu, *J. Bionic Eng.* **2009** ,6 , 143.
- [54] Y. Politi, L. Bertinetti, P. Fratzl, F. G. Barth, *Philos. Trans. R. Soc. A* **2021** , 379 , DOI 10.1098/RSTA.2020.0332.
- [55] S. Nikolov, M. Petrov, L. Lymperakis, M. Friák, C. Sachs, H. O. Fabritius, D. Raabe, J. Neugebauer, *Adv. Mater.* **2010** ,22 , 519.
- [56] N. Suksangpanya, N. A. Yaraghi, R. B. Pipes, D. Kisailus, P. Zavattieri, *Int. J. Solids Struct.* **2018** , 150 , 83.
- [57] F. D. Fischer, O. Kolednik, J. Predan, H. Razi, P. Fratzl, *Acta Biomater.* **2017** , 55 , 349.
- [58] N. Suksangpanya, N. A. Yaraghi, D. Kisailus, P. Zavattieri, *J. Mech. Behav. Biomed. Mater.* **2017** , 76 , 38.
- [59] S. Yin, H. Chen, R. Yang, Q. He, D. Chen, L. Ye, Y. W. Mai, J. Xu, R. O. Ritchie, *Cell Reports Phys. Sci.* **2020** ,1 , 100109.
- [60] K. Wu, Z. Song, S. Zhang, Y. Ni, S. Cai, X. Gong, L. He, S. H. Yu, *Proc. Natl. Acad. Sci. U. S. A.* **2020** , 117 , 15465.
- [61] L. Rossetti, L. A. Kuntz, E. Kunold, J. Schock, K. W. Müller, H. Grabmayr, J. Stolberg-Stolberg, F. Pfeiffer, S. A. Sieber, R. Burgkart, A. R. Bausch, *Nat. Mater.* 2017 166 **2017** ,16 , 664.
- [62] A. Tits, D. Ruffoni, *Bone Reports* **2021** ,14 , 100742.

- [63] A. Tits, E. Plougonven, S. Blouin, M. A. Hartmann, J. F. Kaux, P. Drion, J. Fernandez, G. H. van Lenthe, D. Ruffoni, *Sci. Reports 2021 111* **2021** , 11 , 1.
- [64] Y. X. Liu, S. Thomopoulos, V. Birman, J. S. Li, G. M. Genin, *Mech. Mater.* **2012** , 44 , 83.
- [65] L. Zorzetto, D. Ruffoni, L. Zorzetto, D. Ruffoni, *Adv. Funct. Mater.* **2019** , 29 , 1805888.
- [66] R. P. Behera, H. Le Ferrand, *Matter* **2021** ,4 , 2831.
- [67] M. L. Reaka, *J. Morphol.* **1975** , 146 , 55.
- [68] M. Doube, M. M. Klosowski, I. Arganda-Carreras, F. P. Cordelières, R. P. Dougherty, J. S. Jackson, B. Schmid, J. R. Hutchinson, S. J. Shefelbine, *Bone* **2010** , 47 , 1076.
- [69] N. Otsu, *IEEE Trans Syst Man Cybern* **1979** ,SMC -9 , 62.
- [70] A. Mjaavatten, **2022** .
- [71] C. A. Schneider, W. S. Rasband, K. W. Eliceiri, *Nat. Methods 2012 97* **2012** , 9 , 671.
- [72] C. Sachs, H. Fabritius, D. Raabe, *J. Mater. Res.* **2006** , 21 , 1987.
- [73] W. C. Oliver, G. M. Pharr, *J. Mater. Res.* **1992** ,7 , 1564.
- [74] T. S. Lumpe, J. Mueller, K. Shea, *Mater. Des.* **2019** , 162 , 1.
- [75] V. Slesarenko, S. Rudykh, *Int. J. Eng. Sci.* **2018** , 123 , 62.
- [76] L. Zorzetto, L. Andena, F. Briatico-Vangosa, L. De Noni, J. M. Thomassin, C. Jérôme, Q. Grossman, A. Mertens, R. Weinkamer, M. Rink, D. Ruffoni, *Sci. Reports 2020 101* **2020** , 10 , 1.