Influence of Structural Principles on the Mechanics of a Biological Fiber-Based Composite Material with Hierarchical Organization: The Exoskeleton of the Lobster *Homarus americanus*

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The cuticle of the lobster *Homarus americanus* is a nanocomposite, such as most structural biological materials. It consists of a matrix of chitin-protein fibers associated with various amounts of crystalline and amorphous calcium carbonate in the rigid parts of the body, and is organized hierarchically at all length scales. One prominent design principle found in the hierarchical structure of such biological fibrous composite materials is the twisted plywood structure. In the lobster cuticle, it is formed by superimposing and gradually rotating planes of parallel aligned chitin-protein fibers. To adjust the mechanical properties to the requirements on the macroscopic level, the spatial arrangement and the grade of mineralization of the fibers can be modified. A second design principle of lobster cuticle is its honeycomb-like structure, generated by the well-developed pore canal system, whose twisted ribbon-shaped canals penetrate the cuticle perpendicular to its surface. Due to the hierarchical structure, the mechanical properties of the lobster cuticle have to be investigated at different length scales, which is essential for the understanding of the structure–mechanical function relations of mineralized tissues (e.g., potentially also bone and teeth). In order to investigate the influence of the structural principles on the mechanical properties on the macroscopic scale miniaturized tensile, compression, and shear tests were carried out to obtain integral mechanical data. Characterization of the microstructure included scanning electron microscopy (SEM) combined with energy dispersive X-ray (EDX) measurements.

1. Introduction

Structural materials have always been a key factor for the development of human society, starting with the use and modification of natural materials and, nowadays, culminating in production of a multitude of highly specialized synthetic materials, based on a broad variety of raw materials. The increasing demand for new, sophisticated multifunctional materials has brought natural structural composites into focus, since these have been optimized in their functions in a long selection and adaptation process during evolution. Biological structural materials differ fundamentally from most man-made structural materials, in being inherently structurally heterogeneous. This heterogeneity arises from a multitude of different constituents already at the molecular level, including mainly various organic molecules, such as proteins or sugars, but also inorganic matter, mostly in the form of calcium-based minerals.[1] The constituents are produced and/or provided by the organisms, which also control the formation of subassemblies. These can have different compositions, shapes, sizes, and spatial distribution, leading to the hierarchical organization found in most structural biological materials throughout the flora and fauna, such as wood, vertebrate bones and teeth, mollusk shells, arthropod exoskeletons, and skeletal elements of other invertebrates.[2,3] The complexity of these materials is further increased by different chemical compositions in different positions of the same structure, as the degree of mineralization, fluid content, and the resulting variation in the type and density of internal interfaces. This structural heterogeneity implies that the mechanical properties of such materials are also heterogeneous at different length scales. The highest level of hierarchy in biological structural materials can be either the whole organism, such as, for example, a tree, its smaller subunits, like the stem or single branches, or, like in the case of animals, a single bone of a vertebrate or an appendage segment of an arthropod. These functional units as a whole have ideal mechanical properties for their individual purposes, which are optimized for the occurring loads. The overall mechanical properties of a functional unit rarely reflect the bulk properties of the material ingredients constituting them, but rather depend on their geometry and

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topological arrangement. In some biological materials, such as bone or teeth, the mechanical properties of the bulk material are well investigated. Already at this macroscopic scale significant variations have been observed between different sampling positions within a specimen, and correlations have been shown to specific loading requirements.[4] At the macroscopic level, the mechanical response to external loads represents the integral result of the mechanical behavior of the individual composite constituents, and their hierarchical structural arrangement on different length scales in the bulk material. This structural heterogeneity and the decreasing size of the constituents at the lower hierarchy levels are exactly the point where the study of biological structural materials becomes very challenging. It also implies that the mechanical properties that can be measured in such materials depend strongly on the observed length scale, and may vary from one hierarchical level to another. Depending on the structure, mechanical anisotropy also occurs on different hierarchical levels.

This progress report focuses on microstructure and the macromechanical properties of the mineralized parts of the exoskeleton of an arthropod, our model organism *Homarus americanus*, the American lobster, as an example for a hierarchically structured biological material designed to withstand mechanical loads.

2. Microstructure of the Exoskeleton of the American Lobster *Homarus americanus*

The Arthropoda are one of the most successful and diverse groups of organisms, and their members, such as insects, crustaceans, and chelicerates, have adapted to virtually every habitat on earth. One common feature of all arthropods is the presence of exoskeletons, formed by their outer integument, which is referred to as cuticle, whose basic material is the polysaccharide chitin. In every arthropod species, this cuticle is a functional unit designed and differentiated to perform all the functions required for survival in its specific biological environment.[5] Among other functions, such as acting as a selective chemical barrier between body and environment, the basic function of the exoskeleton is to provide stability to the body, and enable movement through the formation of joints and attachment sites for muscles. In order to grow, arthropods have to shed their old exoskeleton and replace it with a new, larger one, frequently in a process termed molt. In most crustaceans, body stability is achieved by the formation of a rigid cuticle in the load-bearing parts, which is often reinforced by the incorporation of nanoscopic biominerals.

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The American lobster *H. americanus* is a large-sized crustacean. Its body can be divided into the head (cephalon), the thorax, and the tail (abdomen). The first three pairs of walking legs, which are attached to the thorax, end in true pincers.[6] Characteristically, the first pair is enlarged to massive and flattened claws, which are referred to as crusher claw and pincher claw, according to their biological function (Fig. 1a).[7,8] In the rigid parts of the exoskeleton of *H. americanus*, one can distinguish at least five levels of hierarchy, ranging from the macroscopic to the nanoscopic scale (Fig. 1b and c). Macroscopically, the cuticle consists of three layers, forming a functional unit (Figs. 1b and 2a).[9] A fourth thin membranous layer is present between the three main layers and the epidermal cells during intermolt, the period between two molts.[10] The external epicuticle is thin and waxy, and serves as a permeability barrier to the environment. It consists mainly of long-chain hydrocarbons, esters of fatty acids, and alcohols, and normally contains no chitin or

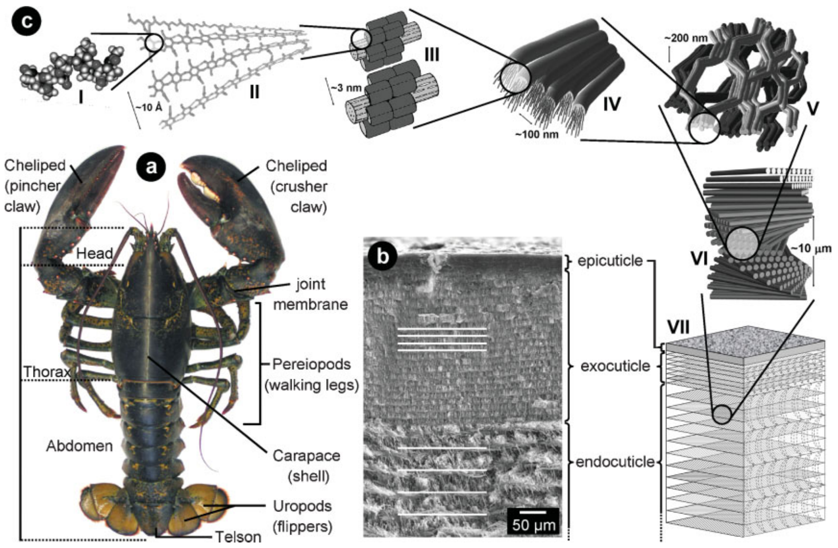

Figure 1. Microstructure of lobster cuticle. a) Morphology of the American lobster *Homarus americanus*. b) SEM image of a cross-section through the cuticle, showing the epi-, exo-, and endocuticle. c) Hierarchical organization, starting with the N-acetyl-glucosamine molecules (**I**) forming anti-parallel chains of α-chitin (**II**). 18 to 25 chitin molecules wrapped with proteins form nanofibrils (**III**), which cluster to form chitin-protein fibers (**IV**) that are arranged in horizontal planes, where the long axes of the fibers are all oriented in the same direction (**V**). The chitin-protein fibers form the typical twisted plywood structure (**VI**) of the three-layered cuticle (**VII**). Modified with permission from [24]. Copyright 2006 Elsevier.

minerals.[8,9,11] The mechanically relevant procuticle comprises the exocuticle and the endocuticle.[9] Both layers consist of planes formed by parallel arrays of mineralized chitin-protein fibers. These planes are stacked with the long axis of the fibers in every plane rotating gradually around the normal axis of the cuticle, thereby creating a twisted plywood structure.[12–15] The stacking height of the twisted plywood is defined by the distance in which the superimposed fiber layers complete a 180° rotation (Fig. 2). In the exocuticle (Fig. 2b), the stacking height is considerably smaller than in the endocuticle (Fig. 2c), most probably caused by a larger rotation angle of the chitin protein fibers. The 50–250 nm thick fibers are composite structures themselves, as they are composed of clustered nanofibrils with diameters between 2 and 5 nm, and lengths of about 300 nm, with various amounts of nanoscopic calcite and amorphous calcium carbonate (ACC) particles located between them.[16,17] The nanofibrils consist of 18–25 chitin molecules wrapped by proteins.[18,19] The sugar molecule chitin is the insoluble linear polymer of β-1,4-linked *N*-acetylglucosamine residues. Chitin occurs in three different polymorphic forms, which differ in the arrangement of the molecular chains in the crystal cell. In α-chitin, which is the most abundant crystalline variant, the chains are arranged in an anti-parallel fashion. In β-chitin, the chains are parallel. γ-chitin is a mixture of α- and β-chitin, with two parallel chains in one direction and the third one in the opposite direction. The three forms can be found in parts of the same organism. Chitin is the second most abundant natural polymer on earth after cellulose, and the basic constituent not only of the crustacean cuticle but of the arthropod exoskeleton in general, including insects, chelicerates and myriapods. It also occurs in mollusk shells, fungal cell walls, and various other organisms.[20–22] The crystalline α-chitin typically predominates in the exoskeleton of large crustaceans. Besides the twisted plywood structure, a second design principle can be found in the lobster cuticle. Due to a well-developed pore canal system, a honeycomb-like structure is generated, as numerous canals penetrate the cuticle perpendicular to its surface.[16,23,24] Each pore canal contains a long, soft, and probably flexible tube, which has an elliptical-like cross-section with the long axis of the ellipse parallel to the fiber orientation in each plane (Fig. 3a and b). Additionally, the pore canals contain chitin-protein fibers oriented perpendicular to those forming the twisted plywood structure. Due to the rotation of this structure, the outer shape of each tube resembles a twisted ribbon (Fig. 3c). Similar pore canals also occur in the cuticle of crabs such as *Carcinus maenas*.[25] The combination of a honeycomb-like structure and a twisted plywood structure with tightly connected lamellae should

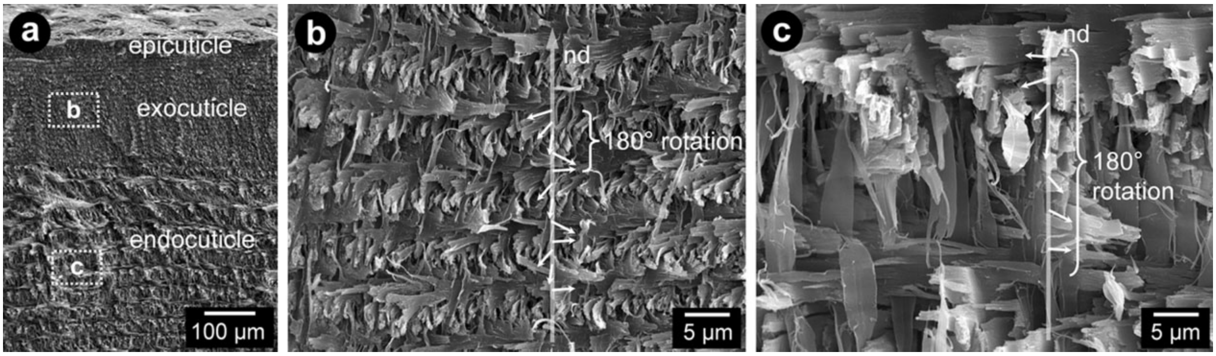

Figure 2. Microstructure of lobster cuticle. a) SEM image of a cross section through the cuticle, showing the three structurally different layers: the thin, waxy epicuticle, and the mechanically relevant exocuticle and endocuticle. b,c) Chitin-protein fibers are arranged in horizontal planes, with their long axes all oriented in the same direction. Superimposed planes gradually rotate around the normal axis (nd) of the cuticle, creating a typical twisted plywood structure. The stacking height is smaller in the b) exocuticle than in the c) endocuticle.

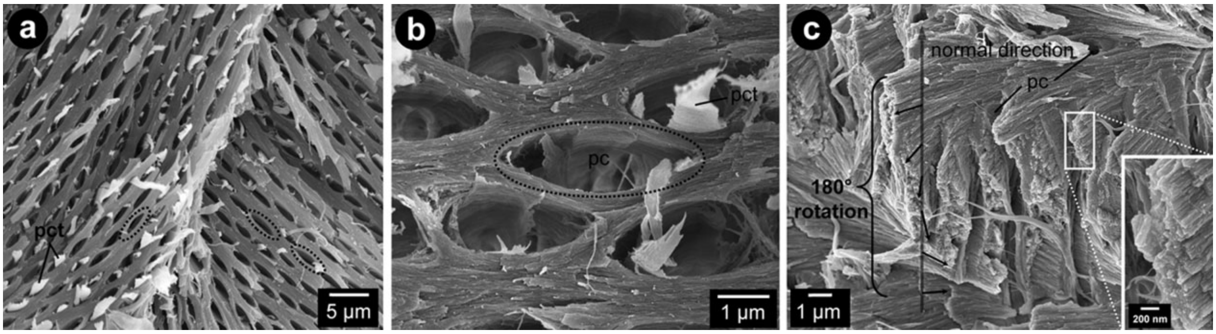

Figure 3. Construction principles of lobster cuticle. a) Twisted plywood structure and pore canal system, containing long, flexible tubes (pct) in cuticle fractured parallel to the surface. b) Mineralized fibers arranged around the cavities of the extremely well-developed pore canal system (pc) give the structure a honeycomb-like appearance. c) Planes of parallel fibers rotating around the normal direction in the twisted plywood structure. Modified with permission from [16] (b) and [30] (a, c). Copyright 2006 Elsevier.

lead to remarkable mechanical properties, and an anisotropic deformation behavior.

3. Mechanical Properties of the Exoskeleton of *H. americanus*

3.1. Mechanical Testing of Biological Materials

Standardized methods for the investigation of mechanical properties of materials require a specific geometry and certain well-defined minimum dimensions of the test specimens, in order to render the results comparable. In general, most species of Arthropoda are too small to fulfil this condition, but due to its size, thickness, and morphology, the cuticle of lobsters is very suitable for miniaturized mechanical testing. In biological composite materials, the mechanical properties are not only determined by the internal material composition, including its structure on all levels of organization, but also by the physiological state of the sample, and its artificial or preparation state, respectively. The structure of the material can be described in terms of a number of internal variables, such as its mass density, stacking height of planes, porosity, crystallographic texture of its crystalline constituents, average diameter of the chitin fibers, or particle size of the minerals, to name just a few.[16,23,26,27] Beyond these *internal* structural and composition measures, the actual state of a biological material can also be described in terms of a number of essential *physiological* parameters. The physiological state of the organism plays a particularly important role for the material, such as, for example, the actual stage of the molting cycle, or the general environmental and living conditions, like pH, salt content, temperature, nutrition, or diseases.[28,29] A third category affecting the mechanical state of the biological material under inspection, which profoundly differs from the two others, is its *artificial* state, which is obtained after sample preparation owing to the *externally* imposed boundary conditions. Parameters belonging to this group are storage conditions, the grade of hydration, and effects caused by the natural decomposition of certain components. All these parameters can influence each other, and they collectively define the structural state of the material, and hence its mechanical properties. For the experimental examination of the mechanical properties of biological materials, it is crucial to establish a defined state of the test specimens, preferably as close as possible to its original natural state, in order to obtain authentic values for the material in its incipient functional state. In our experiments we took these aspects into account by performing identical tests on dry and wet claw endocuticle, since it is particularly the water content that strongly affects the properties of biological composites.

3.2. Macroscopic Mechanical Properties

3.2.1. Tensile Tests

The difference in the material response between the wet and dry states becomes apparent in the global stress-strain behavior of lobster endocuticle, and the membranous cuticle that were both investigated using uniaxial tensile tests (Fig. 4, Table 1).[30] In the dry state, the pincher and crusher claws display a linear elastic behavior, without much irreversible deformation typical for a brittle response, whereas the wet specimens show an onset of plastic deformation, which begins at a yield strain of 0.5% and extends to a strain to fracture of about 1.8% (Fig. 4). The comparison to the strain to fracture of 0.7% achieved by the dry samples shows the absence of mechanisms allowing irreversible deformation and stress reduction in dehydrated endocuticle. In the wet samples, the maximal achieved strain and stress correspond nearly to the strain and stress to fracture, which indicates that significant necking does not occur in the tensile test specimens at the end of deformation. While the absence of necking is typical for brittle materials, it is untypical for more ductile materials, such as the wet samples. Similar differences in deformation behavior were observed for untreated cuticle in the dry and wet state, taken from the walking legs of the crab *Scylla serrata*, and the carapace of the prawn *Penaeus mondon*.[31,32]

For the elastic behavior, the resulting structural stiffness values differ only slightly between the samples taken from the different claws, but the difference is more pronounced between the dry and the wet state (Table 1). The structural stiffness of the dry samples lies between 5.8 and 7.0 GPa, which are values higher than those of wet samples, with about 4.8 GPa.

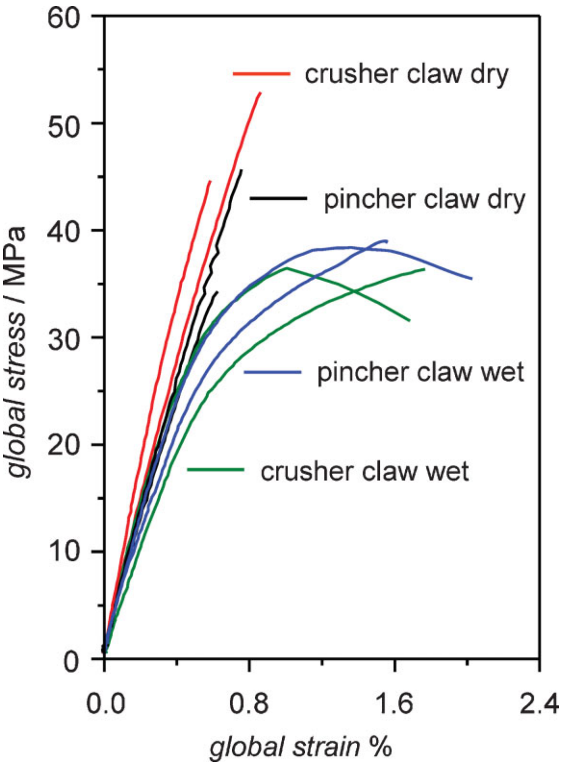

Figure 4. Global stress-strain behavior of lobster endocuticle from the pincher and crusher claw, both in the dry and in the wet state. Modified with permission from [30]. Copyright 2006 Elsevier.

The comparably small difference in structural stiffness between the dry and wet endocuticle in the lobster can probably be explained with the relatively high content of minerals in the claws, whose elastic properties are less affected by the water content than the organic constituents.[33] The effect of water acting as a *plasticizer* for the irreversible deformation behavior also becomes apparent by comparing the fracture surfaces of the samples tested in the dry and in the wet state.[34] In the dry mineralized test specimens, the failure is caused by cracks, which may propagate through the twisted plywood structure in two different ways: first, by cleaving of fibers, which were oriented more parallel to the fracture surface longitudinally along their junctions, or, second, by cutting the fibers, which were oriented more perpendicularly to the fracture surface along their cross-section (Fig. 5a and b). Due to the rotation in the stacked fiber planes and the fixed fracture plane, the fracture surface subsequently shows an alternation of these two types of fracture modes. The residual overlapping platelets display smooth facets, corresponding to a brittle catastrophic failure. Features that would be characteristic of plastic deformation or gradual delamination effects, which impede crack growth, are not visible. The cleaved cuticular pores oriented in the normal direction, which recur on the fracture surfaces, probably act as natural defects in the material. Additionally, during the drying process, internal stresses can generate microcracks, which possibly add up to these naturally present defects. Such an agglomeration of defects and microcracks can lead to a critical crack size, resulting in a brittle failure, as also known from various ceramic materials.[35] The combination of these effects leaves the original microstructure below the fracture surface almost unaltered.

On the other hand, fracture surfaces of wet mineralized test specimens reveal distinct deformation features, indicating a different type of fracture mechanism when compared to the dry state (Fig. 5c and d). In these samples, the twisted plywood structure appears strongly distorted, and split up with its fibrous components aligned in tensile direction. The bundles of fibers, which were originally oriented more or less perpendicular to the fracture surface, slide and are torn apart, which is an indication for the delamination inside the twisted plywood layers. Additionally, the junctions between the fibers at the torn ends of the bundles are separated by flexible pore canal tubes, which are stabilized by the mainly parallel oriented parts of adjacent twisted plywood layers, and thus remain located in their original position. Those pore canal tubes, which are pushed out of the honeycomb structure, protrude out of the fracture surface. The obliquely oriented parts of the honeycomb structure become arranged along the tensile direction during elongation, most likely caused by delamination, rotation, and deflection, which suggests the occurrence of microplasticity. Simultaneously, the honeycomb structure is divided into bundles, in which the pore canals appear slightly compressed and elongated. The fibers oriented parallel to the fracture plane seem not to be deformed plastically, but appear to delaminate in a similar way as in the dry samples, although probably less pronounced. The described mechanisms indicate the occurrence of toughening effects, such as crack deflection and bridging of the cracks, which both lead to a stepwise crack propagation before the actual fracture.

3.2.2. Compression Tests

Under compression, the lobster endocuticle displays a strong structural anisotropy in its global stress-strain behavior. While the samples tested in normal direction show a large linear elastic region, and a subsequent onset of plasticity, the samples tested in transverse direction show an extended plateau region after a relatively small elastic region, and at the end a steeply rising portion of the stress-strain curve (Fig. 6). In the dry state, the structural stiffness in the normal direction and in transverse direction are almost equal (Table 2). In the wet state, the structural

Table 1. Mechanical properties derived from the global stress-strain curves. The properties determined are the average values of the samples from each location and testing condition, including their standard deviation: structural stiffness s_{st}, Poisson's ratio ν, yield strain ε_y, yield stress σ_y, strain to fracture ε_f and stress to fracture σ_f. The standard deviation is given in brackets.

sample	s_{st} [GPa]	ν [–]	ε_y [%]	σ_y [MPa]	ε_f [%]	σ_f [MPa]
pincher claw dry	5.8 (0.4)	0.43 (0.00)	–	–	0.7 (0.09)	40.1 (7.9)
crusher claw dry	7.0 (0.8)	0.34 (0.01)	–	–	0.7 (0.19)	48.8 (5.8)
pincher claw wet	4.9 (0.6)	0.33 (0.03)	0.5 (0.04)	26.6 (3.2)	1.8 (0.33)	37.2 (2.5)
crusher claw wet	4.8 (0.6)	0.34 (0.06)	0.5 (0.05)	25.8 (5.9)	1.7 (0.06)	34.1 (3.6)

stiffness in normal direction is higher than in transverse direction, and only slightly lower than the value obtained for the dry samples. Apparently, dehydration shows only little effect on the structural stiffness in normal direction, while in transverse direction the structural stiffness increases by a factor of 1.4 from the wet to the dry state. The effect of dehydration on the Poisson's ratio is about equal, as its value is increased by a factor of about 1.3 in normal direction and nearly the same for the observation direction I in transverse direction. The difference in Poisson's ratio between the observation directions I and II is remarkable. Both in dry and wet states, the Poisson's ratio is about a factor 3.6 higher for observation direction I than for observation direction II. Compared to Poisson's ratios obtained in the tensile tests, which amounted to about 0.33 in the dry and in the wet state, the values for observation direction I are slightly lower. It is remarkable that, when compressed in transverse direction, the elastic deformation in the normal direction of the cuticle is much smaller than in the transverse direction. This behavior can be explained in terms of the honeycomb-like structure of the pore canal system and the direction of the applied stress. When the cuticle is loaded in transverse direction, the pore canals are compressed and broaden in transverse direction, which corresponds to the cross-section of the pore canals. In normal direction, which corresponds to the long axis of the pore canals, the increase in length during compression is negligible. The deformation behavior is illustrated schematically in Figure 7.

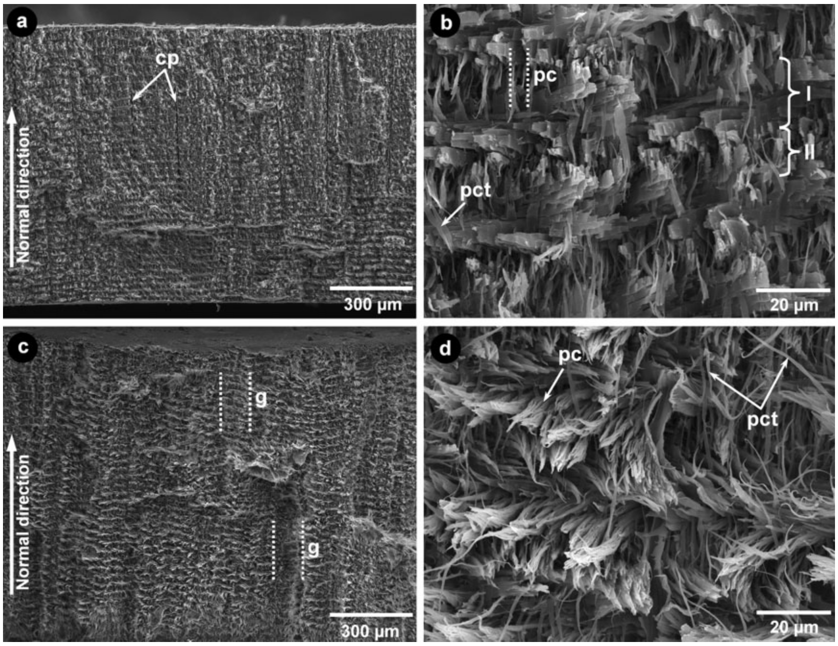

Figure 5. Fracture surfaces of the crusher claw tested in a,b) dry and c,d) wet state. a) Overview of the dry crusher claw, showing the smooth surface and numerous cleaved cuticular pores (cp). b) Detailed image displaying the fracture modes of fibers oriented more parallel (I) and closer to perpendicular (II) to the fracture surface. c) The corrugated and uneven surface of the wet crusher claw with numerous deep irregular grooves (g, indicated by dashed lines). d) Detailed image showing the distorted twisted plywood structure with drawn-out pore canals (pc) and irregularly protruding pore canal tubes (pct). Modified with permission from [30]. Copyright 2006 Elsevier.

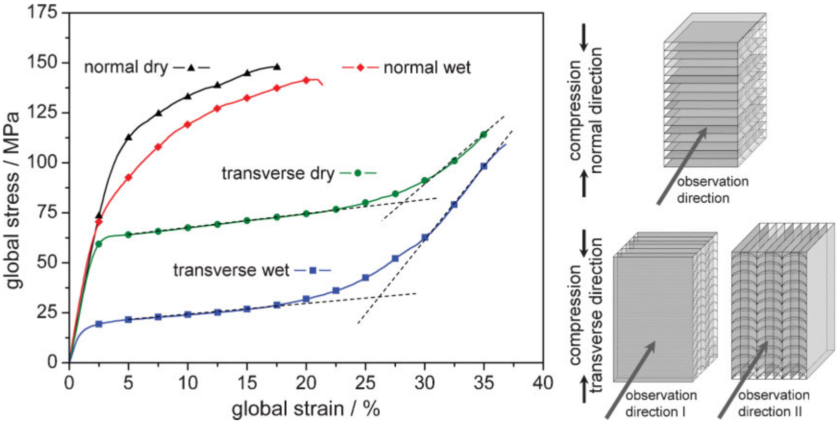

Figure 6. Compression tests of mineralized endocuticle and schematic figure of the compression tests. The graph depicts the global averaged stress-strain behavior under compression of the endocuticle, obtained in normal and transverse directions both in dry and wet state. The interception points of the dashed lines fitted to the curves obtained for compression in transverse direction give the values for strain (ε_d) and stress (σ_d) at densification. The vertical dotted line indicates the strain range used for determining Poisson's ratio (ν) and structural stiffness (s_{st}). For compression in normal direction of the cuticle, the cross section is observed. For compression in transverse direction of the cuticle, the two possible observation directions are parallel to the surface (observation direction I) and the cross section (observation direction II). Modified with permission from [40]. Copyright 2008 Elsevier.

The beginning of plastic deformation is defined by the yield strain and the yield stress. The yield strain amounts to higher values in the normal direction than in the transverse direction, and increases from the wet to the dry state (Fig. 6, Table 2). The same trends can be observed for the yield stress, which is in both, the dry and the wet state, higher in the normal than in the transverse direction. In normal direction, the difference in the yield strain and stress is less pronounced between the dry and wet state, while in transverse direction, dehydration has a slightly higher effect on the values of the yield strain and yield stress. In contrast to the normal direction, the yield point marks the beginning of the plateau region in the transverse direction. After reaching a critical stress level, the structure deforms continuously, without a strong increase in stress. This deformation behavior is similar in the dry and the wet state, but the stress is shifted to much higher values in the dry state. Considering the honeycomb-like structure, the observed stress values are the threshold at which the pore canals probably start collapsing. A similar behavior has been described for polycarbonate honeycombs.[36] After the densification, which is reached at a strain of about 30% and a stress of 87 MPa in the dry state, and of 25% and 35 MPa in the wet state, the stress

Table 2. Average mechanical properties of mineralized endocuticle from two different lobsters, derived from compression tests. s_{st} structural stiffness; ν Poisson's ratio; ε_y yield strain; σ_y yield stress; ε_d strain at densification; σ_d stress at densification; ε_f strain to fracture; and σ_f stress to fracture. The standard deviations are given in brackets. A set of six samples was tested in the normal and 12 samples in the transverse direction, both in the dry and in the wet state.

direction	s_{st} [GPa]	ν [–]	ε_y [%]	σ_y [MPa]	ε_d [%]	σ_d [MPa]	ε_f [%]	σ_f [MPa]
normal dry	4.7 (1.6)	0.13 (0.01)	2.4 (0.7)	96.8 (16.0)	–	–	18.7 (5.5)	147.5 (39.3)
normal wet	4.1 (1.4)	0.10 (0.03)	1.8 (0.6)	56.4 (25.6)	–	–	19.4 (3.5)	124.7 (53.8)
transverse dry observation direction I/II	4.6 (1.6)	0.29/0.08 (0.02/0.03)	1.6 (0.7)	54.9 (12.3)	30.2 (3.4)	87.0 (12.3)	42.3 (2.6)	163.4 (14.7)
transverse wet observation direction I/II	3.0 (1.3)	0.28/0.08 (0.08/0.02)	0.7 (0.3)	14.0 (4.0)	24.6 (2.6)	35.1 (6.7)	35.6 (3.7)	93.4 (5.4)

increases stronger in the dry state than in the wet state. The final strain and stress to fracture values are higher for dry cuticle than for wet cuticle. In normal direction, the strains to fracture are much lower compared to the transverse direction, whereas the corresponding stresses to fracture are higher for wet but lower for dry cuticle. A fundamental difference was observed in the failure of the samples. While the samples tested in the normal direction failed by cleavage in the compression direction, the samples tested in transverse direction showed a distinct fracture plane, oriented perpendicular to the cuticle surface and 45 ° to the compression direction (Fig. 7d), which is equal to the direction of the maximum shear stress. This can be explained by failure of the structure along the long axes of the pore canals, resulting in the propagation of the crack from one pore canal to the next. To investigate the origin of these phenomena, shear tests were performed on dry cuticle, to evaluate the fracture energy needed to create distinct fracture planes. In the twisted plywood structure of the cuticle, two different shear planes exist, which are oriented either parallel to the cuticle surface (mode I) or perpendicular to the cuticle surface (modes II and III, Fig. 8). The difference between mode II and III is the shear direction, which in this case can be in the normal or transverse direction of the cuticle. A fracture of mode II has the highest fracture energy, because the planes of mineralized fibers are displaced against each other longitudinally with respect to cuticle geometry, and the fibers have to be fractured (Fig. 8). In mode I, the fracture leads to delaminating of the superimposed mineralized fiber planes and lateral movement. Additionally, the pore canal tubes are sheared off. The fracture energy required for this mechanism is slightly lower than in mode I. In mode III, the fracture energy is reduced by about 40%. In this case, neither the pore canals nor the mineralized fiber planes have to be sheared off. In a unidirectional fiber reinforced composite, the fracture plane is also oriented 45 ° to the compression direction, or parallel to the fiber axis, if the composite is loaded perpendicular to the fiber orientation.[37] The numerous pore canal tubes may play an important role, as they act like the unidirectional fibers in this load case, and likely stitch the mineralized fiber planes together, improving the resistance against delamination.[38] Presumably, in crustaceans, the original function of the pore canal system is the transport of minerals used for hardening the new exoskeleton after the molt.[39] Our data suggest that the pore canal system also performs structural functions in the lobster's exoskeleton. Moreover, previous studies have shown that the pore

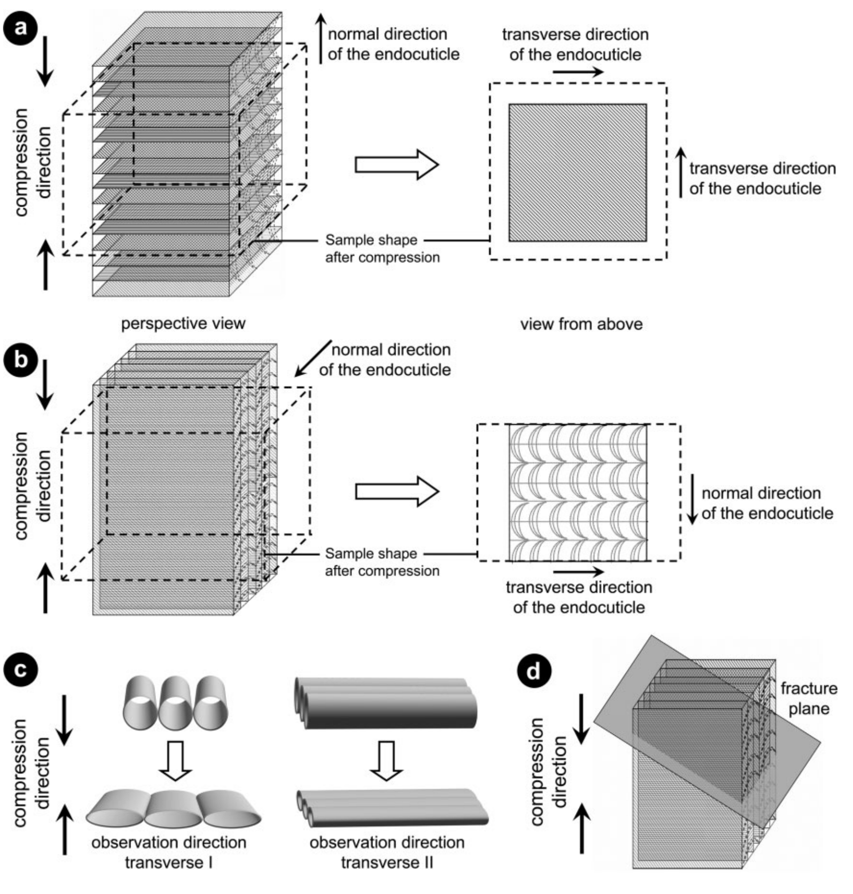

Figure 7. Schematic illustration of the elastic–plastic deformation of the endocuticle under compression in the a) normal and b) in the transverse direction. The dashed lines mark the shape of the specimens after deformation. c) Schematic figure of the deformation of pore canals compressed in the transverse direction. When observing the cuticle surface (transverse I), lateral broadening caused by collapsing pore canals is visible. During observation of the cuticle's cross section (transverse II), this broadening is also occurring, but does not become visible. d) Schematic sketch showing the orientation of the fracture plane in samples tested in transverse direction. Reproduced with permission from [40]. Copyright 2008 Elsevier.

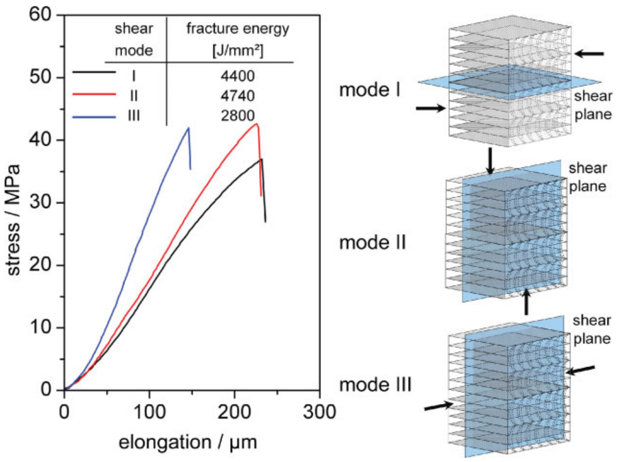

Figure 8. Averaged displacement-stress curves for the three different shear modes I, II, and III. In mode I, the shear plane is parallel to the cuticle surface. In modes II and III, the shear plane is perpendicular to the cuticle surface, but the shear direction is either in the normal (mode II) or in the transverse direction (mode III). Modified with permission from [40]. Copyright 2008 Elsevier.

canals also contain chitin-protein fibers, which are probably reinforcing them.[16,24]

By performing the compression tests combined with a detailed local strain analysis via digital image correlation, the heterogeneity of the elastic–plastic deformation of the endocuticle at the microscopic scale can be observed with high resolution.[40] The local strain analysis revealed significant differences in the plastic deformation behavior between samples that were compressed in normal and in transverse direction. By comparing the two observation directions in the transversally compressed cuticle, a strong anisotropy in the plastic deformation can be found. While in the samples tested in normal direction a relatively homogeneous strain distribution was observed in longitudinal direction, the samples tested in transverse direction developed band-like regions of high strain, which expanded into the adjacent areas. Their evolution corresponds to the plateau region in the global stress-stain curve, indicating that in these zones the honeycomb-like structure of the pore canal system is already collapsed, while the adjacent areas are still intact. During compression in transverse direction, the samples become shorter in longitudinal direction, due to collapsing pore canals that are responsible for the honeycomb-like structure, analogous to the elastic behavior of the samples described above (Fig. 7). In lateral direction, two deformation modes are observed, depending on whether observed at the surface of the sample (observation direction I) or at the cross-section of the sample (observation direction II). In the first case, the collapsing pore canals lead to a broadening of the sample. This broadening of the sample cannot be observed in observation direction II, because the pore canals are parallel to the observation direction. Distinct differences of the local deformation cannot be observed between the dry and the wet state. The underlying deformation mechanisms become visible by examining the microstructure of test specimens after compression. Due to the complex microstructure, it is difficult to monitor signs of deformation in the samples tested in the normal direction in the dry and the wet state, as they were merely deformed about 10%. In contrast, in the transverse testing direction, two forms of deformation mechanisms can be observed (Fig. 9). In the layers where the fibers are oriented perpendicular to the compression direction, the pore canals are collapsed (Fig. 9a and c), while in the layers where the fibers are oriented parallel to the compression direction the fiber bundles buckle into the cavities of the pore canals (Fig. 9b and d). Noteworthy, the pore canal tubes appear hardly damaged, and are obviously able to prevent delamination of the layers when these are distorted against each other. Consequently, the samples do not expand in this direction. The buckling of fibers demonstrates their ability to undergo large plastic deformation in the wet and also in the dry state. However, in the dry state, microcracks are visible, which do not lead to a brittle failure due to the compression loading (Fig. 9a). The deformation described is likely to take place in the plateau region of the stress-strain curve. When a critical stress level is reached in transverse direction, the honeycomb-like structure starts to collapse, followed by progressive failure of the adjacent structure. There is no significant difference in the deformation behavior in the transverse direction in the dry and wet state, except that the

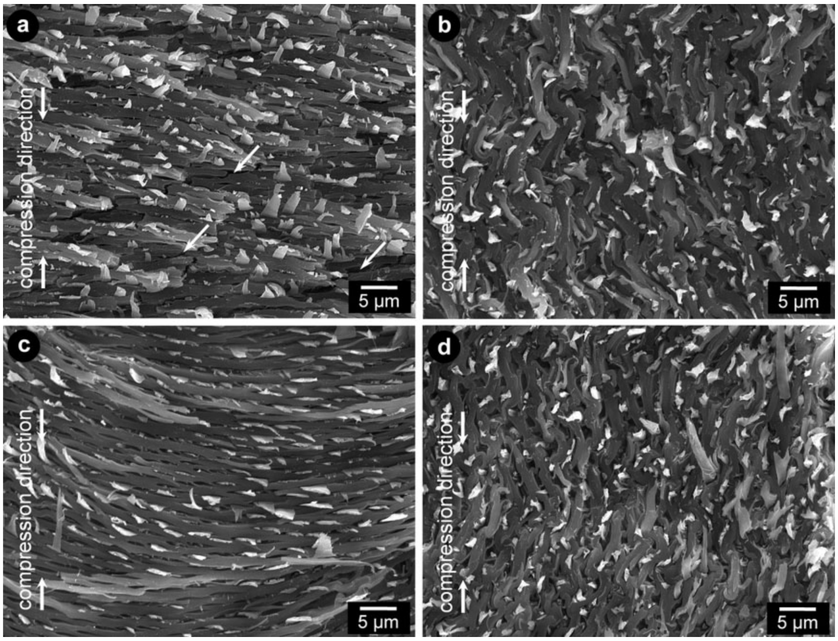

Figure 9. SEM images of compression-test specimens fractured after being tested in transverse direction in the a,b) dry and the c,d) wet state. In layers where the fibers are oriented perpendicular to the compression direction, the pore canals are collapsed, and microcracks (arrows) have formed in the a) dry state but not in the c) wet state. In layers where the fibers are oriented parallel to the compression direction, the fiber bundles have buckled irregularly into the cavities of the pore canals (b,d). Modified with permission from [40]. Copyright 2008 Elsevier.

stress threshold is much higher in the dry than in the wet state. During compression, hydration does not seem to affect the deformation and fracture behavior of lobster cuticle in the same dramatic way than during tension. This is due to the fact that beyond the yield point, the endocuticle undergoes a densification instead of a separation of fibers. This hinders crack opening and propagation, and thus delays the failure of the test specimens. Nevertheless, hydrated samples are more ductile and support higher strains to fracture than dry samples. Additionally, hydration seems to impede the formation of microcracks in the structure during compression, which supports the role of water as a plasticizer.[34] Generally, the observed characteristics in the deformation are typical for honeycomb structures. The observed and measured properties in the transverse direction of lobster endocuticle are quite similar to the mechanical response of a classical honeycomb in its in-plane direction. The same is valid for the normal direction, which corresponds to the out-of-plane direction of a honeycomb structure.[41]

4. Outlook

In contrast to the highly anisotropic elastic–plastic deformation behavior, the elastic properties of the endocuticle, such as the elastic moduli, are relatively isotropic, which is rather untypical for honeycomb-like structures. In order to understand this observation, it is necessary to examine the mechanical behavior of lobster cuticle on the next lower level of the hierarchical organization, the mineralized chitin-protein fibers forming the plywood- and honeycomb-like structure. The orientation dependence of the elastic properties and the hardness and the influence of the grade of mineralization of fibers can be examined by probing cross-sectional areas of the endocuticle and the exocuticle using nanoindentation techniques. Nanoindentation experiments on human and bovine osteonal bone on a similar length scale have shown heterogeneous responses of the probed microstructure, although a direct correlation to the orientation of the mineralized collagen fibers could not be shown, due to the much thinner plywood lamellae and periodic variations in the mineralization occurring in these materials.[42,43] The stacking heights of about 10 μm in exocuticle and 30 μm in endocuticle plywood lamellae in the lobster cuticle, and its much simpler layered structure in comparison to osteonal bone, would be very suitable for such experiments. Previous investigations using nanoindentation on air-dried cuticle have shown that the elastic properties seem to be mainly affected by the grade of mineralization.[44] Such experiments also present a considerable experimental challenge, since obtaining reliable results for the natural hydrated state of the material involves overcoming problems like dehydration during probing.

Owing to the hierarchical nature of the structure of biological matter, it is useful to develop corresponding multiscale modeling approaches. They may help to better understand the mechanical properties of natural materials at each hierarchical scale, and also the coarse graining mechanisms that produce the final global mechanical response to external loads. Multiscale modeling also allows for a more detailed analysis of experimental results, as has been shown recently for the elastic properties of bone at sub-micrometer scales.[45] Developing such a model for lobster cuticle would allow the calculation of the anisotropic properties of bulk mineralized tissues in terms of the orientation of the chitin-protein fibers and the mineralization grade.

5. Conclusions

The cuticle of the American lobster is an ideal model material for studying mechanical properties of hierarchically organized fiber-based biological composites. This is due to its suitable dimensions and its microstructure, which is less complex and better defined when compared to other materials, such as bone. In biological structural materials, like arthropod cuticle, the mechanical properties vary on different length scales, due to the hierarchical organization of these materials. Tensile tests show the influence of mineralization and hydration on the deformation and fracture behavior of the cuticle. While unmineralized hydrated cuticle deforms plastically, a higher grade of mineralization leads to an increase in stiffness and reduced plasticity. Dehydration of the organic matrix leads to an increase in stiffness, and to a complete loss of plasticity. Due to the well-developed pore canal system, the mineralized material deforms like a honeycomb structure under compression. However, the twisted plywood arrangement of the fibers makes the in-plane direction stiffer than the normal direction. The flexible tubes in the pore canals increase fracture resistance in transverse direction, as shown by shear testing. The macroscopic mechanical properties demonstrate that the combination of the twisted plywood and the honeycomb structure results in a material with better properties at lower weight, and economical usage of raw materials and energy. This interaction of different construction principles can be used as a template for the design of novel high-performance materials.

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