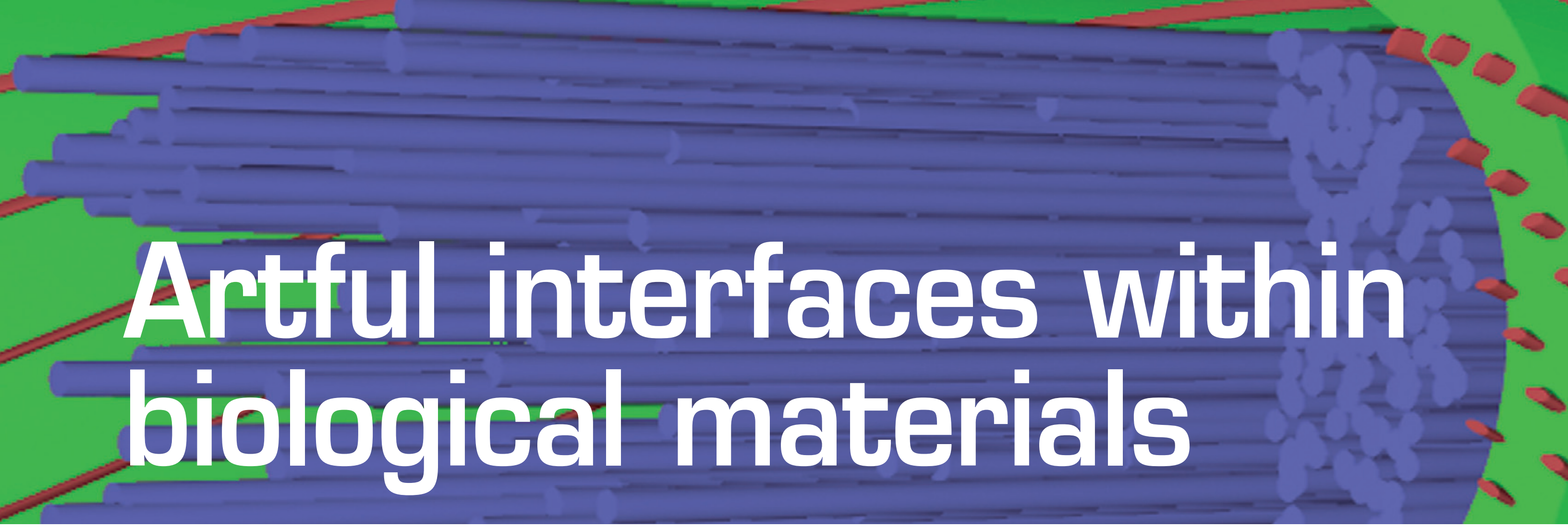

Artful interfaces within biological materials

Biological materials have a wide range of mechanical properties matching their biological function. This is achieved via complex structural hierarchies, spanning many length scales, arising from the assembly of different sized building blocks during growth. The interfaces between these building blocks can increase resistance to fracture, join materials of different character, make them deform more easily and provide motility. While they represent only a tiny fraction of the overall volume, interfaces are essential for the integrity and function of the overall tissue. Understanding their construction principles, often based on specialized molecular assemblies, may change our current thinking about composite materials.

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Biological materials are usually complex macromolecular assemblies, often surrounding the cells in an organ in the form of an extracellular matrix. Hard biological materials, such as bone, enamel or sea shells contain, beside soft organic material, large fractions of inorganic mineral. Remarkably, they possess mechanical or other physical properties by far superior than their constituents[1,2]. Bone, for example, is a fracture-resistant composite of collagen, a comparatively soft fibrous protein, and brittle calcium phosphate mineral[3]. Since the pioneering work of D'Arcy Thompson[4], it is known that the shape and internal arrangement of the components largely determine the functionality of biological materials[5]. They grow by the successive assembly of larger and larger elements synthesized and organized by living cells. The resulting structure is generally hierarchical[6], spanning many length scales. For materials scientists, there is much to be learned from studying how the structural organization of biological materials relates to their extraordinary properties[7-10].

How the geometric distribution of bonds in a hierarchical structure controls the mechanical behavior is an unsolved problem. Theoretical considerations have shown that introducing a sufficient number of hierarchies in a multi-scale composite material based on stiff and soft components, may increase the toughness and defect-tolerance almost arbitrarily[11]. A hierarchical structure implies that building blocks of different sizes have to be joined to make up a useful material. Nature excels particularly in generating interfaces of various types which govern to a large extent the overall properties of the hierarchically structured biological material.

In this short review, we suggest a classification of internal interfaces in biological materials into four categories according to their mechanical function as highlighted schematically in Fig. 1 and in Table 1:

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Table 1 Examples of biological tissues containing internal interfaces following the classification given in the text.

Interface category	Examples of biological materials containing these interfaces
(a) Fracture resistance	Silica sponge skeleton[12,39-41], bone[3,32-36], nacre[13,28-30,38,45-53], bio-inspired examples [31,54-56].
(b) Bridging materials properties	Bone-ligament junctions[57], cartilage-bone junctions[58,59], bone porosity gradients [63], osteons in bone[66], Humboldt squid beak[61,62], fiber gradients in palm trees[64,65], mussel byssus[14,75-80], tissue junctions in teeth[15,67-74,106].
(c) Deformability	Turtle suture[16], skull suture[92], bone[17,90,91], wood cell wall[84-88], tesserae in mineralized shark cartilage[94-96], armor plates of stickleback fish[93].
(d) Actuation and stress generation	Pine cone[18,102], wheat awn[19,101], seed capsules[21], tension wood[103], contractile roots[104].

a) Interfaces which improve the fracture resistance of inherently brittle materials, e.g. the protein layers found in the skeleton of Euplectella[12] and in nacre[13].

b) Interfaces that act as bridges or joints between materials having a high contrast in materials properties, e.g. the gradient in mechanical properties found along the mussel byssus connecting the soft body of the mussel to a hard rocky substrate[14] or in the dentine-enamel junction in teeth[15].

c) Interfaces that allow materials to deform easily, e.g. the suture of the turtle shell[16] or at smaller length scales, in the non-collagenous protein layers found in bone[17].

d) Interfaces that allow materials to act as actuators upon external stimuli allowing for the development of motion and forces, e.g. the motion of the pine cone[18] or the wheat awn[19] upon changes in humidity.

Of course this list is incomplete and material interfaces in biology can also be discussed beyond this classification scheme. Biological interfaces may also be designed to break in controlled ways, for example allowing the self-sharpening of the sea-urchin tooth[20], or in the design of the joint in seed pods, which ruptures to allow for explosive seed dispersal upon drying[21]. Temporary interfaces may form, such as attachment pads in insects[22] or the gecko foot[23], or external connections mediated by a glue secreted by the organism, like in the attachment of mussels[24] or of ivy[25] to rocky substrates. In the present review, we focus on internal interfaces. The versatility of such interfaces seems to control the properties of biological materials to a greater extent than generally thought. This is particularly significant since the amount of molecules involved in the fabrication of interfaces is a relatively small fraction of the total mass of the bulk material. As a consequence, comparatively "expensive" substances may be used in the design of

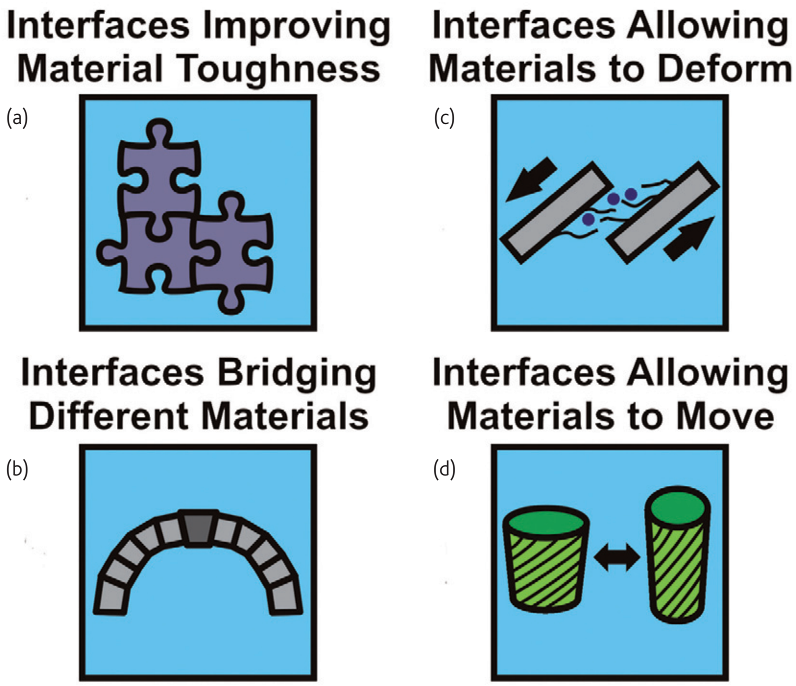

Fig. 1 Four different examples of how interfacial design can influence the mechanical behavior of biomaterials are addressed: (a) interfaces which improve the fracture resistance of a material by introducing soft interfaces (e.g. nacre), (b) interfaces that act as bridges between materials of highly different mechanical properties (e.g. mussel byssus), (c) interfaces that allow materials to deform plastically (e.g. bone and wood), and (d) interfaces that allow materials to act as actuators of motion/stress (e.g. the scales of the pine cone).

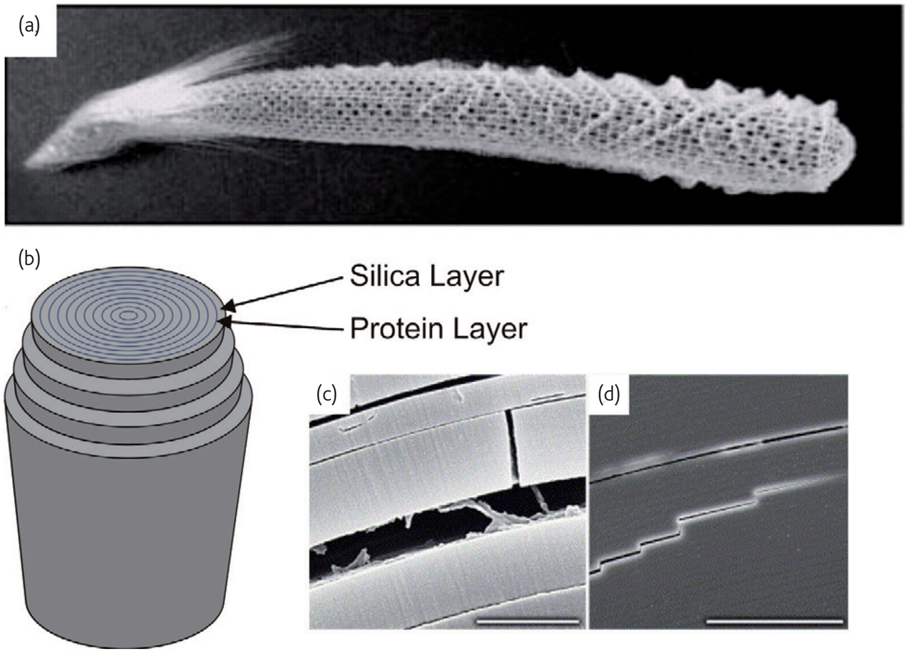

Fig. 2 (a) The glass spicules which make up the skeleton of the deep sea sponge Euplectella sp. are examples of a system in which the interfaces play an important role in improving the fracture resistance of the brittle silica. Reprinted from[12] with permission from AAAS. (b) A sketch of the cross section of one of the spicules, illustrating the concentric layers of silica separated by thin protein layers. (c) SEM image of a fracture within a layer showing the presence of soft interfacial proteins (scale bar 500 nm). (d) A macroscopic SEM micrograph of the fracture path showing crack deflection due to the soft layers (scale bar 50 μm). (c) & (d) Reprinted from[39] with permission.

functional interfaces within composite materials. It is likely that a better understanding of their governing principles may significantly impact the way we think about composite materials today.

Enhancing the fracture resistance of brittle materials

Hard biological materials are often based on ceramic phases, common examples being silica, calcium phosphate and calcium carbonate[26]. As the ceramic phases are inherently brittle, a specific control of the structure of these phases and their interfaces inside the composite is required to produce a reliable structural material. To do this, Nature uses several strategies, to avoid the formation of cracks by controlling particle size and structure, to facilitate (irreversible) deformation, and to design structures that hinder crack propagation.

The materials can be made tolerant to defects by reducing the dimensions of the hard and brittle phases. If the length scale of mineral particles is kept to a size smaller than the critical Griffith size, small cracks and defects will typically stay below the critical size for crack propagation[27]. In addition it is also now becoming clear that the presence of proteins occluded within the minerals themselves may lead to toughening[28-30]. Incorporated proteins can introduce internal stresses into the mineral which may hinder crack propagation through the interface[30], much akin to pre-stressed reinforcement in concrete. Crack propagation can be hindered by a large interfacial area within the material which traps or deflects the cracks[31]. Mechanisms of this kind as well as bridging by un-cracked ligaments are clearly seen in the example on crack propagation in bone[32-34]. Alternating layers of the mineralized fibrils with different orientations[35], as well as cement lines in compact bone[32] provide a huge reservoir of mechanically active internal interfaces at all scales[36]. While weak interfaces can stop or deflect cracks[31], it has also been shown that a sufficient variation in elastic modulus can also stop crack propagation even with strong interfaces[37]. Such variations have recently been measured in nacre using nano-dynamic mechanical analysis (DMA)[38].

The glass spicules making up the skeleton of the deep sea sponge, *Euplectella*, (Fig. 2a) are natural materials in which the incorporation of soft interfaces greatly improves the fracture resistance of the inherently brittle silica[12,39]. Each spicule is made up of micron thick concentric rings of nanoparticulate silica separated by 5 – 10 nm thick layers of proteinaceous "glue"[39] (Fig. 2b). This soft glue (Fig. 2c) although not yet fully characterized, consists of chitin and collagen like proteins[40,41], the properties of which are strongly modified by the presence of water[42]. The consequence of a structure of soft and hard multilayers can be seen in (Fig. 2d) in which crack propagation perpendicular to the layers is deflected along the interface[43]. The presence of the protein interfaces leads to an improvement of fracture resistance with respect to the bulk material by around 2.5 times, which is remarkable keeping in mind the small quantities of protein present (< 1 %)[44].

A similar principle of interfacial design for improving fracture resistance of brittle materials can also be found in the well studied

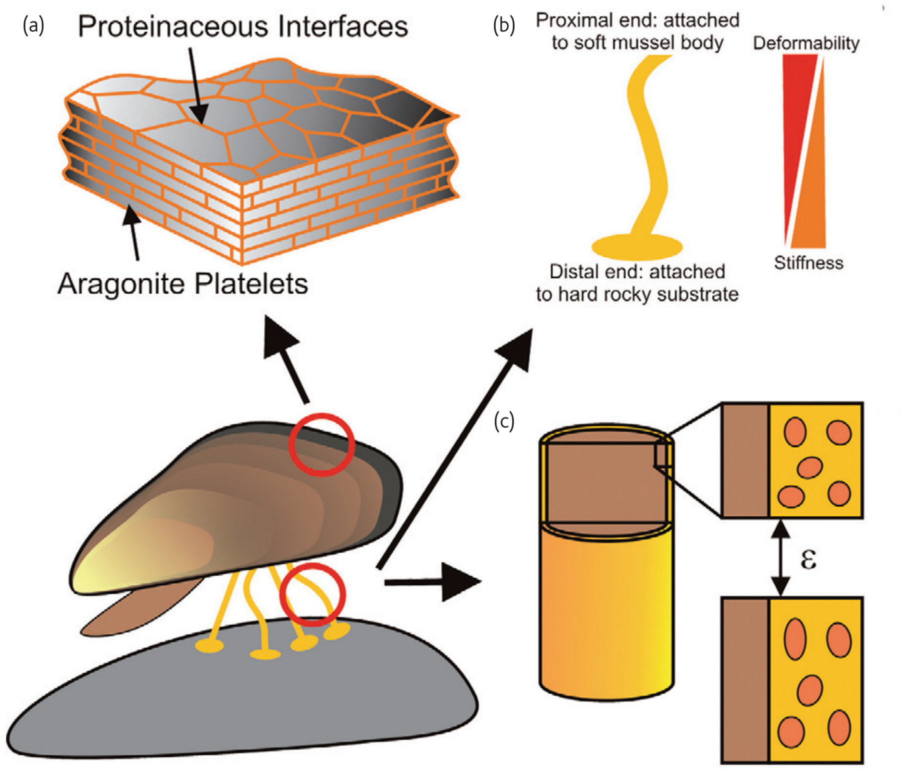

Fig. 3 The mussel has several examples of different materials whose performance is improved through interfacial control. (a) The shell is made of tablets of calcium carbonate in a brick-and-mortar arrangement, glued together by a thin protein layer (figure based on[46]). (b) The mussel attaches itself to rocky substrates via a collection of byssus threads. The byssal thread has a gradient in mechanical properties, being soft and flexible close to the mussel body and stiffer close to the rock surface (figure based on[14]). (c) In some species the byssus is coated by a stiff abrasion resistant coating. This coating is a composite of micron sized granules stiffened by a higher amount of cross-linking with the surrounding matrix. The structure of the interface allows the coating to co-deform to large strains with the underlying material (figure based on[80]).

system of nacre (Fig. 3a)[45-51]. Nacre is an assembly of ~500 nm thick calcium carbonate tablets 5 – 15 μm in diameter, glued together by nanometer thick layers of soft protein[28-30] in a brick and mortar arrangement. Like with the glass sponge and bone, crack propagation is hindered and deflected at the soft protein interfaces. In addition to crack deflection there are a variety of other mechanisms at work in the interface, which are thought to improve overall fracture resistance. The platelets do not have completely smooth surfaces, they are partially in contact with each other through mineral bridges[52] and nano-asperities[53], which could allow for some stress transfer between platelets. Fracture of the asperities could also lead to more energy dissipation and therefore improved toughness[48]. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) measurements indicate that the protein layer consists of highly folded protein chains, which upon deformation unfold giving rise to energy dissipation and improved toughness[13].

This concept of introducing soft interfaces into brittle materials has inspired a variety of artificial ceramic-based composites[54-56]. Thin layers of graphite, for example, can greatly improve the fracture resistance of silicon carbide[31] and layers of polymethyl methacrylate (PMMA) can do the same with alumina[56].

Bridging materials properties

Joining materials of widely different properties is a frequent requirement in load-bearing tissues and organs. A typical example is the insertion of ligaments into bone[57] or the interface between comparatively soft cartilage and bone in the joints[58,59]. An abrupt change in material properties results in stress and strain incompatibilities and may give rise to contact failure when the structure is loaded. One design strategy to mitigate these incompatibilities is to gradually change materials properties across the interface[60]. Such a gradient can be achieved by different means: in the beak of the Humboldt squid, for example, this is realized by a gradual change in water content[61,62] giving rise to stiffness gradients between the hard cutting surface and the softer underlying tissue. Another example is the gradual change in porosity between trabecular and cortical bone[63] or in the variation of cell diameter and cell wall thickness in the fibrous tissue of the Mexican Fanpalm (*Washingtonia Robusta*)[64,65]. A gradual change in fiber orientation was found to modulate the mechanical properties in the osteonal tissue surrounding blood vessels in compact bone[66]. A different strategy is fiber anchoring. This typically happens for the insertion of tendons and ligaments into bone[57], where fibers are anchored within the stiff bony substrate

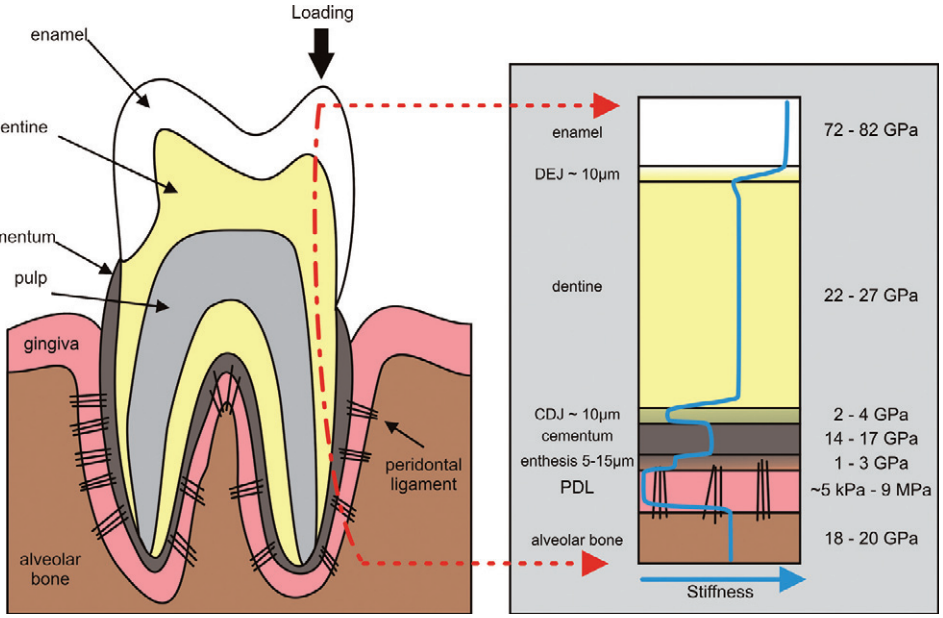

Fig. 4 Left, schematic cross-section of a human tooth indicating the major tissues and the interfaces between them (figure based on[68]). Right, a sketch (not to scale) of the stiffness variation passing through the different tissues (red line in left image) using the same color coding. In addition the interfaces between the tissues are sketched with the approximate widths of the layers indicated: the dentine enamel junction (DEJ), the cementum-dentine junction (CDJ), and the enthesis attaching peridontal ligaments (PDL) to the cementum (figure based on[106], also using data from[107]).

material. With this "blending" of two materials, a clear distinction of where one material ends and the new one starts gets lost.

Multiple examples of both types of strategies, grading and fiber anchoring, can be found within a single organ, such as the tooth[67]. The huge loads produced by chewing (up to 1 kN in humans[68]) have to be taken up by a hard and wear resistance coating (enamel), and transmitted through the tooth body, mainly consisting of dentin, to the alveolar bone in which the tooth is fixated. Several internal interfaces join the relevant tissues: a periodontal ligament (PDL) holds the tooth within its bone cavity and is anchored into the bone on one side and into the tooth cementum on the other[69] (Fig. 4). Further interfaces are the dentin-cementum junction and the dentin-enamel junction (DEJ)[15,70,71]. A key parameter in controlling the stiffness is the amount of mineral incorporated in the materials ranging from more than 95 % of the volume in enamel to roughly 50 % in dentin, but also collagen fiber orientation, particle size and other factors contribute[15,68,72]. The change in stiffness from the hard enamel to the softer dentin goes through a small minimum at the DEJ re-echoing ideas for crack stopping presented in the previous section. Indeed, the DEJ appears to be softer than both enamel and dentin, which contributes to arresting cracks less than 10 µm beyond the (optical) interface between these two tissues[15,70,73]. The presence of the soft periodontal ligament between tooth and bone allows for a small relative movement between tooth and bone. Despite the high loads appearing at this interface, the PDL is solidly anchored into both bone and cementum by 1 – 2 µm deep inserts. For both interfaces the stiffness is graded due to structural (ligament orientation) and chemical changes[74].

A completely different example is the attachment of the mussel (Fig. 3) to a rocky substrate via byssal threads glued to the surface by adhesive plaques[75]. The byssal thread itself has a gradient in mechanical properties along its length (Fig. 3b). The stiffness increases by almost a factor of 50 from the soft and flexible proximal region of the byssus up to 900 MPa at the stiffer distal portion of the byssus close to the rock surface[76]. Likewise there is a gradient in deformability of the byssus with the proximal region having around twice the extensibility of the distal region[76]. This gradient in mechanical properties is mediated by composition gradients in collagen-containing proteins[14,77]. In some species (such as *Mytilus galloprovincialis* found in the turbulent inter tidal zone) the byssus is also coated by a stiffer abrasion resistant coating[78,79]. This coating is a composite of micron sized granules stiffened by a higher amount of iron-mediated cross-linking than the surrounding protein matrix[80] (Fig. 3c).

Deformability through soft interfaces

A widespread design principle of biological materials is to build a composite material[81] consisting of stiff, often fibrous, reinforcements embedded in a soft deformable matrix. On deformation, the reinforcement carries the load and provides stiffness to the material. As stiff materials are often brittle, the reinforcement must be protected against large deformations, which is the task of thin and soft interface layers. These flow plastically before the fracture strain in the reinforcements is reached. The result is a stiff material, which can undergo large irreversible deformations[82]. The bonding by the

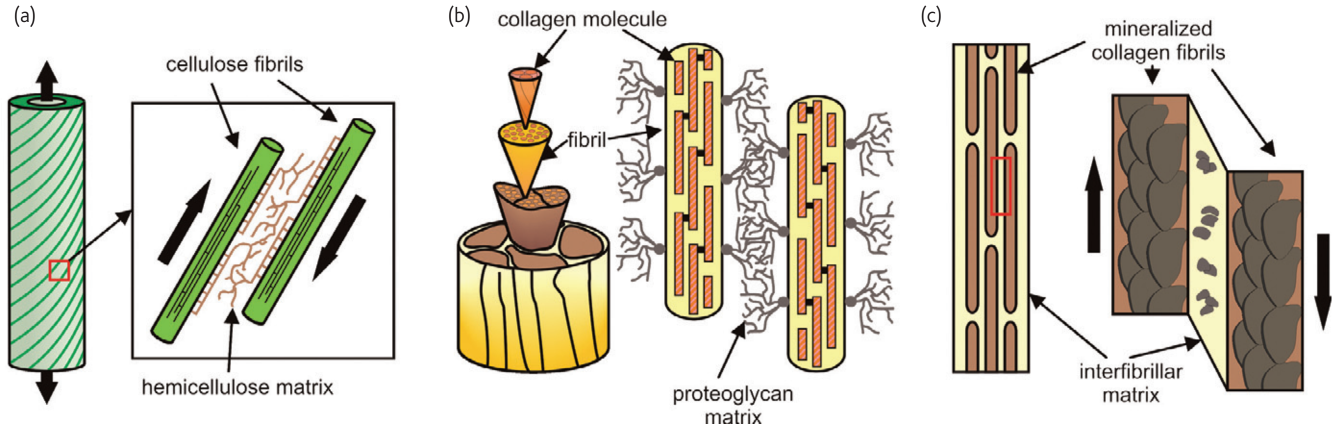

Fig. 5 Interfaces providing plastic or viscoelastic deformation between (a) cellulose fibrils in the wood cell wall (figure based on[84]), (b) collagen fibrils in the tendon (figure based on[89]), and (c) mineralized collagen fibrils in bone (figure based on[90]).

interface makes frequent use of a large number of comparatively weak bonds, based on electrostatic forces or hydrogen bonds, for example. Under load, these "sacrificial bonds"[13,17] can break, but are then reformed in a different arrangement, thereby dissipating energy during the slippage of the reinforcements. This results in an irreversible (plastic) deformation which depends on the spatial arrangement of the bonds[83].

Fig. 5 shows three different systems – wood cell wall, tendon and bone – in which the same principle of such a "Velcro-like[84]" interface is acting, albeit based on a quite different biochemistry. In wood the stiff cellulose fibrils run helically within the cell wall (Fig. 5a). When stretched uniaxially, the tilt of the fibrils adjusts itself towards the loading direction. For such a reorientation, the cellulose fibrils have to slide against each other. This is made possible by the hydrogel-like matrix of hemicellulose between the fibrils, where a breaking and reforming of hydrogen bonds probably occurs during the flow of the hemicellulose matrix[84-88] (Fig. 5a). The surfaces of collagen fibrils within a tendon are covered in proteoglycans, which are branched molecules negatively charged under physiological conditions, allowing for plastic flow of the matrix[89] (Fig. 5b). Plastic flow in the matrix has also been demonstrated in bone[90]: less than half of the externally applied deformation is experienced on the scale of the mineralized fibril (Fig. 5c), and roughly only a sixth at the level of the nanometer-sized mineral particles[90]. The interfibrillar glue layer is probably composed of negatively charged polyelectrolytes and reinforced by mineral particles. Measurement of an activation enthalpy of about 1 eV within a typical volume of 1 nm^3 indicates that the electrostatic bonding is mediated by divalent calcium ions[91].

A very different example is the complex bony sutures found in the ribs making up the shell of the turtle[16] (Fig. 6). The suture of the red slider turtle is highly convoluted and consists of interdigitating fingers of bone separated by a soft collagenous interface (Fig. 6b). The soft interface permits small deformations under bending and confers flexibility of the shell (Fig. 6c); however upon larger deformations the fingers interlock

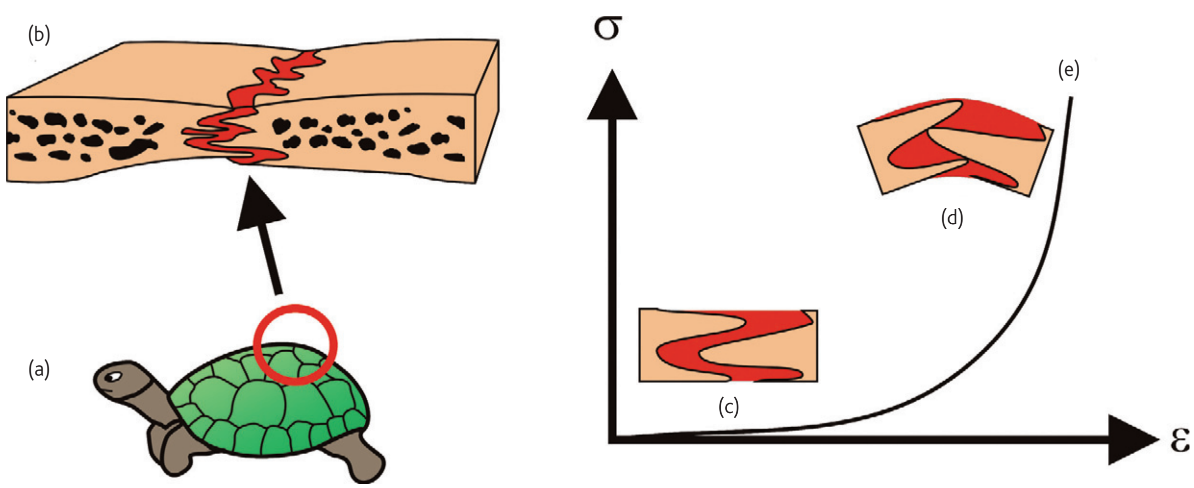

Fig. 6 (a) The shell of the red slider turtle is made up of (b) modified ribs which are linked together by a suture consisting of interdigitating protrusions of bone separated by a soft collageneous layer. For small amounts of bending (c), deformation is concentrated in the soft layer, however upon large deformations (d) the interdigitations of bone interlock resulting in a significant stiffening of the composite, as illustrated by a schematic stress-strain curve (e) (figure based on[16]).

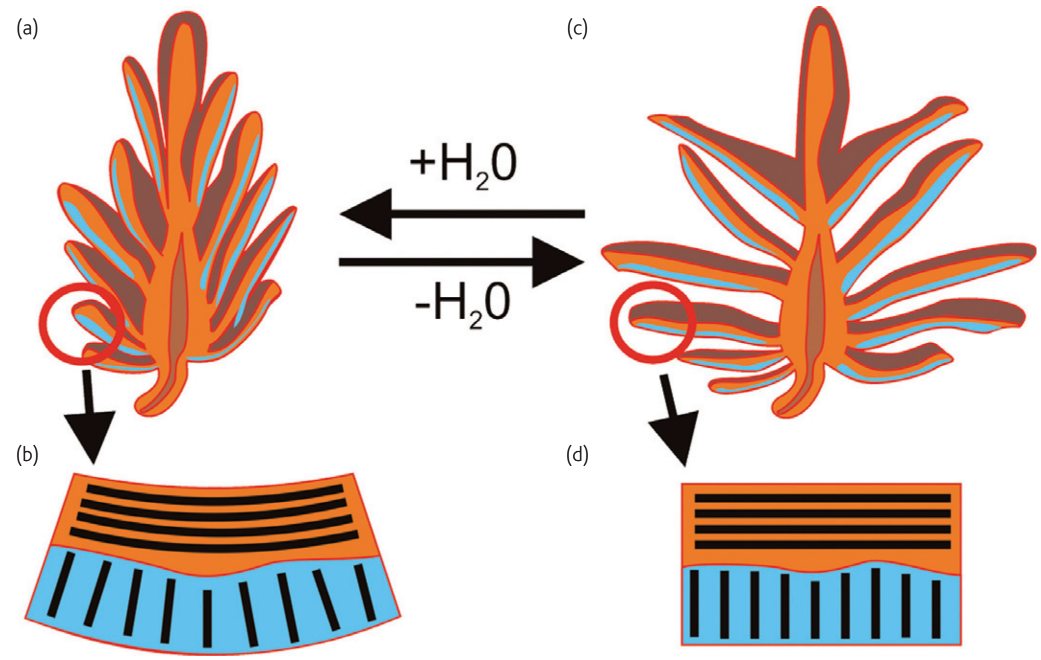

Fig. 7 A sketch of actuation of scales in a pine cone upon (a) drying and (b) wetting. Each scale can be viewed as a bilayer structure (c,d) in which the swelling/shrinking direction in each layer is constrained by the orientation of the stiff cellulose microfibrils. The bottom layer (blue) upon shrinking will contract along the length of the scale however, the stiff upper layer will not contract, thus causing the pine cone to open (figure based on[18] and[86]).

(Fig. 6d) giving rise to a marked stiffening of the interface as schematized in Fig. 6e. Other examples of similar interlocking structures include the bony sutures found in skulls[92], the bony armor-plates of the three-spine stickleback[93] that provide a flexible protective system, and the interlocking mineralized tesserae found in sharks and rays[94-96]. How the free space between interlocking elements provides flexibility and hinders crack propagation has also been demonstrated by materials engineered by "prefragmentation"[97,98]. Structures can be made by specially shaped bricks which geometrically interlock such that the failure of an individual brick does not propagate into the rest of the structure[99].

Interfaces to develop forces and motion

Many plant organs move or develop stresses upon changes in humidity, in contrast to the fast movements of the well-known Venus fly trap[100] which requires that active chemical energy be provided by the organism[86]. Plant organs that move upon hydration include the wheat awn[19,101], the pine cone[18,102], the seed capsules of Acanthaceae[21], and examples of stress generating organs include tension wood[103] and contractile root systems[104]. The governing principal behind all of these hydroscopic actuators is that they consist of at least two types of tissues which can contract or expand with differing amounts upon hydration or drying[9,105]. Possibilities are that two materials with different swelling properties constrain each other over a tight interface like in a bimetallic strip. An alternative is to make use of external geometric constraints to control the direction of swelling of a tissue.

Two examples from actuating plants, where swelling of different tissues is mediated by the architecture of the stiff cellulose fibrils, are the pine cone and the wheat awn. The hygroscopic component of the cell walls is the hemicellulose matrix responsible for the plastic deformation of wood encountered in the previous section (Fig. 5a). The pine cone (Fig. 7) is a well-known natural actuator in which the dead tissue that makes up the scales moves upon changes in humidity allowing the seeds inside the pine cone to be released[18]. Each scale consists of two types of tissues, one consisting of cells in which the cellulose microfibrils are aligned along the length of the scale and the other in which they are perpendicular[102]. Upon drying the interfacial matrix between the fibers shrinks on the lower half of the scales (Figs. 7c and d). The presence of the fibers leads to anisotropic contraction which is hindered by the stiffer surrounding tissue, thus leading to a bending of the scales (Figs. 7a and b). A similar principle to the bilayer is observed in wheat awns; the "antenna-like" structures attached to the wheat grain[19,101]. A cross-section through the awn again reveals two tissue types with different cellulose organization. One with cellulose fibrils aligned along the awn, thus constrained to swell anisotropically, and the other with more randomly oriented cellulose fibrils, which swell more isotropically. This allows the awns to open and close in a "swimming movement", thus propelling the seed along the ground.

Another example of geometric constraints on a swelling tissue is found in the red clover. This has a contractile root system that enables it to actively pull the foliage buds into the ground as the plant grows[104]. It is thought that this contractile process helps protect underground organs from external environmental changes as well as facilitate vegetative spreading of the root system. The active root contraction

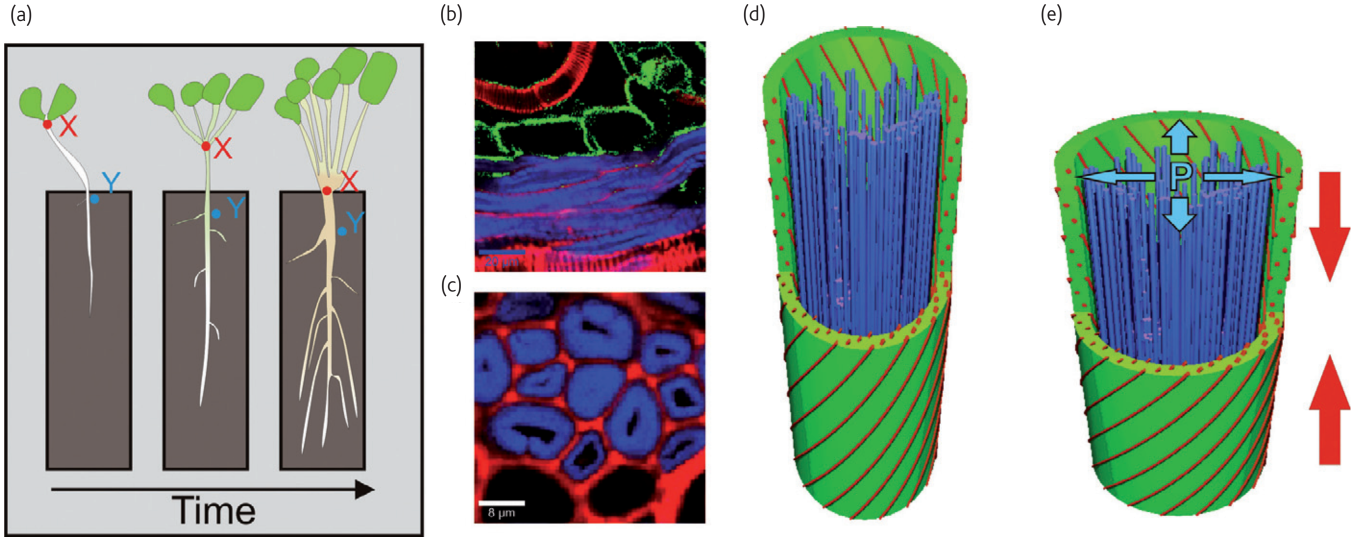

Fig. 8 (a) A sketch of the early stages of growth of Trifolium pratense (red clover). As the root develops with time it actively pulls the plant into the ground (see motion of points X and Y with time) through active contraction of the root system (see relative displacement between X and Y). Raman images of (b) longitudinal and (c) transverse cross-sections of the root highlight the presence of the specialized contractile tissues pectin (green), cellulose (blue), and lignin (red). (d, e) A sketch of how contraction works: parallel fibred cellulose layers push against the cell wall which then contracts, pulling the plant into the ground. Permission to reprint the Raman images was kindly granted by Nicole Schreiber and Burgi Gierlinger.

that occurs during the early stages of growth is schematized in Fig. 8a. Note that during growth both points X and Y move downwards and that the relative distance between them decreases indicating a shortening of the root itself. A specialized tissue (the G-layer), is thought to be responsible for contraction. The microstructure of the G-layer containing tissue is highlighted in Figs. 8b and 8c in Raman images taken of longitudinal and transverse cross-sections. In this tissue the lignified plant cell wall (marked red in Figs. 8b and c) is filled with thick hygroscopic layers of parallel fibred cellulose (blue in the Raman image). A schematized version of the model of the G-layer filled cell is given in Fig. 8d. It is postulated that the interfaces between the cellulose fibrils absorb water upon hydration, and swell. However, the stiff fibrils constrain swelling in an outward direction and the G-layer then pushes out against the cell wall. As the cell wall is reinforced with spirally wound cellulose microfibrils this outward swelling is converted into a contraction of the entire cell, thus pulling the tissue into the ground. A similar mechanism was also recently hypothesized to be responsible for the development of tensile stresses in the tension wood of poplar, allowing the branch to counteract increasing gravitational loads due to growth[103].

Outlook

Nature has evolved many different principles for constructing interfaces with diverse properties. Just subdividing a brittle material into thin layers (or bricks) by thinner soft layers (usually organic) can dramatically improve the fracture resistance, as in glass sponge skeletons, for example. A similar strategy but with a different geometric arrangement of the interfaces will allow the material to deform much more by shearing of the soft inter-layers. This principle operates in tendon, bone and the wood cell wall. Strong interfaces can be designed by inter-digitation of the surrounding material, by fibers crossing from one side to the other and, most interestingly, by the use of special "amphiphilic" molecules. These molecules strongly bind one end to the material surface and use the other end to form a thin matrix layer between two material pieces. Nature seems to have evolved highly specialized molecules for this task which may serve as inspiration for new types of composite materials, where the matrix surrounding hard particles or platelets is the minority component. Investing a greater research effort into better understanding the very elaborate and specialized molecules making up this matrix could be particularly rewarding. This matrix often takes only a minute fraction of the total volume of the material and yet seems to control many of the material properties. The small volume fraction makes this research challenging, but has great potential to inspire new synthetic materials. mt

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