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Functional gradients and heterogeneities in biological materials: Design principles, functions, and bioinspired applications

Zengqian Liu [a,b], Marc A. Meyers [c,d], Zhefeng Zhang [b], Robert O. Ritchie [a,*]

[a] Department of Materials Science and Engineering, University of California Berkeley, CA 94720, USA
[b] Shenyang National Laboratory for Materials Science, Institute of Metal Research, Chinese Academy of Sciences, Shenyang 110016, China
[c] Department of Nanoengineering, Materials Science and Engineering Program, University of California San Diego, La Jolla, CA 92093, USA
[d] Department of Mechanical and Aerospace Engineering, Materials Science and Engineering Program, University of California San Diego, La Jolla, CA 92093, USA

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ABSTRACT

Living organisms have ingeniously evolved functional gradients and heterogeneities to create high-performance biological materials from a fairly limited choice of elements and compounds during long-term evolution and selection. The translation of such design motifs into synthetic materials offers a spectrum of feasible pathways towards unprecedented properties and functionalities that are favorable for practical uses in a variety of engineering and medical fields. Here, we review the basic design forms and principles of naturally-occurring gradients in biological materials and discuss the functions and benefits that they confer to organisms. These gradients are fundamentally associated with the variations in local chemical compositions/constituents and structural characteristics involved in the arrangement, distribution, dimensions and orientations of the building units. The associated interfaces in biological materials invariably demonstrate localized gradients and a variety of gradients are generally integrated over multiple length-scales within the same material. The bioinspired design and applications of synthetic functionally graded materials that mimic their natural paradigms are revisited and the emerging processing techniques needed to replicate the biological gradients are described. It is expected that in the future bioinspired gradients and heterogeneities will play an increasingly important role in the development of high-performance materials for more challenging applications.

Contents

* Corresponding author.
E-mail address: roritchie@lbl.gov (R.O. Ritchie).

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1. Introduction

The advancement in modern technologies continues to impose more stringent requirements for engineering materials with improved mechanical performance; additionally, there is a pressing need for materials to be more energy-efficient and environmentally-friendly. To address this challenge, there is a continual quest to seek new materials with unprecedented combinations of properties and functionalities, such as stiffness, strength, ductility, toughness and formability, with minimal weight and cost. Unfortunately, many of these properties – prime examples are strength and toughness – tend to be mutually exclusive within individual materials [1,2]. Consequently, the development of new structural materials is invariably an exercise in compromise; this is particularly the case for the traditional design and fabrication strategies which primarily result in homogeneous materials with relatively uniform composition and microstructure, despite the fact that many practical applications encompass heterogeneous states of stress, strain and temperature.

A rational solution to this problem is to adapt the local properties of materials to fit their specific requirements, by locating optimized compositions and architectures in appropriate regions, thereby generating multiple advantages within a single material to create improved global properties. For instance, the mechanical performance of structural materials can be intelligently enhanced by designing superior strength and ductility in those regions experiencing the highest levels of stress and strain, respectively [3–5]. In such a scenario, materials can be made to demonstrate site-specific attributes, *e.g.*, composition, architecture and resulting properties, through the inclusion of varying degrees of heterogeneities or gradients. The term "gradient" is employed here in its broad sense to describe the non-uniform nature of materials, frequently near surfaces or internal interfaces, as opposed to a strict homogeneity; it also represents gradual transitions, rather than abrupt changes, between dissimilar nano-/micro-structural features. It has long been recognized that the introduction of spatial gradients can effectively enhance the mechanical and functional performance of materials by, for example, alleviating stress concentrations or singularities, improving interfacial bonding and enabling new functions [3–10]. Indeed, the creation of gradients, or more specifically the development of so-called functionally graded materials (FGMs), offers one promising pathway towards answering the demands for emerging applications of materials.

With respect to such gradients and heterogeneities, Nature provides a rich source of inspiration for the design and fabrication of high-performance synthetic materials and components [5,11–18]. Natural (or biological) materials are generally composites with spatially heterogeneous and tunable properties. These materials have unique characteristics that distinguish them from synthetic ones, which have been referred to as the seven features of the Arzt heptahedron (Fig. 1a) [11,13,16]: (i) self-assembly, (ii) self-healing, (iii) evolutionary and environmental constraints, (iv) importance of hydration, (v) mild synthesis conditions, mostly at temperatures of ~300 K and pressures of ~1 atm, (vi) multi-functionality, and (vii) hierarchy of structure at nano-, micro-, meso- and macro-levels. Despite the vast diversity of biological materials, the building blocks employed by living organisms are limited principally to hard (bio-mineral) and soft (bio-polymer) phases, which invariably display significantly inferior mechanical properties to many synthetic materials. Nevertheless, natural materials, such as seashells, fish scales, bone and teeth, can possess extremely impressive combinations of properties far surpassing those of their constituents; many even outperform their man-made counterparts [11–18]. Such remarkable performance

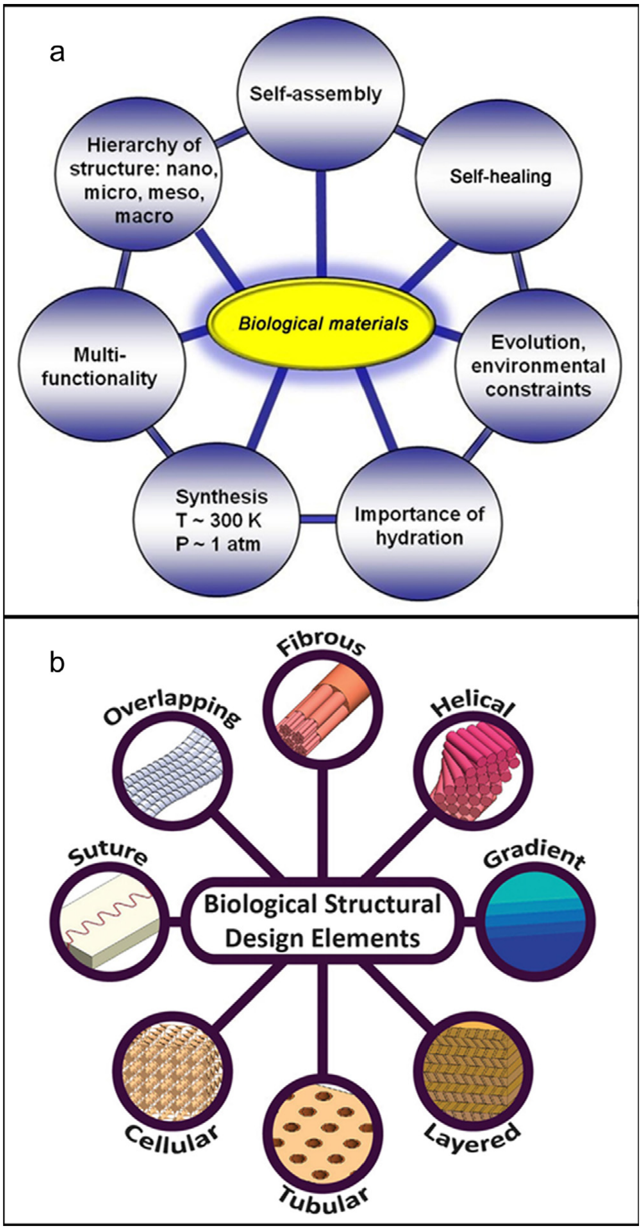

Fig. 1. Characteristics and structural design elements of biological materials: (a) Seven unique characteristics that distinguish biological materials from their synthetic counterparts represented using the Arzt heptahedron [11,13,16]; (b) diagram of the eight most common structural design elements used in biological materials [19].

essentially evolves from the ingenious manner that the building blocks are assembled over multiple length-scales. Indeed, different organisms have developed similar materials design strategies to address their natural challenges as a result of the convergent evolution (Fig. 1b) [11,19], *i.e.*, common features arise during independent evolution. In this context, functional gradients, which have been identified as one basic structural element in Nature [19], are broadly employed in biological materials with diverse architectures [5,11–19]. These gradients have evolved to efficiently fulfill their functions within their specific environmental constraints and, as such, represent ideal paradigms from which meaningful guidelines can be drawn for synthetic materials.

The translation of the adoption of naturally-occurring gradients into synthetic materials offers a spectrum of opportunities to improve their performance for a wide range of applications. Such a strategy relies fundamentally on clarifying the detailed chemical/structural factors responsible for the gradients, understanding the composition/structure-property relationships, and further extracting the underlying design principles. Moreover, the replication of biological gradients in syn-

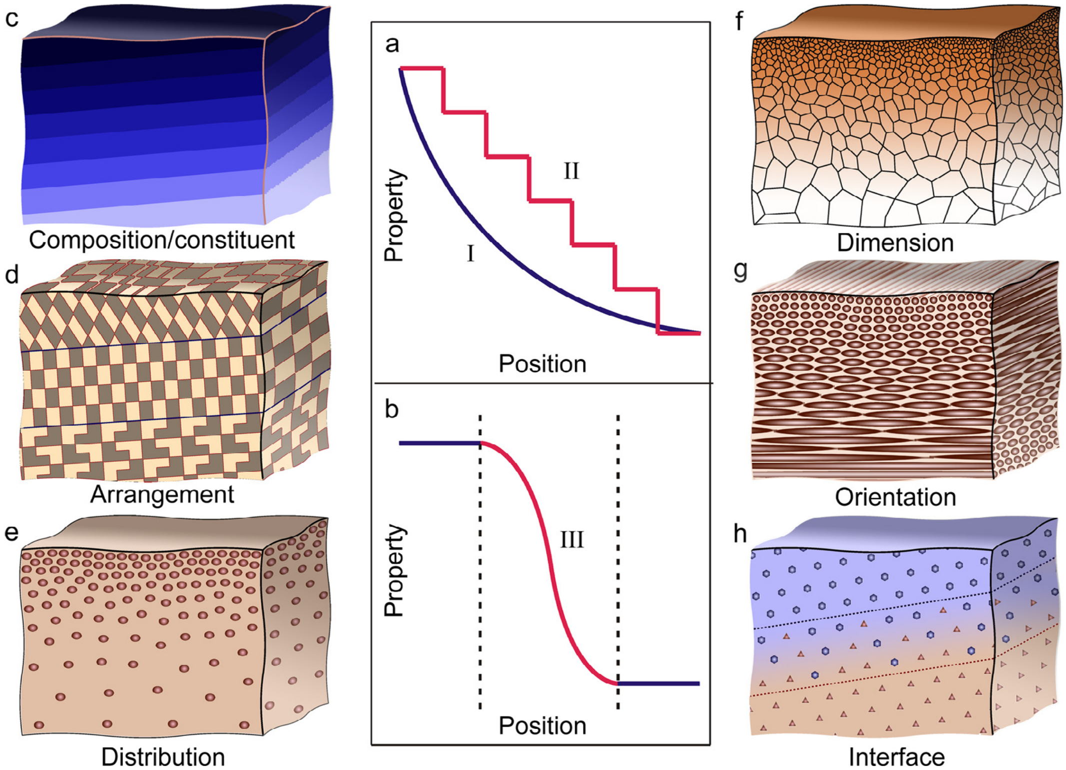

Fig. 2. Local property profiles and basic forms of gradients in biological materials: (a) Local properties change either gradually (I) or in a stepwise manner (II) through the entire material volume; (b) Local properties vary continuously across the interface between dissimilar components; (c–g) The gradients in biological materials are fundamentally associated with the changes in chemical compositions/constituents (c) and structural characteristics, including the arrangement (d), distribution (e), dimensions (f), and orientations (g) of building units; (h) Gradient interface in biological materials.

thetic materials necessitates an effective control of the fabrication processes that for synthetic materials differ markedly from the genetically controlled self-assembly in organisms. Indeed, biomimetics and bioinspiration have become an exciting approach for the development of new materials with unprecedented combinations of properties [18–31]; unfortunately though, there have been few realistic successes with structural materials. One notable achievement is the fabrication of a freeze-cast alumina ceramic containing an infiltrated PMMA phase in the image of nacre (red Abalone shell), which displays marked R-curve behavior with a very high fracture toughness over 30 MPa $m^{1/2}$ due to its brick-and-mortar structure that acts to stabilize the subcritical crack growth [20,21]. Other examples include the burr-inspired Velcro that uses mechanical interlocking between hooks and loops [27], and gecko-inspired reversible attachment devices that use van der Waals forces in nanoscale pillars [16,28]. Although it is still very much of a challenge to create gradients and heterogeneities in synthetic materials in such an intricate manner as in biological materials, the development of new materials processing technologies, as represented by the additive manufacturing [30–35], has enabled a more effective control of the composition and structure at several length-scales, thus making the implementation of bioinspired gradients increasingly practical. In particular, the translation of the design principles underlying biological gradients and heterogeneities offers new pathways towards unprecedented properties and functionalities of materials.

In this review, the functional gradients and heterogeneities developed in biological and bioinspired materials are reviewed and discussed in terms of recent advances in experimental, theoretical and modeling studies. Attention is paid to the general nature of gradients in materials (Section 2), the specific design forms and principles of gradients in biological materials (Section 3), the functions and benefits that they contribute to organisms (Section 4), and the lessons that can be learned from their adoption in the bioinspired design and application of synthetic materials (Section 5). Finally, emerging synthesis and fabrication techniques that may enable the creation of bioinspired gradients are highlighted (Section 6).

2. General nature of gradients

Functional gradients are essentially featured by the creation of site-specific properties distributed within a material that originate from variations in such factors as composition, microstructure and geometry. The basic structural units of gradient-involved materials are referred to as material ingredients [7]; as such, the gradients can be described and interpreted in terms of their changes. Spatially, the ingredients may vary across a wide range, *e.g.*, throughout the entire material volume,

Table 1
Design forms and principles of gradients in biological materials.

Gradient		Example materials	Description	Reference
Chemical compositions/constituents	Mineralization	Chiton radular tooth, elasmoid fish scale, crayfish mandible	Higher mineralization degree towards surface	[40–44]
	Inorganic irons	Spider fang, *Glycera dibranchiate* jaw, *Nereis virens* jaw	Increasing ion concentration towards tip and periphery	[45–51]
		Byssal thread	Inorganic ions enriched in cuticle	[62,63]
	Biomolecules	*Nereis virens* jaw	Higher concentration of histidine residues towards tip	[45,51]
		Tarsal setae of ladybird beetle	Decreasing proportion of elastic protein resilin from tip to base	[53,54]
		Squid beak	Decreasing content of histidine-rich proteins from tip to base	[57–60]
		Byssal thread	Stiff and compliant proteins predominating in distal and proximal portions, respectively (length direction)	[45,61]
			Dopa-rich protein enriched in cuticle (radial direction)	[62,63]
	Hydration	Squid beak	Increasing hydration degree from tip to base	[57–60]
		Horse hoof	Decreasing hydration degree along inner-to-outer direction	[73,95]
Structural characteristics	Arrangement	*Saxidomus purpuratus* shell	Porous and crossed-lamellar arrangements in middle and inner layers, respectively	[67–69]
		Haliotis laevigata abalone shell	Amorphous layer existing at the surface of aragonite platelet	[70–72]
		Alligator osteoderm	Comprising four regions with different structural organizations	[74,75]
		Wheat awn	Well aligned fibrils in the cap and random organization at the ridge	[76]
		Pine cone scale	Fibrous sclerenchyma region and porous sclerid region	[77,78]
	Distribution	Elk antler	Decreasing porosity from cancellous bone to compact bone	[80,81]
		Squid sucker ring	Decreasing pore fraction from core to periphery	[82,83]
		Wood stem	Decreasing porosity from earlywood to latewood	[87–89]
		Bamboo stem	Increasing density of vascular bundles towards surface	[90,91]
		Horse hoof	Increasing tubular density along inner-to-outer direction	[73,95]
	Dimensions	Lobster/crab exoskeleton	Increasing layer thickness from exocuticle to endocuticle	[96–98]
		Sponge spicule	Decreasing layer thickness from near the core to periphery	[100,101]
		Equine superficial digital flexor tendon	Small and large fibrils enriched in myotendinous and osteotendinous junction regions, respectively	[102]
	Orientations	Softwood branch	Decreasing microfibril angle from lower side to upper side	[15,105–107]
		Pangolin scale	Continuous tilting of lamellae towards exterior	[108,109]
		Arapaima gigas fish scale	Inclination of fibrils between neighboring lamellae	[110,111]
		Lobster/crab exoskeleton	Periodic helical arrangement of fibrils (Bouligand structure)	[96–98]
Gradient interfaces		Dentin-enamel junction	Gradual transition in mineral content, collagen fibril orientation, and size and morphology of mineral crystals; continuous fibril bridges	[121–124]
		Cementum-dentin junction	Hypomineralized collagen fibers; higher content of polyanionic molecules; fibrous joint	[125,126]
		Tendon/ligament	Continuous variation in mineral content, collagen fiber orientation, and crystalline ordering; fibrous joint	[127–129]
Integration of multiple gradients	Parallel combinations	Tooth Mantis shrimp appendage	Mixture of diverse chemical/structural gradients and graded interfaces	[121–138] [104,113–116]
	Hierarchical gradients	Bone	Gradients over multiple length-scales consistent with structural hierarchy	[140–151]

or within a limited zone, *e.g.*, near the interface between dissimilar components. These represent the extremes in gradients, as illustrated in Fig. 2a and b, respectively. In addition, the variations of ingredients may follow different trends, leading to diverse dependences of local properties on location. For example, considering the broad-scale gradient depicted in Fig. 2a, the properties may change either gradually or in a discontinuous manner as exemplified by the stepwise mode [3,4,7]. In the former case, two classical relationships, that are pertinent to a wide range of materials, have been established to describe the position-local property correlations [3,8]:

(i) a power-law relation, $P = P_0 z^k$, and
(ii) an exponential relation, $P = P_0 e^{az}$,

where P_0 is the reference initial property and P denotes the spatially changing property, *e.g.*, the elastic modulus or hardness, at the position denoted by distance z. The parameter k is a non-dimensional exponent varying from 0 to 1 and a has a unit of length^{-1}. The power-law relation becomes linear when k equals to unity; for the exponential relation, $a > 0$ indicates an increase of local property with distance, while $a < 0$ represents a negative variation.

3. Design principles of gradients in biological materials

Although the idea of functionally graded materials (FGMs) is relatively new in engineering design (the concept was first proposed in 1980s in Japan [7,36]), the design motif has long been utilized in biological materials, as mentioned above [5,11–19,37–39]. Nature provides a rich toolbox for living organisms to create gradients in materials considering the innumerable chemical, structural and geometrical variables that can be adapted (see Table 1). In spite of this, the basic designs of natural gradients are remarkably similar among the vast spectrum of biological materials, indicative of convergent evolution. In the context of materials design and evolution processes, the gradients are fundamentally associated with the changes in two sorts of ingredients, *i.e.*, chemical compositions/constituents, and structural characteristics which further involve the arrangement, distribution, dimensions and orientations of structural building units, as discussed below (Fig. 2c–g). Additionally, interfaces play a critical role in maintaining structural integrity and supporting the specific functions of biological materials [17,37]. Indeed, it is a common design motif to incorporate gradual transitions across these interfaces (Fig. 2h); such localized gradients will be discussed separately. Moreover, the extraordinary properties of biological materials are generally the product of an integration of multiple gradients in different forms which are ingeniously organized over a series of length-scales. These complexities will also be clarified with reference to the prime examples of the tooth, the mantis shrimp appendage and bone.

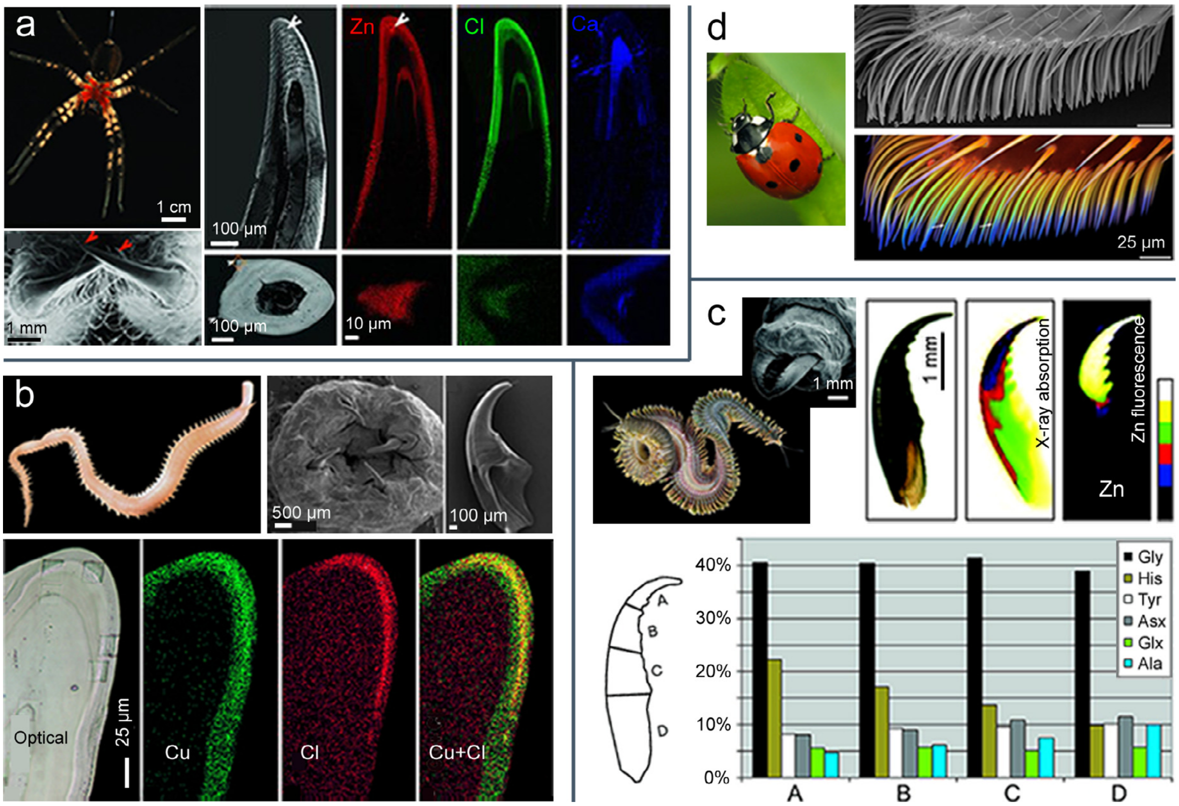

Fig. 3. Gradient chemical compositions/constituents of inorganic ions and biomolecules in biological materials: (a–c) Inorganic ions that participate in the formation of inter-molecular coordination complexes are more concentrated approaching the tip and peripheral regions, yet deficient at the base and interior, in the spider fang [48] (a) and the jaws of bloodworm *Glycera dibranchiate* [49,50] (b) and marine polychaete *Nereis virens* [45,46,51] (c). The biomolecules that interact with the ions display a non-uniform distribution of similar trend in *Nereis virens* jaw [51] (c); (d) The proportion of elastic protein resilin is the highest at the tips and decreases gradually towards the bases in the adhesive tarsal setae of ladybird beetle *Coccinella septempunctata* as revealed by confocal laser scanning microscopy analysis [52,53].

3.1. Chemical compositions/constituents

The majority of gradients in biological materials are linked to a varying degree to the variations in local chemical compositions or constituents. A range of chemical factors, such as the type and concentration of bio-minerals, inorganic ions and biomolecules, as well as the hydration level, can be tuned for spatial manipulation of local properties [5,38]. Among the multiple sources of chemical gradients, the degree of mineralization can be readily regulated within biological materials by preferentially locating the hard and stiff bio-minerals at regions experiencing considerable mechanical stress and abrasion. This is particularly important for surfaces where higher hardness and stiffness are required to promote enhanced contact and wear resistance. Such approach, which basically relies on the biomineralization process, is widely employed by a multitude of highly mineralized tissues; notable examples are chiton radular teeth [40,41], elasmoid fish scales [42,43] and crayfish mandibles [44].

Aside from the degree of mineralization, chemical gradients can also be generated in a more ingenious manner through much finer modulations in local compositions and constituents; this is especially true for biological materials with moderate or low mineralization levels. Given that the process of biomineralization can be limited by the scant availability of minerals, organisms are adept at controlling the local material properties by adjusting the bonding states of their biopolymers at the molecular level; specifically, inorganic ions can play a critical role in forming the coordination-based cross-links between neighboring protein side chains [45–47].

Gradients in ion contents are used in a variety of biopolymer-based materials to optimize their function; prime examples are spider fangs [48] and worm jaws [49–51], as shown in Fig. 3a–c. Inorganic ions that participate in the formation of intermolecular coordination complexes, such as those of Zn, Cu and Cl, are more concentrated in these tissues at locations near the tip and peripheral regions, yet deficient at the base and interior; this has the effect of generating a resulting gradient in the cross-linking density [45–48,50,51]. The ion-rich regions, which generally correspond well to those experiencing higher stresses during predation and mastication, are accordingly hardened owing to the strong bonding between the ions and biopolymers, which is beneficial to an enhanced resistance to impact and wear. Meanwhile, the biomolecules that interact with these ions, as represented by the histidine residues, always display a non-uniform spatial distribution of similar fashion, thereby causing the same trend as exemplified by the *Nereis* jaw (Fig. 3c) [45,51]. The coexistence of such chemical variations leads to a synergistic effect in offering site-specific bonding states and gradient in resulting properties.

In view of the trace concentrations of inorganic ions, the type and contents of biomolecules usually play a dominant role in controlling the properties of non-mineralized biological materials. A good example are the adhesive tarsal setae of ladybird beetle *Coccinella septempunctata* [52–54], as shown in Fig. 3d. The proportion of the elastic protein resilin is highest at the tips of the setae and decreases gradually towards their bases, which are dominated by materials other than resilin (other proteins and very likely mainly chitin). As a result, the local Young's modulus exhibits a major increase, from 1.2 MPa to 6.8 GPa, along the distal-to-proximal direction, representing a stiffness gradient that endows the setae with an effective

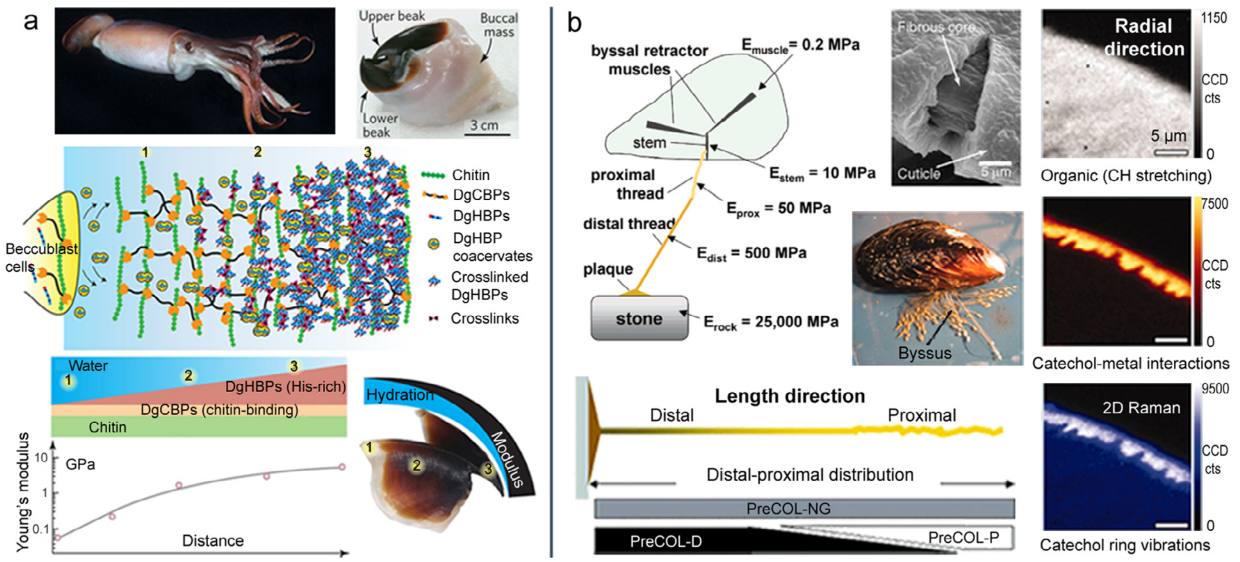

Fig. 4. Simultaneous adaptation of biomolecules and hydration in biological materials and chemical gradients along different directions: (a) In the squid beak, the content of histidine-rich proteins decreases from the tip to the base, while at the same time the hydration degree varies inversely, leading to a gradient in Young's modulus [57–60]; (b) The proteins that contain stiff and compliant domains in their primary sequences are enriched, respectively, in the distal and proximal portions along the length direction of the byssal thread [45,61]. In the cross-sectional direction, the granular cuticle contains more dopa-rich protein and inorganic ions, notably Fe^{3+}, that are involved in forming dopa-metal cross-links, as compared to the core [62,63].

adaptation to rough surfaces for an enlarged contact area (from the compliant tip) while simultaneously preventing lateral collapse and condensation (from the stiff base). The result is that the attachment capacity of the adhesive pads is improved.

The degree of hydration is another important variable that can be tuned to induce graded properties, particularly considering the strong dependences of the properties of biopolymers on water content [11–15,18,55,56]. A simultaneous adaptation of both biomolecules and hydration is represented by the squid beak which is among the hardest and stiffest wholly organic materials known [57–60]. The squid beak, which is composed of chitin, water and proteins with chemical cross-links, has evolved to exert damage to the prey during predatory activity while mitigating self damage at the junction between the base of the beak and the buccal mass. The content of histidine-rich proteins, which are hydrophobic and participate in forming chemical cross-links and coacervates that impregnate the chitin network, decreases from the tip to the base, while at the same time the degree of hydration varies inversely [58–60] (Fig. 4a). At the same time, the beak exhibits a visible variation in pigmentation ranging from translucent along the wing edge to black at the tip. A 200-fold stiffness increase is thus created across the beak from the hydrated chitin base to the dehydrated distal rostrum, enabling a gradual transition between the stiff implant and soft tissue.

Many biological materials display distinct chemical gradients spatially along different directions. This is the case, for instance, of the byssal thread of marine mussels [45,61–63], as shown in Fig. 4b. The byssus functions to provide mussels with secure attachment to rocks and pilings by mediating contact between a very stiff insert material, *e.g.*, rock, and very soft living tissue. Along the length direction of the thread, the protein, which contains stiff silk-fibroin-like domains in its primary sequence, is enriched in the distal portion, whereas the protein that contains compliant elastin-like domains

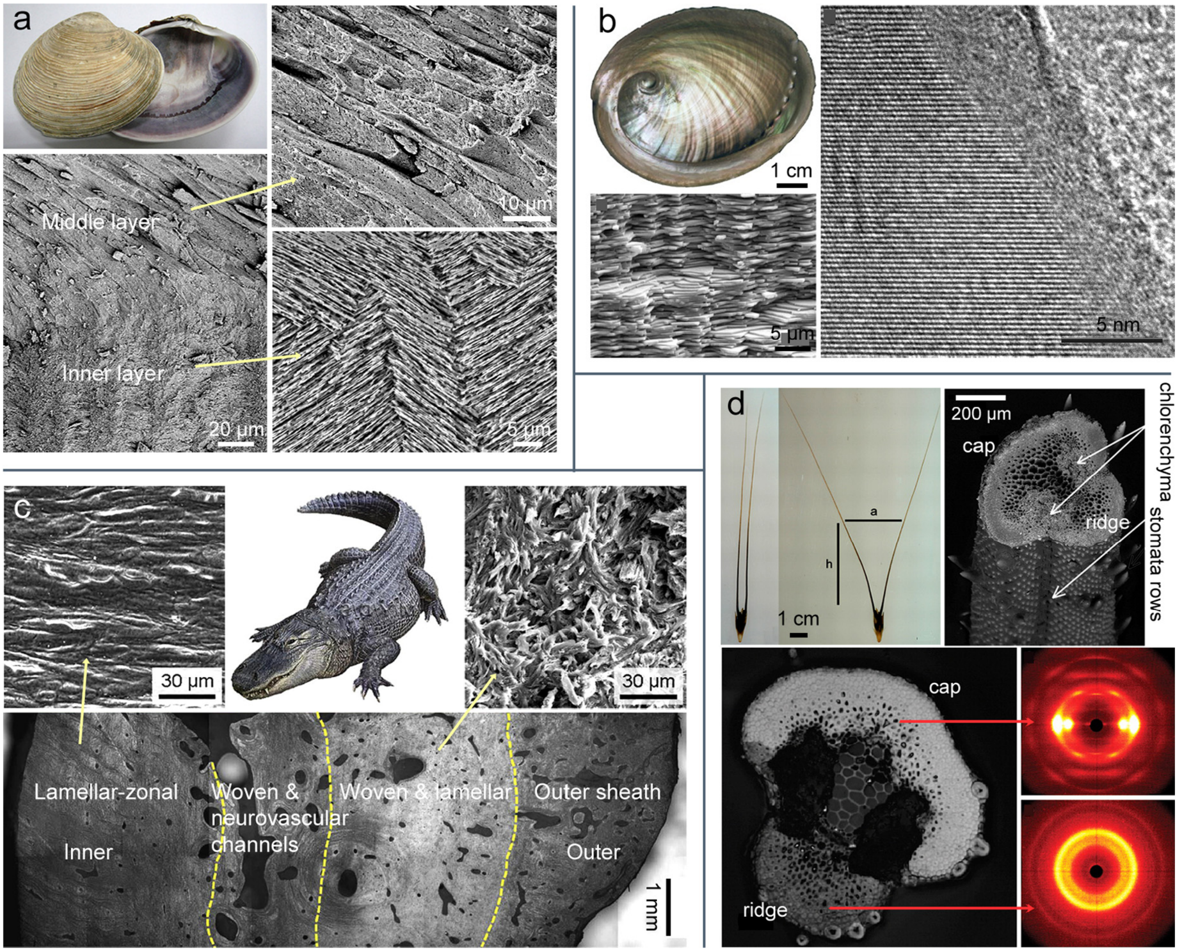

Fig. 5. Gradient structural arrangements in biological materials: (a) The aragonite constituents are stacked in a loosely-packed porous form and with a dense crossed-lamellar arrangement in the middle and inner layers, respectively, in bivalve *Saxidomus purpuratus* shell [67–69]; (b) amorphous $CaCO_3$ layers of thickness of 3–5 nm exist at the surfaces of aragonite platelets in *Haliotis laevigata* abalone shell [70–72]; (c) the alligator osteoderm is composed of four regions with different structural organizations and displays a transition from a hard and stiff dorsal cortex to a compliant ventral base [74]; (d) the cellulose fibrils are well aligned along the long axis in the cap yet randomly organized at the ridge at the lower part of wheat awns, leading to non-uniform expansion between different regions when exposed to daily humidity cycles [76].

predominates in the proximal portion. Such an inverted distribution of these proteins leads to a 10-fold reduction in stiffness of the thread from distal to proximal which serves to compensate the mismatch between rocks and retractor muscles [45,61]. In the cross-sectional direction, conversely, the threads from several species, such as *M. galloprovincialis*, possess granular cuticles containing a dopa-rich protein and inorganic ions, notably Fe^{3+}, that are involved in forming dopa-metal cross-linking complexes, as compared to the core [62,63]. Here the density of such cross-links is relatively higher within the granules than the matrix. As a consequence, the cuticle of byssal thread is endowed with a combination of high hardness conferred by the granules and extraordinary extensibility provided by the less cross-linked matrix.

3.2. Structural characteristics

Given definite chemical compositions/constituents, the properties of materials are principally dictated by their nano/microscale structure or architecture. The critical role of structure is notably represented by the well-known Hall–Petch relationship in physical metallurgy [64,65], *i.e.*, the yield strength correlates inversely with the square root of the grain size in crystalline materials. A grain size gradient from the surface to the inside has recently been created to endow materials with a combination of strength and ductility not achievable in conventional homogeneous counterparts [6]. Biological materials demonstrate rather complex structural diversity and hierarchy [11–19,66], and as such, have flexible choices to tune their detailed structure. In spite of this, the generation of structural gradients is basically associated with four aspects of elementary characteristics, *i.e.*, the arrangement, distribution, dimensions, and orientations of structural units (Fig. 2d–g).

3.2.1. Arrangement

By tuning the local arrangement of constituents, biological materials are able to generate site-specific properties for enhanced performance even without alteration of chemistry. In the bivalve *Saxidomus purpuratus* shell, for example, the constituent aragonite units are stacked in loosely-packed porous form and dense crossed-lamellar arrangement in the middle and inner layers, respectively (Fig. 5a) [67–69]. This results in graded mechanical properties along the thickness direction of shell, *i.e.*, the middle layer is weak and prone to collapse so as to dissipate energy and relieve stress concentrations, while the inner layer is strong and tough against damage owing to the efficient crack deflection and twisting induced by the crossed-lamellar structure [69]; taken as a whole, this all contributes to the improved protection of the mollusk.

With respect to length-scales, gradient arrangements can also occur over atomic or molecular levels in biological materials. This is exemplified by another highly mineralized tissue – the nacre from the shell of *Haliotis laevigata* abalone [70–72]. Amorphous $CaCO_3$ layers of thickness ~3–5 nm are formed at the surfaces of micrometer-sized aragonite platelets (Fig. 5b), producing a smooth transition between the organic and inorganic components. This nanoscale gradient is intended to lower the interfacial energy between the two phases by substituting one "costly" interface by two with much lower energy to provide better adhesion that benefits the mechanical performance of the hybrid material.

Likewise, varying structural arrangements have been widely incorporated in a multitude of less-mineralized protective armors, such as the fish scales and reptile osteoderms [42,43,73–75]. For instance, the alligator osteoderms, which are interconnected by sutures and collagen fibers to provide the alligator with both protection and flexibility, are roughly composed of four regions with different structural organizations, as shown in Fig. 5c [74,75]. These osteoderms are featured by a sandwich structure combining an inner porous core and an outer dense cortex with woven bone in the dorsal region and lamellar-zonal bone in the ventral region. A gradual transition is thus created from the hard and stiff dorsal cortex to a more compliant ventral base; such a gradient induces a more efficient load redistribution and energy absorbance of the tissue, leading to enhanced protective effect.

Additionally, how biological materials arrange their constituents enables a practical means to realize functional adaptations to various environmental stimuli [15,18]. A typical example is the biopolymer-based wheat awn that plays a critical role in seed dispersal by balancing the dispersal unit as it falls and propelling the seed on and into the ground [76]. At the lower part of awn, the cellulose fibrils are very well aligned along the long axis in the cap yet randomly organized at the ridge (Fig. 5d). The asymmetry in this arrangement leads to non-uniform expansion between different regions when exposed to daily humidity cycles to induce periodic movement of the awns, *e.g.*, expanding in length with humidity to push the awns together and contracting with drying to pull the awns apart, thereby providing the mobility to drive the seed into soil for its dispersal.

Pine cones, which have non-uniform arrangement of cellulose microfibrils in their scales, demonstrate similar mechanisms of seed dispersal that is enabled by the opening of the scales through dehydration; this process is also reversible as rehydration of the cone re-closes the scales [15,18,77,78], as shown in Fig. 6. The scales are composed of two regions with distinct structural organizations, *i.e.*, a fibrous sclerenchyma region and a porous sclerid region, with the latter comprising mainly cellulose which can absorb water easily. The structure of the porous region is also graded from its interface with the fibrous one, where it is more solid, to the external surface, where its porosity is at a maximum [77,78]. The length of the fibrous component remains constant, but the porous component shrinks with drying and swells with hydration. This enables the local strain to increase monotonically with the distance from the fibrous layer in the sclerid region. Such a strain gradient leads to the reversible deformation of the pine cone induced by changes in humidity, *i.e.*, the scales are closed when the humidity is high to protect the seeds and, once the cone dries, the scales open to release the seeds.

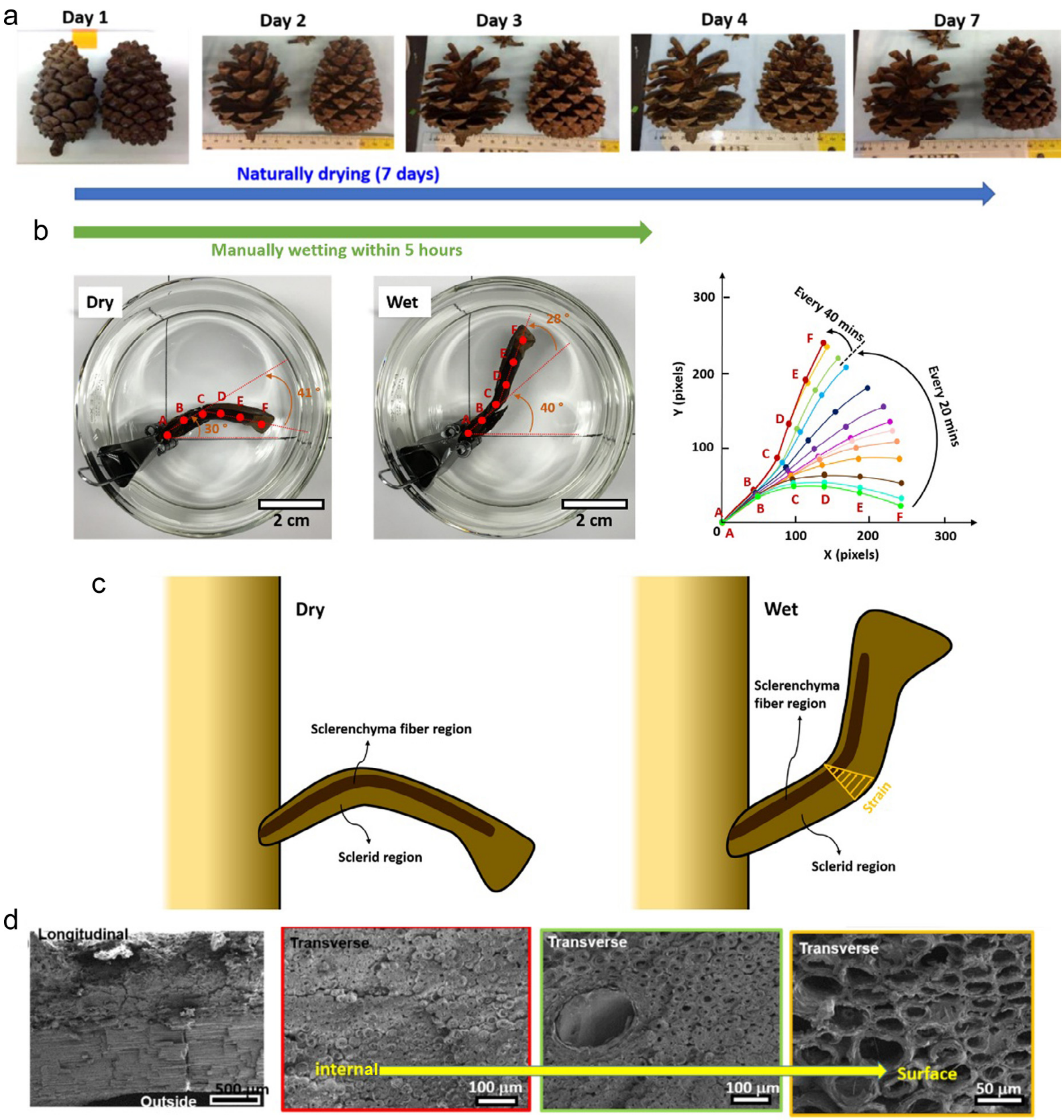

Fig. 6. Reversible deformation of the pine cone induced by changes in humidity due to gradient structural arrangement: (a) Natural drying, which occurs over 4 days, opens the cone to release the seeds; (b) the scales can reversibly re-close by hydration, as indicated by the shape change of the scale after immersing in water over 5 h; (c) a strain gradient is created in the sclerid (porous) region to induce shape change, while the sclerenchyma (fiber) retains its length, when the scale is hydrated; (d) the scale is composed of a fibrous sclerenchyma region (left) and a porous sclerid region with the local structure of the latter changes gradually from the interface with the fibrous one, where it is more solid, to the external surface, where its porosity is maximum [77,78].

3.2.2. Distribution

Biological materials have evolved multiple structural units in forms of cellular, fibrous, tubular, etc., in ecological conditions to meet their particular functions. The spatial distribution of these units is tightly related to local properties and has been widely adjusted to produce gradients. This is featured by the site-specific porosity involved in a broad range of biological materials through which the stiffness, which generally scales with the square of relative density for an open-cell foam [79], is exquisitely tuned. Prime examples of tissues comprising porosity gradients are antler, the squid sucker ring and sea urchin spine [80–85]. For example, the porosity decreases continuously with the transition from inner cancellous (trabecular) bone to outer compact (cortical) bone in the elk antler [80,81], leading to an increase of stiffness from interior to surface.

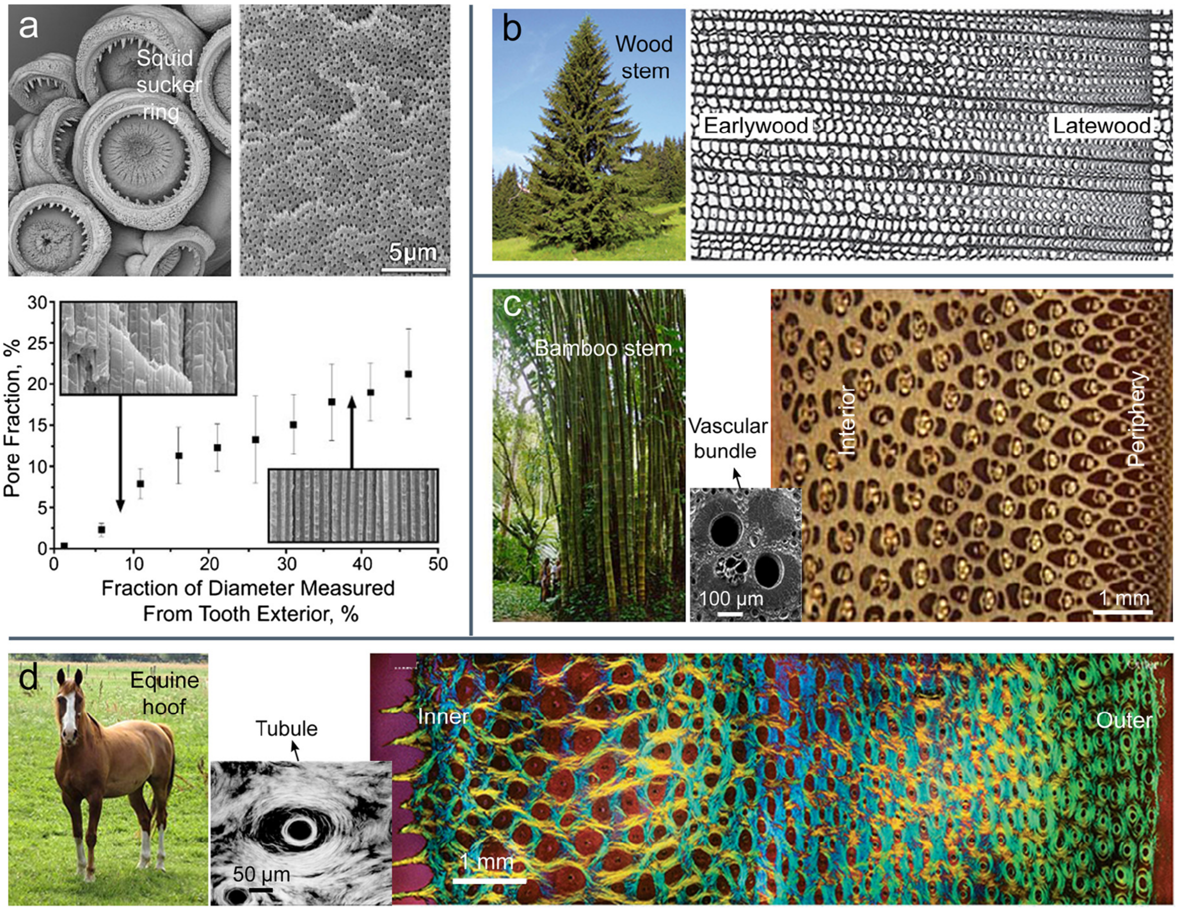

Fig. 7. Gradient distribution of structural units in biological materials: (a) The pore fraction decreases from the core to the periphery in the nanoporous sucker ring tooth of squid [82,83]; (b) the stem of the Norway spruce displays decreasing porosity from the earlywood to latewood within the growth ring [86–89]; (c) the density of vascular bundles that are rich in cellulose fibers decreases from the outermost periphery to the interior in bamboo stems [90,91]; (d) the tubular density increases along the inner-to-outer direction over the wall thickness in horse hoof [94,95].

Similar porosity gradients also exist in the squid sucker ring which functions to provide the squid with additional gripping power during prey capture and handling [82,83]. The pore fraction decreases from the core to the periphery of the ring tooth (Fig. 7a), resulting in distinct mechanical gradients of decreasing modulus and hardness along opposite directions; such a motif represents a common design in Nature in the form of a porous core supporting a denser periphery for high bending stiffness [11–13]. In particular, the modulation of porosity becomes a major source of gradients for cellular materials. With wood stems, for instance, the growth ring is featured by a gradual decrease in porosity from the earlywood to latewood (Fig. 7b) [86–89]. As a consequence, gradient of the opposite fashion is generated in apparent density and stiffness, which is beneficial to water transport and mechanical stability during the growth period.

In contrast to pores, fibers or fiber bundles normally have a strengthening effect, but their distribution is also widely regulated in biological materials. This can be seen in the decreasing density of vascular bundles, rich in cellulose fibers, that occurs from the outermost periphery towards the interior in bamboo stems (Fig. 7c) [90,91]. Such a gradient yields functionally graded properties across the thickness, *e.g.*, the local stiffness decreases from the surface inwards, conferring enhanced flexible rigidity to the stem. In addition, many biological materials, especially those used for energy absorption such as sheep horns, horse hooves and tooth dentins [19,92–95], contain tubules comprising inner cavities often surrounded with more mineralized walls. The performance of these materials is frequently optimized via a spatial adjustment of the distribution of tubules. With horse hooves, for example, the tubular density increases along the inner-to-outer direction over the wall thickness, as shown in Fig. 7d [73,94,95]. Such a non-uniform distribution, in conjunction with a reverse change in the degree of hydration, results in varying mechanical properties including strength, stiffness, fracture toughness, mechanical damping and wave dispersion capacity, in specific regions to fit the function of the hoof.

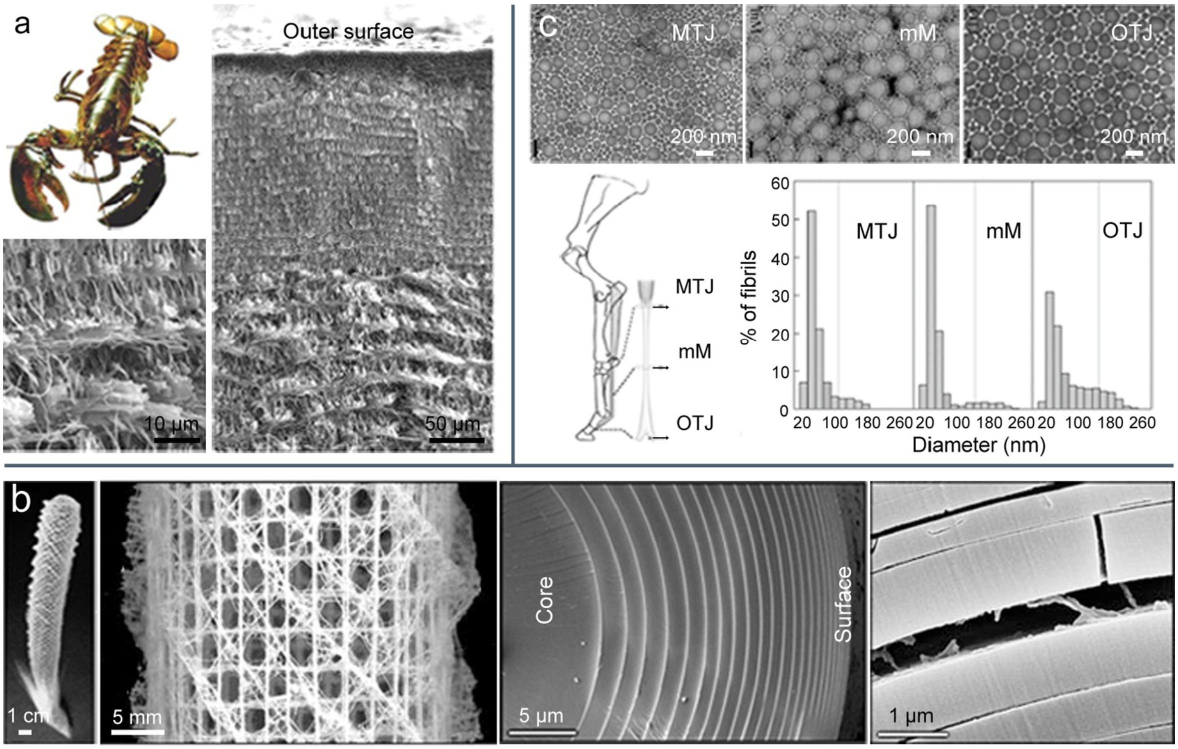

Fig. 8. Gradient dimensions of structural units in biological materials: (a) The thickness of twisted plywood layers increases from the exocuticle to endocuticle in the exoskeleton of *Homarus americanus* lobster [96,97]; (b) the concentric silica layers of sponge spicules display decreasing thickness from near the core to the periphery [100,101]; (c) the superficial digital flexor tendon of horse demonstrates varying diameters of collagen fibrils in different zones; small fibrils are predominantly distributed in the myotendinous junction (MTJ) region whereas the osteotendinous junction (OTJ) region is relatively rich in large fibrils [103].

3.2.3. Dimensions

Many properties and functions of biological materials are determined largely by the dimensions of their constituents, reminiscent of the well-known Hall-Petch relationship in crystals [64,65]. This offers a viable means for the development of gradients in these materials through alterations in their characteristic structural dimensions. Specifically, it is a common strategy for biological materials with a laminated structure to ingeniously tailor the lamellar thickness [96–99]. For example, the thickness of the twisted plywood layers increases from the exocuticle to endocuticle in the exoskeleton of arthropods such as the *Homarus americanus* lobster [96,97] and *Loxorhynchus grandis* crab [98], as shown in Fig. 8a. Such a variation results in graded mechanical properties across the tissue with the exocuticle being harder and stiffer than the endocuticle, in a manner analogous to the Hall-Petch relationship where finer structure leads to higher hardness and stiffness.

Dimensional gradients have also been adopted in biological ceramics that utilize a laminated structure to generate toughness. A prime example are the sponge spicules that are constituted by a central core of hydrated silica surrounded by alternating layers of silica and proteinaceous material. The thickness of the concentric silica layers decreases from near the core to the periphery (from ~1.5 to ~0.2 µm for the case in Fig. 8b) [100,101]. As such, the depth of crack penetration is effectively limited by the thinner outer layers because cracks invariably tend to propagate along the organic interlayers, while the global mechanical rigidity benefits from the thicker inner layers; in this manner, the spicules can achieve an enhanced mechanical robustness.

In addition to laminated structures, dimensional gradients have been utilized in biological materials with a broader variety of architectures, where the relevant dimensions extend over multiple length-scales down to the nanometer level [102–104]. In human tooth dentin, for instance, the thicknesses of the nano-sized mineral (hydroxyapatite) crystals display a decreasing trend from the dentin-enamel junction (DEJ) to the coronal dentin [102], as discussed in further detail in the following section. Likewise, the dimensions of structural units have also been modulated in a range of biopolymer-based materials. This can be seen in the varying diameters of collagen fibrils in different regions of the superficial digital flexor tendon of horse (Fig. 8c) [103]. Small collagen fibrils, with diameters below ~100 nm, are predominantly distributed in the myotendinous junction region whereas the osteotendinous junction region is relatively rich in larger diameter (>200 nm) fibrils; in comparison, the middle metacarpal region exhibits an intermediate distribution of larger and smaller diameter fibrils. This variation leads to a combination of distinct properties along the tendon to facilitate its function; specifically, the myotendi-

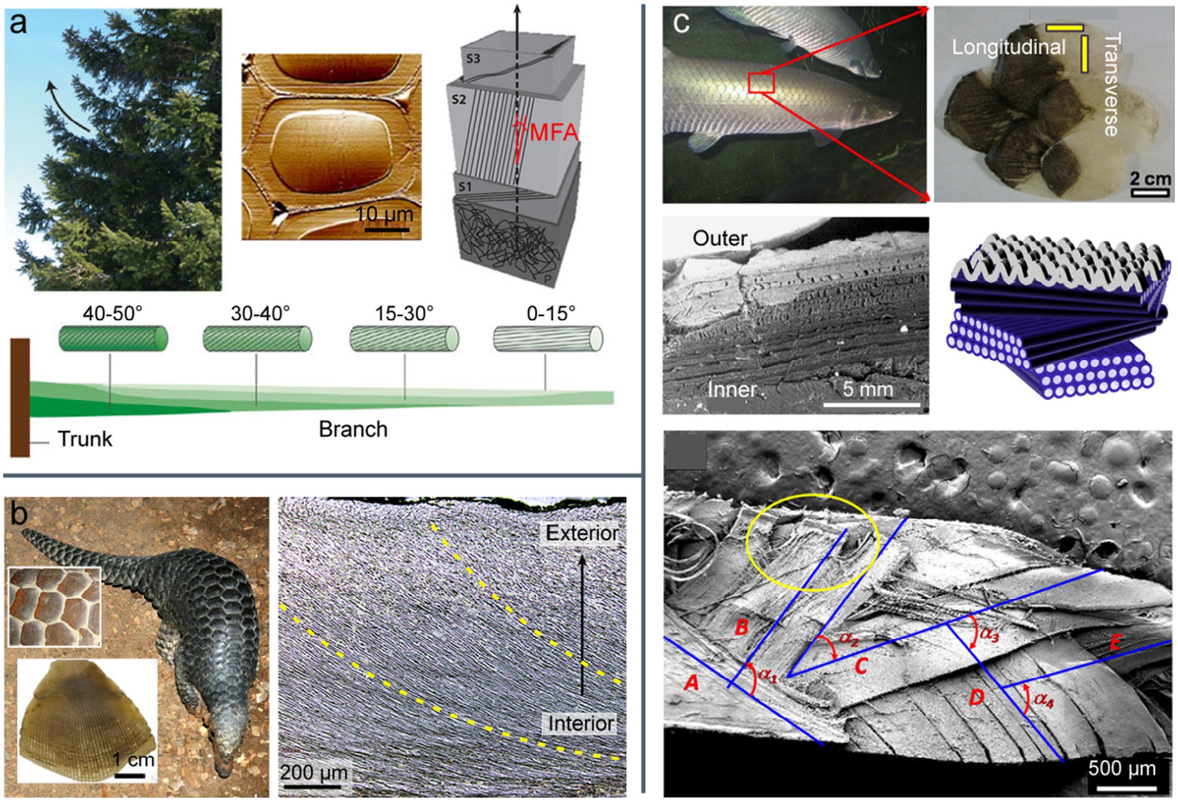

Fig. 9. Gradient orientations of structural units in biological materials: (a) In the softwood Norway spruce, the alignment of cellulose fibrils changes from the lower side near the trunk, where the fibrils deviate markedly from the long axis with a microfibril angle (MFA) of ~40°, to the upper side of the branch where the fibrils are almost parallel to the long axis [15,105–107]; (b) the orientations of keratin lamellae change gradually from being almost parallel to the surface at the base to ~45° tilted at the top in the intermediate layer of pangolin scales [108,109]; (c) the collagen fibrils are inclined by ~35–85° between neighboring lamellae in the inner part of *Arapaima gigas* fish scales [110,111].

nous junction is more elastic because it is associated with the sliding or creeping of the finer fibrils while the osteotendinous junction becomes more rigid due to its higher stiffness [103].

3.2.4. Orientations

The majority of biological materials are composed of anisotropic structural units, such as fibers, lamellae, platelets and tubules, with properties that depend strongly on orientation. Tuning the orientations of these units provides a further route to controlling their local properties, thereby presenting a new source of gradients to confer specific functions. This is apparent in the upward bending of softwood branches [15,105–107], shown in Fig. 9a. The cellulose fibrils run spirally in the major part (S2 layer) of the plant cell wall around the central lumen; the angle of these fibrils relative to the long cell axis, termed as the microfibril angle, reflects the structural adaptation during growth of wood in providing a critical factor that determines the mechanical properties [88,105]. In softwood branches, the alignment of fibrils changes from the lower side near the trunk, where the fibrils show a large deviation with respect to the long axis (with the microfibril angle of ~40°), to the upper side of the branch where the fibrils are almost parallel to the long axis. Such a gradient produces an internal stress through the swelling of the cell walls, during which the length of fibrils is fixed but torsional deformation occurs to actuate directional force. The cells on the lower side of the branch expand in the axial direction on swelling due to their high microfibril angle, whereas the cells on the upper side contract; this helps the bending of the branch upwards to accommodate its own weight [15,105–107].

Gradient orientations have been employed in a wide range of impact-resistant biological materials as well, as seen in natural defensive armors such as pangolin and fish scales [42,43,108–112], and offensive weapons as the mantis shrimp appendage [99,104,113–116]. The general principle here is to maximize the damage imposed on opponents while minimizing injuries to the tissues themselves, again through tailoring local structural orientations. For instance, in the intermediate layer of pangolin scales, the keratin lamellae are arranged to be almost parallel to the surface at the interior, yet are tilted by ~45° at the exterior, with a continuous transition in between (Fig. 9b) [108,109]. In this scenario, the mis-orientation between the primary structural feature and externally applied load, which is generally normal to the tissue surface, gradually increases with the distance from the loading site. Such a gradient leads to higher stiffness and strength towards the surface and an

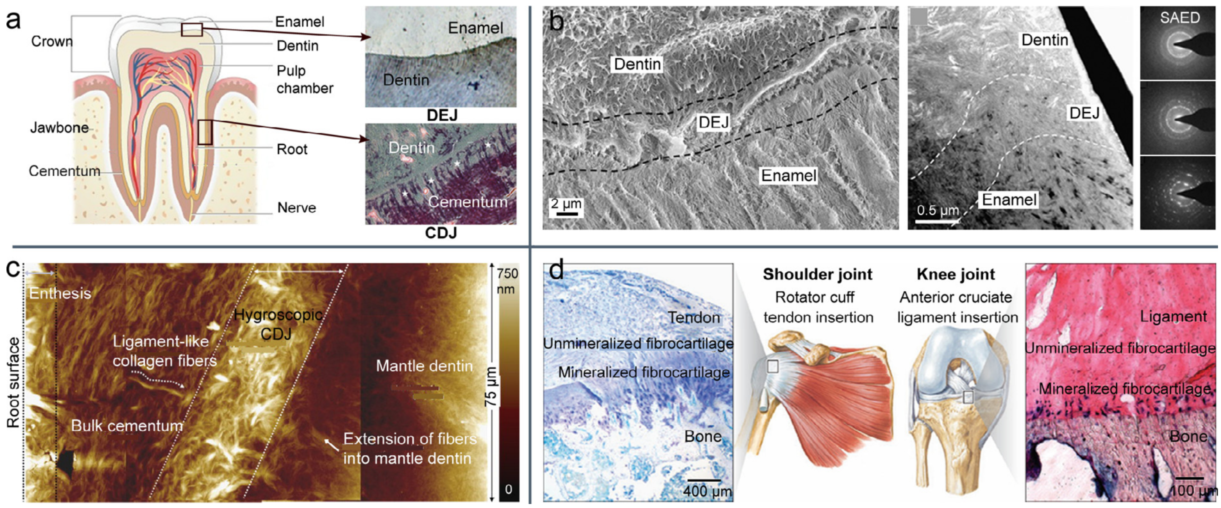

Fig. 10. Gradient interfaces in biological materials: (a) The structure of the human tooth and its graded interfaces, the dentin-enamel junction (DEJ) and cementum-dentin junction (CDJ) [120,122,125]; (b) in the DEJ, a gradual transition in the mineral concentration, collagen fibril orientation, and the size and morphology of mineral crystals exist between the dentin and enamel, with the two tissues bridged by collagen fibrils [121–124]; (c) the CDJ demonstrates a gradient in chemical composition and a fibrous joint between cementum and dentin with the collagen fibers split into individual fibrils and intermingled with the extracellular matrix of mantle dentin [125,126]; (d) the tendon/ligament-to-bone connections are roughly composed of four zones, *i.e.*, tendon/ligament, uncalcified fibrocartilage, calcified fibrocartilage and bone, where both the mineral content and crystalline ordering increase successively [127–129].

increasing fracture resistance with depth due to enhanced extrinsic toughening from continuous crack deflection along the lamellar interface [117]. The synergistic combination of these site-specific mechanical properties gives rise to an improved protective role of the scales; the outer layer is harder and stronger to prevent surface impact damage and penetration, whereas the subsurface layer is more compliant and tougher to accommodate the excessive deformation and arrest the inward propagation of cracks. Indeed, this concept is used in many natural and synthetic dermal armors [3–5,42,117].

Another typical class of architecture encompassing orientation gradients is the successive arrangement of layers with different orientations of constituents, forming a twisted plywood or Bouligand-type structure. The *Arapaima gigas* fish scales, which function to protect the fish from predation by piranhas, are a particularly good example of this [110,111]. As shown in Fig. 9c, the collagen fibrils are inclined by $\sim$35–85° between neighboring lamellae in the inner part of the scales. With this arrangement, remarkable extrinsic toughening mechanisms operate via crack twisting and deflection along the internal interfaces; moreover, the stretching, rotation and delamination of fibrils under load act to dissipate additional mechanical energy. Consequently, the toughness and penetration resistance of scales are markedly enhanced.

An idealized form of plywood structure is that each layer is twisted by a constant angle with respect to the previous one and the rotation completes one or more cycles of full 180°; this is the true Bouligand structure. Such variations of the constituent orientations result in a functional gradient of the in-plane mechanical properties, such as local elastic modulus, which are directly associated with these orientations. The development of microcracks that are preferentially initiated at the interfaces between adjacent lamellae in symmetric, cross-ply composites can also be suppressed by the gradient orientations through eliminating sharp interfaces [3,9]. Such Bouligand structures are particularly common in the arthropod exoskeletons with the appendages of mantis shrimps being a prime example [96–99,104,113–116,118], as described in the following section.

3.3. Gradient interfaces

In addition to property variations over the entire volume of materials, living organisms are adept at designing continuous transitions across the interfaces between dissimilar components [17,37,38]. Such localized gradients, which act to "smooth out" any abrupt changes in composition/structure or properties, are widely employed in a multitude of biological materials. The factors contributing to gradient interfaces may involve gradually varying chemical compositions/constituents and microstructural characteristics, as well as coarse-scale textures of tissues. The introduction of such gradients can be highly beneficial for the global properties of the material by, for example, mitigating stress concentrations, avoiding marked mismatches in properties and preventing crack propagation at the interfaces [3,4,7].

Gradient interfaces have evolved to join dissimilar materials in a variety of organs, the tooth being a prime example. As shown in Fig. 10a, the solid tooth consists of two major parts that perform distinctly different functions [119,120]. The outer enamel, which comprises primarily prisms of highly mineralized collagen fibers, provides hardness and wear resistance to the tooth. By comparison, the inner dentin, which is composed of less mineralized dentin tubules, is much tougher and necessary to maintain the integrity of tooth. In addition, the root of tooth is covered by the cementum that is further attached to the alveolar bone by the periodontal ligament. Both interfaces between these adjacent tissues, known as the dentin-enamel junction (DEJ) and cementum-dentin junction (CDJ), display graded properties to support the tooth's function.

In the case of the human tooth, the DEJ is characterized by a gradual transition in the mineral concentration, collagen fibril orientation, and the size and morphology of hydroxyapatite crystals between the dentin and enamel (Fig. 10b) [121–123]. Additionally, collagen fibrils are extended from the dentin matrix and inserted into the enamel to form continuous bridges across them [121,124]. As a consequence, the DEJ exhibits site-specific hardness and elastic modulus over its certain width and plays an effective role in arresting crack propagation through the tooth. In comparison, the gradient at the CDJ arises mainly from chemical changes, *i.e.*, the CDJ is constituted by hypo-mineralized collagen fibers and contains higher concentration of polyanionic molecules, such as glycosaminoglycans, that are hygroscopic [125,126]. Additionally, the collagen fibers are split into individual fibrils and intermingled with the extracellular matrix of mantle dentin, forming a fibrous joint between cementum and dentin (Fig. 10c). The resulting property variations promote the accommodation of mastication loads at CDJ, and thus allow for better attachment and load-bearing capability of tooth.

Similar continuous transitions in structure and properties have been broadly incorporated into the specialized interfaces of insertion sites or entheses that connect soft tissues to bone, *e.g.*, the tendon and ligament tissues that attach muscle to bone and bone to bone, respectively [127,128]. Several chemical and structural characteristics, such as the mineral content and collagen fiber orientation, change continuously across, and near, these interfaces, leading to graded mechanical properties. With fibrocartilaginous entheses, for example, the tendon/ligament-to-bone connection can be roughly divided into four zones, specifically pure dense fibrous connective tissue, uncalcified fibrocartilage, calcified fibrocartilage and bone, in which both the mineral content and crystalline ordering increase successively [127–129] (Fig. 10d). This results in a gradual transition in local hardness and elastic modulus through the interfaces and, as such, helps to mediate load transfer between the soft and hard tissues while minimizing stress concentrations.

3.4. Integration of multiple gradients

Living organisms generally adopt fairly intricate strategies to generate site-specific properties by combining different classes of gradients over a range of length-scales consistent with their complex structural hierarchy. The integration of multiple gradients enables a more flexible modulation of local properties in order to meet specific mechanical and functional demands and environmental challenges. Such parallel and hierarchical combinations of gradients are discussed below with reference to the specific examples of tooth, mantis shrimp appendage and bone.

3.4.1. Parallel combinations

The tooth represents an organ where parallel combinations of various gradients are employed to fulfill its mastication function. Despite consisting of brittle minerals and weak collagens, the human tooth is able to withstand biting forces up to 1000 N over innumerable cycles during its lifetime [119]. To achieve this, both chemical and structural adaptations and interfacial gradients have been incorporated into its structure and connection to the jaw. The primary property gradation arises across the organ from distinct changes in chemical constitution and structure existing from the enamel, dentin and cementum to the alveolar bone [121–126,130–138]. In the bulk enamel, the organic and water contents increase continuously from the occlusal surface to the DEJ, while both the content and crystallinity of the hydroxyapatite (HAP) minerals change concomitantly in the opposite fashion (Fig. 11) [132,133]. Additionally, the prisms and involved HAP crystals are aligned to be almost normal to the occlusal surface in the outer enamel, yet their orientations become less defined towards

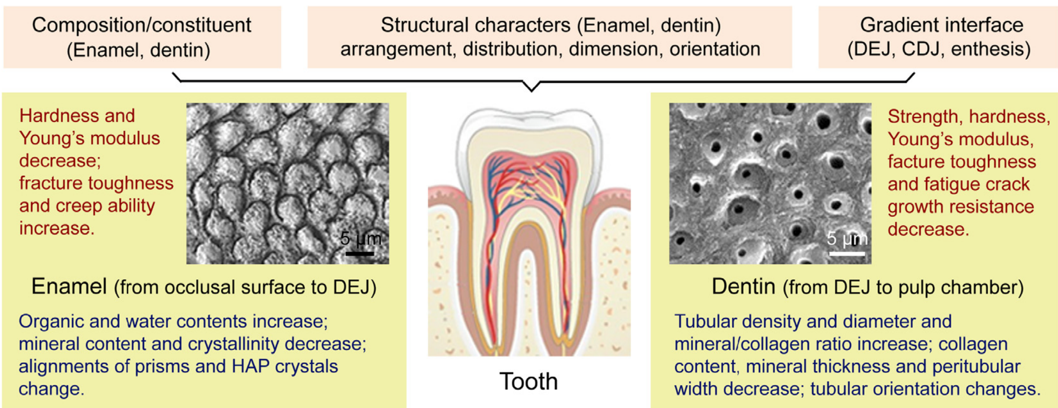

Fig. 11. Parallel combinations of various gradients in the tooth: Gradients in chemical compositions/constituents and structural characteristics, including the arrangement, distribution, dimensions and orientations of structural units exist in both the enamel and dentin, leading to functionally graded mechanical properties across these tissues [102,130–138]. Different regions are connected by specific graded interfaces, the DEJ and CDJ, with the tooth attached to the alveolar bone through the periodontal ligament and fibrous enthesis, all of which represent interfacial gradients [121–129].

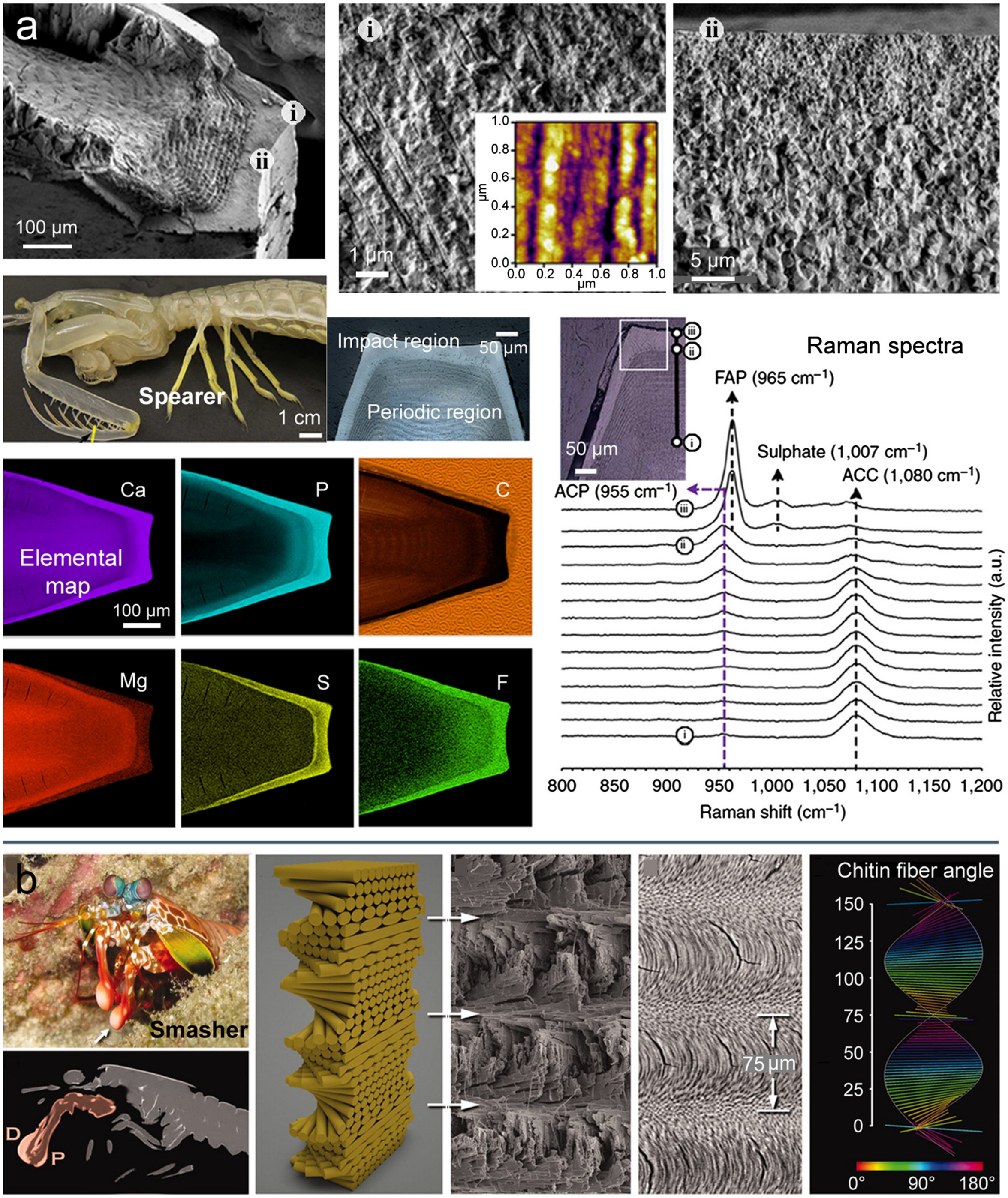

Fig. 12. Multiple chemical and structural gradients in the mantis shrimp appendage: (a) The impact region is abundant in calcium and phosphorous, yet deficient in carbon and magnesium, as compared to the periodic region, with sulphur and fluorine also present in relatively higher concentrations [104,113]. The amorphous apatite is increasingly substituted by fluorapatite towards the outer surface in the impact region; this is accompanied by an increasing crystallinity and decreasing crystallite size as well as a continuous variation of the orientations of apatite crystallites that are preferentially aligned perpendicular to the surface at the extruded edges; (b) the chitin fibrils are helicoidally arranged in the periodic region, demonstrating a Bouligand structure [104,113–116].

the DEJ [131,133–135]. These variations result in decreasing local hardness and Young's modulus yet increasing fracture toughness along the thickness of enamel [131–135]. As a consequence, contact and wear resistance is enhanced at the occlusal surface, whereas the stress transferred to the DEJ is reduced such that the propagation of any cracks from the enamel into the dentin is inhibited.

Likewise, a mixture of chemical and microstructural gradients is utilized in the underlying dentin, in particular with respect to the dentin tubules. The tubular density and diameter, as well as the mineral/collagen ratio, increase gradually from the DEJ to the pulp chamber; this is accompanied by a decrease in the collagen content and the width of peritubular dentin [102,130,136]. The thickness of the nano-sized HAP crystals also displays a decreasing trend along the same direction, presenting an additional dimensional gradient. Besides, the tubular orientations vary continuously as the tubules radiate out from the pulp towards the DEJ and the surrounding cementum [136]. These changes lead to a remarkable simultaneous enhancement in the local strength, hardness, stiffness (Young's modulus), fracture toughness and crack-growth resistance across the dentin along the pulp-enamel direction [102,136–138]. Such property gradients act to provide for the effective arrest of any damage, in the form of cracks, which may develop in the enamel, by generating enhanced protection in the inner soft tissue regions, thus ensuring the structural integrity of tooth. These characteristics are further aided by the fact that the various regions of tooth are connected by the DEJ and CDJ with its attachment to the alveolar bone through the periodontal ligament and fibrous enthesis [121–129], all of which are featured as gradient interfaces, as noted above. The parallel combinations of gradients in both the bulk tissues and their interfaces provide the tooth with optimized performance for mastication.

The appendages of mantis shrimps are another excellent example of a biological material utilizing a combination of multiple gradients. These shallow-water crustaceans have evolved a pair of raptorial appendages, which can be either hammer-like or spear-like devices, to inflict substantial impact forces to their prey during hunting. Such appendages can be essentially divided into three distinct regions, *i.e.*, an outer impact region and an internal periodic region with a surrounding striated region which presumably prevents the expansion during impact [113]. A primary chemical gradient exists between the impact and periodic regions with the former markedly abundant in calcium and phosphorous, which is indicative of a significant extent of mineralization, yet deficient in carbon and magnesium, with sulphur and fluorine also present in relatively higher concentrations (Fig. 12a) [104,113]. The phase chemistry and ordering are further finely tuned within the impact region with fluorapatite increasingly substituting amorphous apatite towards the outer surface. The crystallinity also increases along the same direction, yet the crystallite size decreases concomitantly. Specifically, the impact region of the spearing appendage is additionally featured by a continuous variation in the constituent orientations in such a manner that the apatite crystallites are preferentially aligned perpendicular to the surface at the extruded edges. In comparison, the partially mineralized chitin fibrils are helicoidally arranged in the periodic region of the appendage, forming a unique

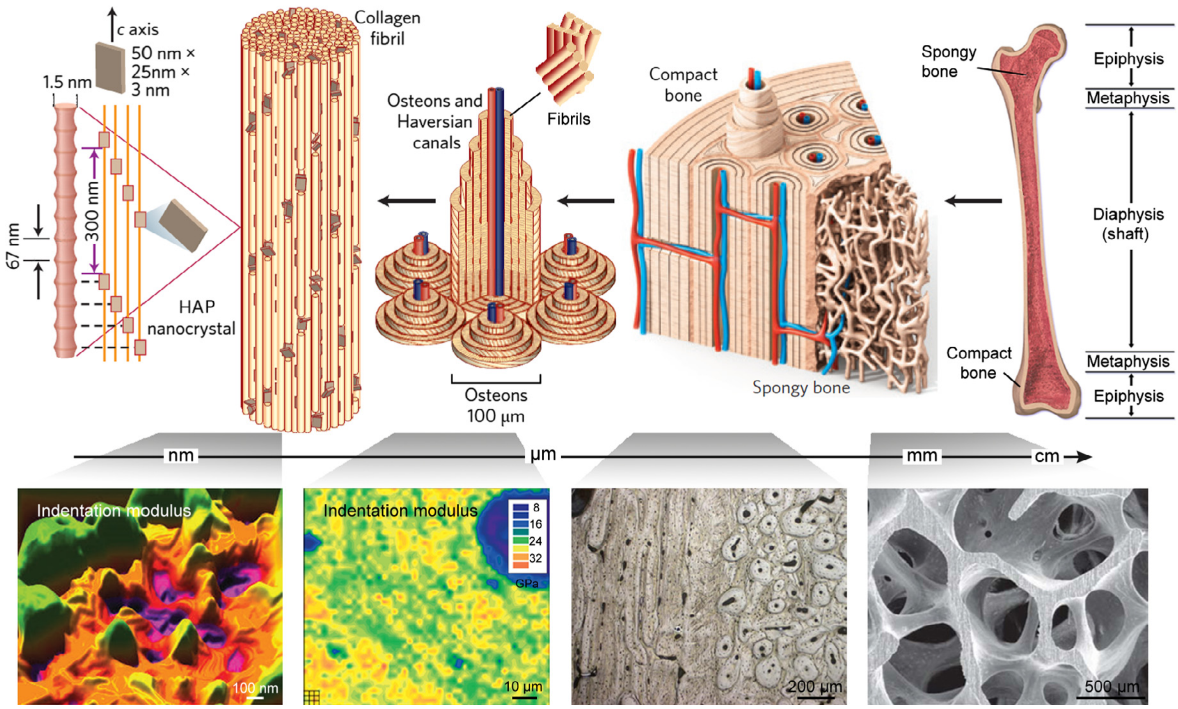

Fig. 13. Hierarchical structure and gradients of bone: Macroscopically, bone displays non-uniform variations in mineral-to-collagen and phosphate-to-carbonate ratios along its length [139–141]. There is also a gradient of increasing density in the radial direction from the interior spongy (trabecular) bone to the exterior compact (cortical) bone [30,142–144]. At the micrometer level, the mineralized collagen fibrils spiral with varying degrees periodically around the central axis with local mineral content changes within the lamellae of osteons [145,146]. At the nanometer level, the collagen fibrils consist of twisted type-I collagen molecules with plate-shaped HAP nanocrystals [143,144,149]. There exist marked heterogeneities at the nano-scale of individual collagen fibrils owing to the underlying local structural and compositional variations [147,148].

orientation gradient in form of Bouligand structure (Fig. 12b) [104,113–116]; this leads to periodical oscillations in local hardness and stiffness and continuously varying paths for the propagation of cracks. The final result of the combination of these multiple gradients is a remarkably resilient and damage tolerant yet extremely effective weapon for use during repetitive predation.

3.4.2. Hierarchical gradients

The outstanding properties of biological materials, especially when compared to those of their constituents, result from their ingeniously designed hierarchical structure which spans multiple length-scales [11–14,66]. Of note here is that functional gradients have been incorporated into each structural hierarchy to systematically regulate properties. Bone is a prime example where hierarchical gradients are effectively employed. Its compositional and structural characteristics are spatially adjusted from nano- to near macro-scales to create optimized local properties at all levels in order to improve its mechanical function.

In long bone, for example, in addition to its macroscopic variation in geometry, both the mineral-to-collagen and phosphate-to-carbonate ratios are non-uniform along its length and tend to decrease towards the flared extremities or epiphyses (Fig. 13) [139–141]. These are accompanied by a gradient in local mechanical properties, *i.e.*, the longitudinal elastic modulus and fracture strength are highest at the mid-diaphysis and display a decreasing trend in the length direction, whereas the radial and circumferential mechanical properties vary in the opposite fashion; as such, the bone changes from being mechanically anisotropic to essentially isotropic. Such alternations represent a set of adaptations of bone at a near-macroscopic level to meet site-specific demands for improved global mechanical performance, specifically to enhance and modulate sagittal bending.

With respect to its structural arrangement, a transition in terms of a decrease in apparent density exists from the exterior compact bone to the interior cancellous/spongy bone [30,142–144]. Both regions display a non-uniform distribution of porosity along the radial direction, with the compact bone further surrounded by dense circumferential lamellar bone. Such structural variations lead to a gradual shift in local bone strength towards the periosteal surface, which correlates positively to the apparent density [142]. At the micrometer level, the compact bone is primarily composed of nominally cylindrical osteons of ~100–200 μm in diameter with inner Haversian canals [142–144]. The osteons possess a lamellar structure with the mineralized collagen fibrils spiraling at varying degrees around the central axis periodically within the lamellae [145]. This orientation gradient is further accompanied by a fluctuation of local mineral content [146]. As a consequence, the elastic modulus demonstrates a periodic variation within the osteon lamellae and increases at the interstitial bone where the mineral content is higher. Such mechanical modulations are thought to help prevent the development of incipient cracks from causing catastrophic failure of bone by lowering the driving force for crack propagation and inducing crack deflection [147,148].

In essence, the fibrils consist of two major nanophases: type-I collagen molecules, which are ~300 nm long and ~1.5 nm in diameter, and plate-shaped HAP nanocrystals, which are a few nanometers in size [143,144]. The collagen molecules are aligned in parallel, with a gap and stagger of 67 nm, and cross-linked to form fibril arrays. The mineral platelets are preferentially located between the heads and tails of molecules with their *c*-axis along the length of fibril, with further mineralization also occurring on the surface of fibrils [143,149]. The mechanical properties of bone, measured by high-resolution nanoindentation, exhibit remarkable heterogeneities at the length-scale of individual collagen fibrils, especially when loaded along the long bone axis [150,151]; this phenomenon has been attributed to the underlying local structural and compositional variations. Such sub-micro- and nano-scale gradients offer new mechanisms to enhance ductility and modulate damage evolution by extending the inelastic deformation over larger areas and inducing non-uniform notch/crack opening and blunting as compared to a more uniform shape change in homogeneous material, thereby allowing more efficient energy dissipation in bone.

4. Functions of biological gradients

The widespread use of gradients provides Nature with a wealth of feasible approaches to optimize the specific functions of biological materials despite their fairly limited choice of components. The performance of biological materials takes distinct advantage of gradients from a variety of aspects. The most common case is to combine a set of superior properties within the same material to enhance its mechanical functions. This has been employed by numerous structural biological materials, where load bearing and support, resisting contact and impact damage, and interfacial strengthening and toughening are particularly critical. The modulation of local chemical and structural characteristics plays an important role in generating a variety of functional properties as well, including light collection and transmission, sensing and actuation to environmental stimuli, and control of liquid flow [15,18,38]. The benefits from gradients are elaborated separately in the following sections with typical biological materials taken as examples.

4.1. Load bearing and support

Many biological materials, *e.g.*, bones and plant stems, have evolved specifically for their mechanical function to be load bearing or to act solely as support. These materials generally utilize various gradients to achieve a high mechanical efficiency

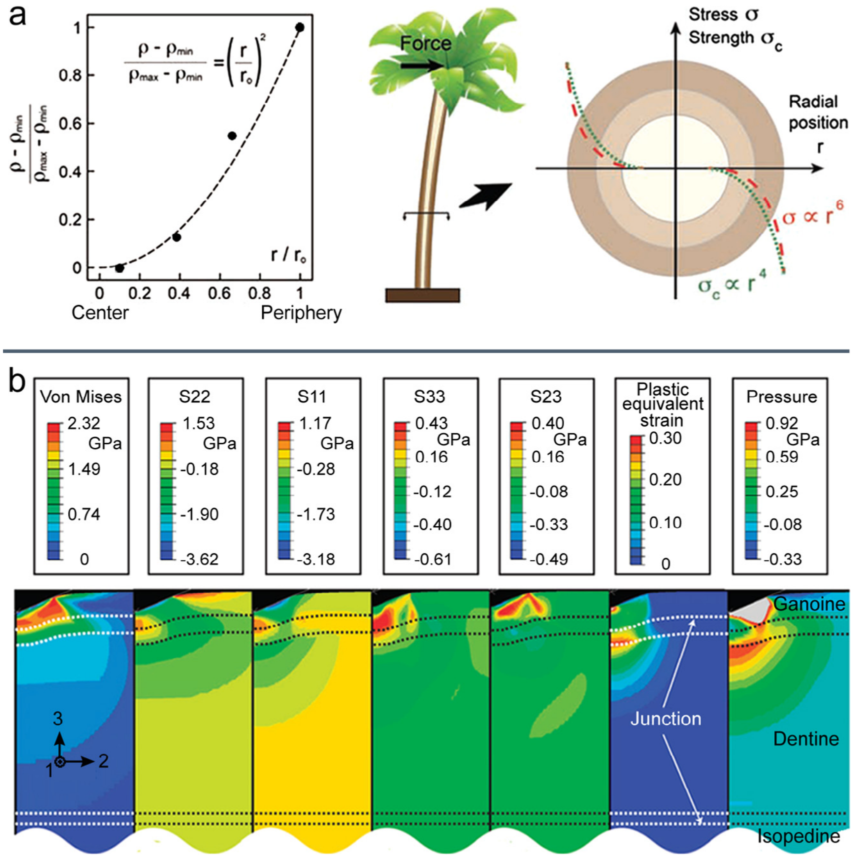

Fig. 14. Gradients in biological materials for load bearing and support and contact damage resistance: (a) The relative density increases with the radius r from center to periphery in the stem of the palm tree *Iriartea gigantean*, leading to non-uniform distributions of both local mechanical strength σ_c and stress σ and a close match between them [38,89]; (b) finite element analysis simulation reveals the contours of stress, plastic strain and pressure in *Polypterus senegalus* fish scales with integrations of diverse gradients (for 1 N maximum load indentation) [43].

via maximizing overall robustness while minimizing their weight. A general design principle is to combine a strong and stiff exterior with a weak yet light interior and to incorporate a gradual transition between them. As a result, the local properties at specific positions can be adjusted to fit their mechanical demands, leading to enhanced global capabilities for load bearing and support using relative meagre constituent materials.

The gradient in the stem of the palm tree *Iriartea gigantea* is a prime example of such function [38,89]. As shown in Fig. 14a, the relative density increases, following a quadratic relation with the radial distance, from the center to the periphery of the palm stem, due to its non-uniform porosity that varies in the opposite fashion. The local mechanical strength σ_c, which scales with the square of density, is thus related to the radius r as $\sigma_c \propto r^4$. Additionally, the local elastic modulus E_c displays a similar varying trend with a scaling function of $E_c \propto r^5$. Consequently, the global flexural rigidity of the stem is improved by a factor of 2.5 in comparison to an equivalent section of the same radius and mass as the stem but with uniform density. Moreover, the radial density gradient leads to a nonlinear distribution in the local stress σ with radius r (following a $\sigma \propto r^6$ relationship) in the stem under bending which resembles the loading state exerted by wind [89]. The distinct dependences of local strength and stress on the radius demonstrate a close match between them; invariably, the former slightly surpasses the latter across the whole section. This again represents Nature's efficient use of materials to fulfill mechanical demands through gradients.

4.2. Contact damage resistance

A wide variety of biological materials are primarily subjected to contact forces, such as impact, indentation and sliding, in their applications; consequently, their mechanical function has been optimized to resist contact damage. This is especially true for materials used for offensive attack, such as the shark tooth [81,152], the mantis shrimp appendage [104,113–116] and spider fang [48,153], and defensive protection, such as the turtle carapace [154,155], armadillo osteoderm [156] and fish scales [42,43,110–112]. The evolutionary "arms race" between predator and prey has led to a widespread utilization of functional gradients in both materials to minimize their contact damage while intensifying the injuries caused to the adversaries or avoiding them by protection.

A prime example of biological weapons is the mantis shrimp appendage which utilizes a combination of multiple gradients to enhance its function [104,113–116], as discussed in Section 3.4.1. Its unique chemical and structural gradients lead to graded mechanical properties from the surface towards the interior with the outer impact region featured by higher stiffness and strength with a clear elastic-plastic transition in its stress-strain curve [113,114]. In particular, this region exhibits a quasi-plastic contact response associated with the interfacial sliding and rotation of fluorapatite crystals. This endows the impact region with remarkable localized yielding before shear-induced circumferential cracks can be initiated, thereby resulting in significant stress redistribution and energy dissipation under the contact point. Moreover, the elastic-plastic transition becomes less obvious in the inner region and an impressive strain-hardening capacity is generated through the densification of the involved microchannels, thus providing additional energy dissipation during impact. These mechanisms, all of which are closely associated with the gradients, impart the appendage with exceptional damage tolerance to resist repetitive impact loading that is offensive enough to smash hard seashells. Other strategies, such as pervasive nanoscale deformation twinning and topological interlocking of building blocks [157–162], are also employed in biological materials to enhance their contact damage resistance.

Another example of the adoption of gradients for improved contact damage resistance is the spider fang which is used to penetrate the exoskeleton of its insect prey. In addition to the chemical variations discussed in Section 3.1, the spider fang utilizes multi-level structural gradients from the arrangement of nano-sized constituents to its macroscale shape and geometry [48,153]. Specifically, both the local radius of venom canal and the wall thickness of the fang increase linearly from its tip to base, such that the fang displays a hollow conical structure expanding approximately along a quarter-circle curve [153]. Such a gradient, in conjunction with its moderate taper, favors improved stiffness compared to a needle-like architecture, shifts the stress maximum from close to the base towards the tip, and lowers the maximum stress by roughly an order of magnitude. As a consequence, both the penetration ability and damage resistance of the fang are improved, effectively contributing to enhanced biomechanical functionality.

As a response, functional gradients have also been broadly adopted in biological armors for optimized protection against such predation threats. These armors are generally characterized by a multi-layered structure encompassing an integration of diverse gradients. The scales of *Polypterus senegalus* fish are a good example here. They comprise four different material layers of organic-inorganic nanocomposite from the outer to inner surfaces, namely, the ganoine, dentine, isopedine and bone basal plate, with respective layer thicknesses of ~10, 50, 40 and 300 μm [43,112]. The indentation modulus of their cross sections decreases from ~62 GPa to ~17 GPa, with a concomitant hardness reduction from ~4.5 GPa to ~0.54 GPa, along the thickness direction. The mechanical properties differ significantly between ganoine, dentine and isopedine regions and display continuous gradations within the first two layers. Additional, gradual transitions in composition and structure also exist at the ganoine-dentine and dentine-isopedine junctions, representing graded interfaces. These spatial variations offer unique mechanisms to enhance the resistance of the scales to penetration by biting attacks, as shown in Fig. 14b [43,112]. The juxtaposition of different material layers enables the combination of their distinct properties, such as the hardness and stiffness of the outer ganoine and energy-dissipation ability of underlying dentine, while maintaining a low weight. The composite structure favors circumferential cracking rather than the more dangerous radial cracking; additionally, deep penetration and further cracking are prevented by the more compliant inner layers of isopedine and bone. Load transfer and stress redistribution between the dissimilar material layers are additionally promoted by their gradient interfaces, which act to help suppress excessive plasticity, arrest cracks, improve adhesion and prevent delamination of the scales.

4.3. Interfacial strengthening and toughening

Another salient mechanical benefit of gradients is to strengthen and toughen the interfaces between dissimilar components; indeed, introducing continuous interfacial transitions provides an effective means to restrict the evolution of damage along or across interfaces. This strategy has been frequently applied in many biological materials, tissues and organs, as exemplified by the DEJ in the human tooth. Due to the intricate modulations of chemical and microstructural characteristics described in Section 3.3 (also see Fig. 10b), the local hardness and Young's modulus decrease from the enamel to the dentin, while the toughness, measured by indentation, varies inversely [121–123], as shown in Fig. 15a. Separation along the interface is effectively suppressed and cracks formed in enamel can be easily arrested within ~10 μm or so at the mantle dentin side of DEJ because of the elastic mismatch between the two phases and efficient crack-tip shielding from uncracked-ligament bridging [121,163]. Indeed, the DEJ layer is endowed with a unique combination of mechanical properties, *i.e.*, high strength comparable to that of enamel and high toughness approaching that of dentin [121,123]. As a result, the distinct components of enamel and dentin are ingeniously joined at the DEJ through interfacial strengthening and toughening by gradients, providing the tooth with remarkable resistance against catastrophic failure during mastication despite the apparent brittleness of its outer enamel.

4.4. Functional benefits

While numerous gradients have been developed to fulfill mechanical demands, many biological materials have evolved to provide non-mechanical functions that are equally vital physiologically for living organisms. Analogously, the generation of a variety of functional properties relies primarily on the spatial regulation of local composition and structure in these materials. This is especially true for the anchorage spicules of the glass sponge *Euplectella aspergillum* which lives in the cold envi-

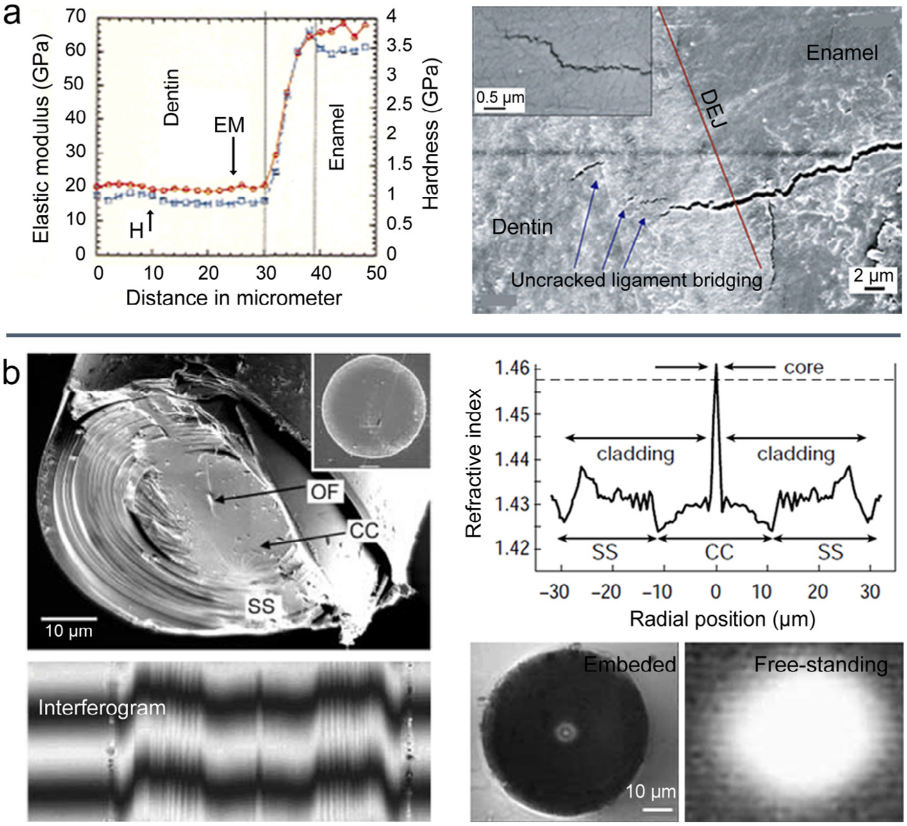

Fig. 15. Biological gradients for interfacial strengthening and toughening and non-mechanical functions: (a) Variations in the local elastic modulus and hardness across the DEJ and the arrest of cracks from the enamel at the DEJ caused by the potent uncracked-ligament bridging in the human tooth [121–123]; (b) the concentric layered structure, non-uniform interferogram and corresponding refractive index profile of the anchorage spicules of glass sponge *Euplectella aspergillum* [164,165]. The spicules act as single- or few-mode wave guides when embedded in epoxide and display unique guiding property for multi-mode light waves at free-standing state [164,165].

ronment of the deep sea, frequently with no ambient sunlight [164,165]. In contrast to the spicules in the skeleton that mainly provide structural rigidity [100,101], these basalia spicules play a role mechanically in securing the sponge to soft sediments. Given the distinct functions, the spicules possess a similar structure of concentric silica layers surrounding the central core. Chemical analysis has revealed that the core is hydrated and heavily doped with higher concentrations of carbon, sodium and mercury; the organic content and hydration level gradually decrease towards the periphery, while the degree of silica condensation increases [164,165]. Such variations, along with the unique architecture of the spicule, lead to a non-uniform refractive index profile across the spicules comprising several characteristic regions, including a high refractive index core with a low index cylinder surrounding the core and an outer portion with progressively increasing index, as shown in Fig. 15b [164,165]. As a consequence, light waves collected by the spicules can be effectively confined to the core, resulting in notable wave-guiding properties reminiscent of those of commercial telecommunication fibers. Such fiber-optical features appear to be vital for the efficient utilization and distribution of light within the sponges in their living environment.

5. Bioinspired applications of gradients

The ingenious design of gradients is clearly critical for biological materials to generate their outstanding structural and functional properties which appear more remarkable considering the limited palette of "building blocks" that Nature has to use. As they are so effective, these biological gradients represent distinctive motifs that need to be translated into engineering design. It is doubtful whether the original inspiration for early graded materials, *e.g.*, the blades of ancient Chinese bronze swords or the Samurai [7,166], came from Nature; rather, these designs may have evolved independently through a process that in Biology is called 'convergence', *i.e.*, a common solution is found in different species. In the case of human tools, this convergence can also be applied, as we established that a hard surface backed by a tough foundation provides in many applications the best combination of properties [3–5,117]. The implementation of bioinspired functional gradients definitely presents a spectrum of opportunities for the development of high-performance synthetic materials, aided by our increasing clarification of naturally-evolved gradients, their contributions to properties, and the principles underlying their mechanisms. Although it is difficult to precisely control, by processing or heat treatment, the composition and structure of synthetic materials over more than a few length-scales, which is the basis of gradients in biological materials, the design principles of some of the biological gradients have been replicated in a number of synthetic materials, as elaborated below.

5.1. Bioinspired FGMs for structural applications

Akin to Nature, gradients have been created in synthetic materials, specifically those that make use of their mechanical properties for structural applications. Typical examples are the widely exploited FGMs as high-temperature structural components or for contact damage resistance [3–9,36]. By modulating the local composition and structure, these materials are designed to combine a unique set of mechanical properties for enhanced performance. For example, polymer-based heterogeneous composites have been recently developed with extreme gradients that imitate biological tissues connecting tendon/ligament to bone (see Fig. 10d) [167,168]. By introducing different types and concentrations of reinforcements, such as hard polymer segments, laponite nanoplatelets and alumina microplatelets, into the soft polyurethane matrix, individual composite films are first produced with elastic moduli varying over almost five orders of magnitude from 4 MPa to 7 GPa. Composites in the form of patches can then be prepared by solvent-welding specific combinations of these films, with particular stiffness, into one single component (Fig. 16a) [167,168]. These materials can be tailored to display critical hard-to-soft transitions from surface to base without apparent internal boundaries between the films; such gradients can simultaneously act to relieve both the maximum strain at the patch surface and the maximum stress at the join when the patch is attached to a soft substrate. As a consequence, the patch-substrate combination can be reversibly stretched up to a global strain of 350% without detachment, endowing the component with unusual stretchability yet high surface stiffness [167]. These graded materials represent a significant potential for a range of challenging applications including as flexible electronics.

5.2. Gradient materials for biomedical applications

The concept of functional gradients in living tissues or organs has also been employed as a fundamental design motif for the synthetic biomaterials used for tissue engineering. By mimicking the gradients of target tissues, these implants can better fulfill their specific mechanical and physiological demands, and thus promote tissue regeneration or reconstruction. Macroporous scaffolds to be used for bone repair, for example, have been designed with similar gradients in pore size and porosity as natural bone [169–175]. Such bioinspired gradients allow a combination of fast osteoconduction process for bone remodeling in the high-porosity regions with larger pore size and good mechanical rigidity in the denser regions. The gradients in pore size and distribution can be further combined with non-uniform chemical compositions in biomaterials; this is exemplified by the mineralized collagen-glycosaminoglycan scaffolds that mimic the composition and structure of articular cartilage on one side and subchondral bone on the other, with a smooth transition in between [172–175]. Such integration of several types of gradients allows the cartilage- and bone-forming cells to grow at specific regions and to develop a natural interface as the bone-cartilage junction, thus demonstrating a good potential for orthopedic applications.

The regulation of cell behavior, *e.g.*, adhesion, migration and proliferation, that is essential for a variety of biological processes, is generally mediated through fine modulations of bioactive cues in the body [171,176,177]. Creating spatial gradients of these cues in biomaterials provides a feasible pathway towards the control of cell behavior for definite purposes. For instance, in an attempt to imitate the biological tissues connecting tendon to bone, reverse gradients in concentrations of dual platelet-derived growth factor-β (PDGF-BB) and bone morphogenetic protein 2 (BMP-2) have been incorporated into a porous polycaprolactone/Pluronic F127 membrane using a diffusion method [177], as shown in Fig. 16b. With the continuous release of these growth factors, the adipose-derived stem cells seeded on the membrane display position-dependent differentiation behavior *in vitro* into different target cells after two weeks of culture. The osteogenesis and tenogenesis processes are increasingly promoted towards the sections with higher concentrations of BMP-2 and PDGF-BB growth factors, respectively; reverse changes in the densities of corresponding target cells are thus generated in accordance with the concentration gradients. Therefore, the membrane demonstrates a potential for biomedical applications in tendon-to-bone regeneration as tendon tends to form towards one end and bone towards the other. Such gradients in growth factors can be further integrated with a graded porous microstructure to better mimic the native tissues for improved biomedical performance [178,179].

5.3. Bioinspired design of gradient interfaces

Interfaces invariably play a dominant role in determining the overall properties of materials in both synthetic and biological systems [17,37,180]. The strength of metals and alloys, for example, often relies on the impeded motion of dislocations at grain boundaries at ambient temperatures; while at elevated temperatures, the grain boundaries become the preferential sites for microstructural damage and eventual failure [2,180]. As a source of inspiration, the widespread gradient interfaces developed by Nature present rich paradigms to be mimicked in synthetic materials. This is represented by the scaffolds that imitate the bone-cartilage junction used for tissue engineering [172–175], as described above.

Another prime example are the TiB/Ti-alloy composites that have been made with a network distribution of branched reinforcements [181,182]. By hot pressing mixtures of spherical titanium alloy powders and smaller prismatic TiB_2 powders, TiB whiskers can be formed via *in situ* reaction and assembled into a network structure at the boundaries between Ti-alloy

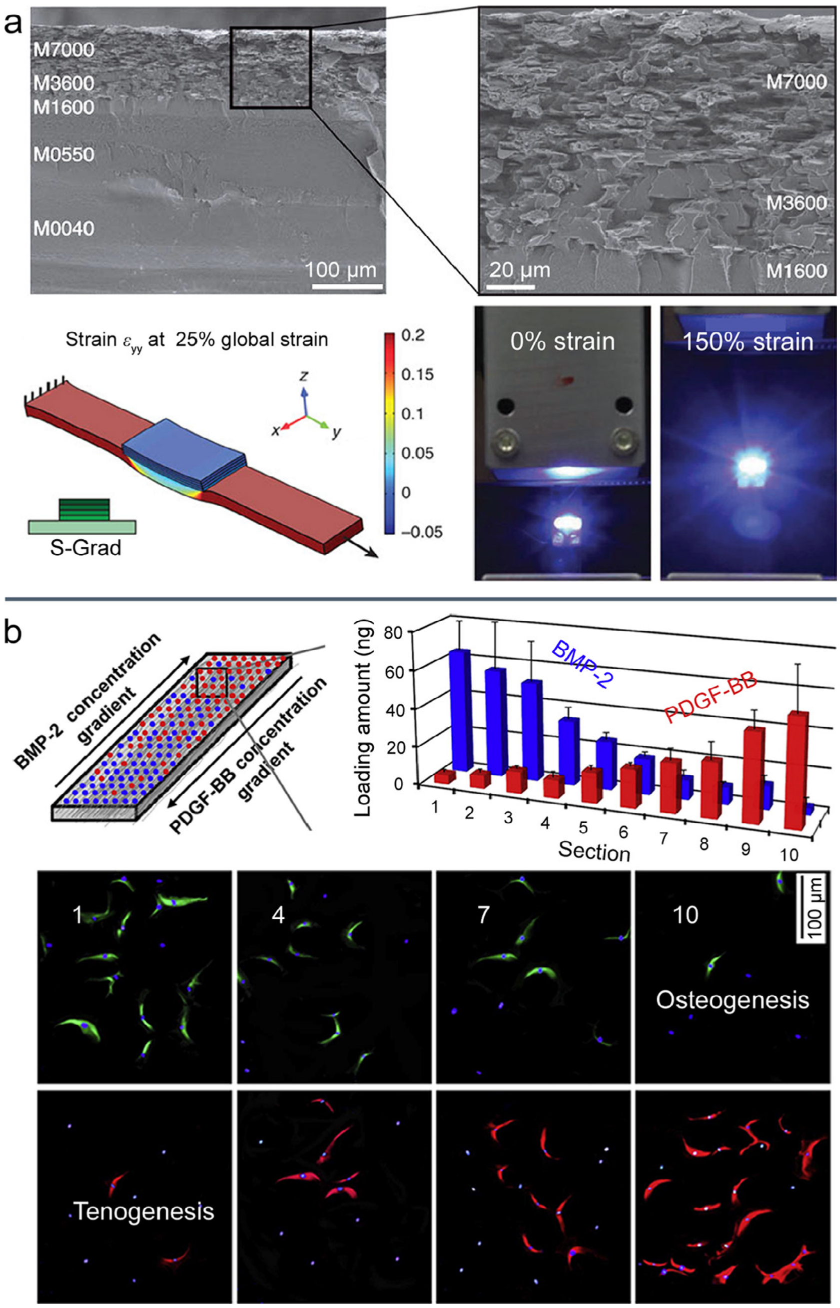

Fig. 16. Bioinspired design of gradients for structural and biomedical applications: (a) A composite patch composed of different layers comprising extreme hard-to-soft transitions from surface to base without apparent internal boundaries between films [167]. Finite element analysis reveals simultaneous alleviation of both the maximum strain at the patch surface and the maximum stress at the interface of patch-substrate module. The substrate can be stretched by more than 150% without failure of the fragile light-emitting diode deposited at the patch surface; (b) reverse gradients in concentrations of dual platelet-derived growth factor-β (PDGF-BB) and bone morphogenetic protein 2 (BMP-2) on a porous membrane and the resultant position-dependent differentiation behavior of seeded stem cells after two weeks of culture [177].

particles, as shown in Fig. 17a. These whisker reinforcements grow with a branched morphology and extend into neighboring particles like dowel connectors, forming broad interconnected boundary regions between particles which in many aspects resemble the gradient entheses at biological interfaces, such as the tendon/ligament-to-bone connections shown in Fig. 10d [127,128]. The boundaries are thus significantly strengthened and stabilized as compared to a homogeneous distribution of reinforcements; the result is that fracture is effectively suppressed by hindered crack propagation along a rather tortuous path [181,182]. Such bioinspired graded interfaces lead to simultaneous strengthening and toughening of

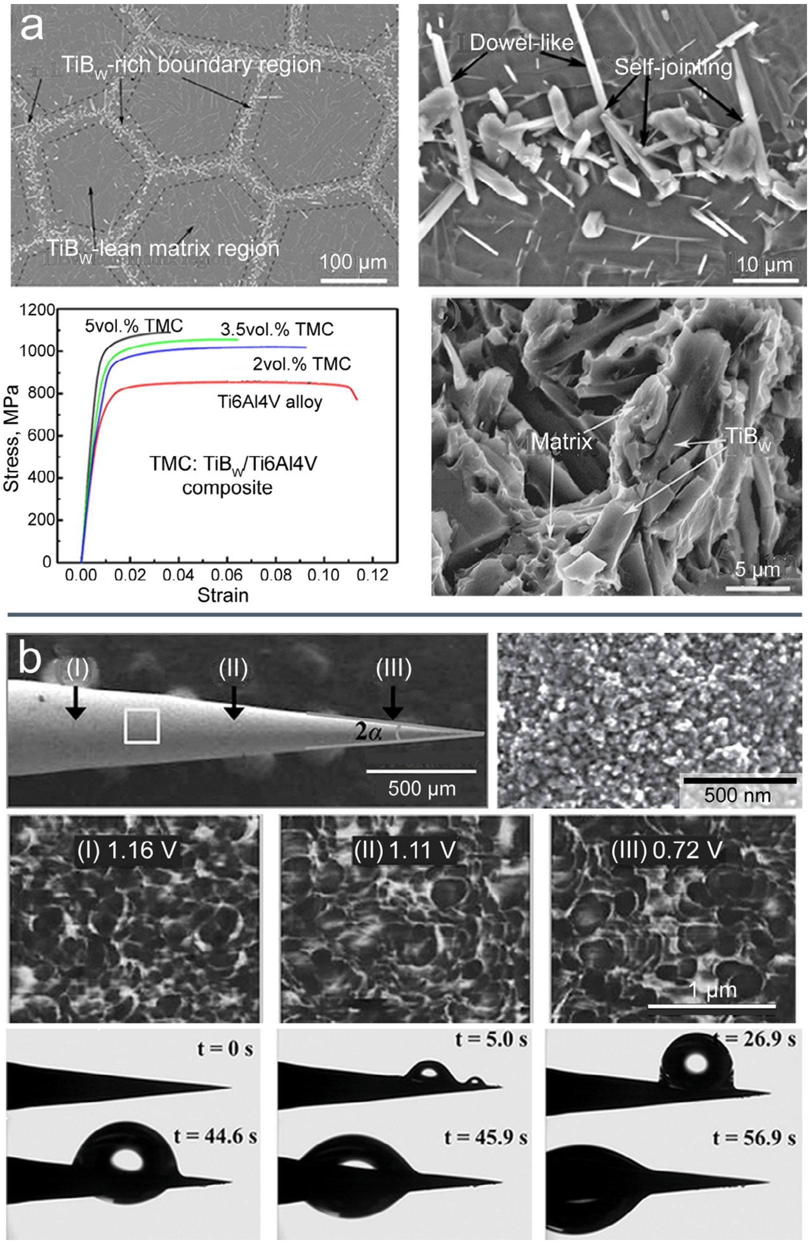

Fig. 17. Bioinspired design of gradients for interfacial strengthening/toughening and functional applications: (a) TiB/Ti-alloy composites with a network distribution of branched reinforcements at the boundaries between Ti-alloy particles exhibit remarkably high strength and ductility and fairly torturous crack propagation paths [181,182]; (b) the surface of bioinspired conical copper wire possesses a homogeneous nanoscale structure and displays a decreasing interaction force with the tip of lateral force microscope from base to tip [183,184]. Microscopic observations reveal quick collection and transportation of water drops on the surface.

the composites, which show especially appealing properties at high temperatures, thereby providing a meaningful route towards high-performance metal-matrix composites.

5.4. Bioinspired gradients for functional applications

A multitude of non-mechanical properties of biological materials also results from their compositional and structural gradients. Replicating the design principles of these gradients offers potent solutions for the generation and enhancement of corresponding properties in synthetic materials to promote their functional applications. One example of such bioinspired design is the conical copper wires with gradient wettability on their surfaces used for continuous and efficient fog collection

[183,184]. The inspiration for this came from cactus plants, which despite living in arid environments are capable of harvesting water from fog using their spines [185]. These spines derive a remarkable combination of functional properties, such as water deposition, collection, transportation and absorption, from a subtle integration of multiple structures, *e.g.*, oriented barbs, conical shapes of spine and barbs, graded grooves and belt-form trichomes; in particular, gradient surface-free energy and Laplace pressure are generated from tip to base from these features [185]. This enables the plants to relieve aridity through efficient fog collection using their spines, a notable function that could be advantageous in many synthetic materials and devices.

By mimicking the design of such cactus spines, conical copper wires have been prepared using graded electrochemical corrosion and then assembled with gradually increasing wettability from tip to base through graded surface modification by varying the ratio between the hydrophobic methyl terminal groups and hydrophilic hydroxy groups (Fig. 17b) [183,184]. The hydrophobic tip ensures a high growth rate of water drops and thus allows rapid water collection; additionally, an efficient transportation of collected water is guaranteed by the improved wettability towards base and the gradient Laplace pressure arising from the conical shape. As such, the bioinspired gradients endow the wires with a distinctive capacity for continuous and efficient fog collection, a functional characteristic that may find applications in future fog collection projects and perhaps even provide a new global means for obtaining drinking water in dry environments.

6. Synthesis and fabrication techniques

The gradients in biological materials are invariably the product of a series of complex self-assembly processes, such as biomineralization, mediated genetically in organisms. The replication of these gradients in synthetic materials necessitates a precise modulation of composition and structure at multiple length-scales, from nano- to macro-levels; unfortunately, this is still beyond the reach of present materials processing technologies. As a result, it is fairly challenging to create intricate gradients that can be comparable to their natural paradigms, especially those demonstrating complex integration of different types of gradients over multiple length-scales, as exemplified by the tooth, the mantis shrimp appendage and bone, as discussed in Section 3.4. By comparison, limited bioinspired gradients can, and have been implemented at one or a few length-scales in synthetic materials, which play an effective role in enhancing their mechanical and functional performance. Such achievements are represented by the creation of diverse gradients mimicking those in the chemical and structural characteristics of a series of biological materials, described in Section 5, and by the development of engineering FGMs that have been used for several applications, such as for aerospace projects and medical implants [3–7,36].

Processing methods to create such gradients in synthetic materials remains a challenge. There two principal types of methods that have been traditionally used to fabricate such gradients in the form of FGMs [7,186]. One is through "constructive processing" which denotes the selective stacking of starting components in some specific sequence; prime examples here are vapor deposition and solid-state powder metallurgy. The second type is "transport-based processing" which mainly utilizes natural transport phenomena, *e.g.*, heat and mass diffusion, to create chemical and structural gradients in materials. However, despite their widespread use in engineering design, these methods suffer from marked constraints in terms of how feasible it is to precisely control the hierarchically local properties in the manner achieved in biological materials.

Recent progress in materials processing technologies has enabled an increasing control of material structure and hence local properties even down to the nanoscale, and this bodes well for the effective processing of bioinspired gradients in synthetic materials. Simple "top-down" procedures can work here. For example, the surfaces of metals and alloys can be plastically deformed with graded distributions of strain and strain rate through mechanical surface grinding treatments (or other surface modification methods), as shown in Fig. 18a [6,187,188]. The result is a graded structure that varies continuously with increasing depth into the samples from a layer of nanostructured grains at the surface, a layer with ultrafine grain-size beneath, followed by a deformed (work-hardened) layer containing abundant defects, down to the undeformed region in the bulk of the sample. This can result in marked ductility in the bulk of the material by effectively suppressing the onset of strain localization near the surface. Moreover, a strongly textured nanoscale laminated structure can be formed in metals, such as nickel, that have been treated by this method, leading to a ductile material incorporating a stable, ultra-hard surface layer [187]. Such strain-induced gradient architectures have been used for several applications, in particular for bulk materials where the synergy of strength and ductility needs to be coupled with wear and fatigue resistance.

Biological gradients can also be mimicked in synthetic materials by clever control of materials processing or by deliberately designed self-assembly. A good example of this is by coating ceramic reinforcement microparticles (such as alumina) with superparamagnetic nanoparticles (such as iron oxide) by dispersing them in water with a pH value where their surfaces are oppositely charged to generate magnetic responsivity in the reinforcements [18,29,31,32,189–191]. Once suspended in a fluid, the spatial distribution and orientation of these reinforcement particles can be readily manipulated by applying tunable magnetic fields as low as 1 mT (Fig. 18b). In particular, the reinforcements can be concentrated towards specific regions, such as surfaces or weak sites, through the application of gradient magnetic fields [29]. As such, synthetic composites with elaborate structures can be created after consolidation of the fluid, *e.g.*, through polymerization reactions. Such magnetically-assisted control of the composite structure during processing represents significant progress in the quest to enable the high-fidelity replication in synthetic materials of the functional gradients that work so effectively in biological materials.

The development of novel fabrication approaches, such as additive manufacturing [30–35,190–198] and freeze casting [20,21,24,199–201], further permits the potential of creating intricate bioinspired gradients in synthetic materials. Additive

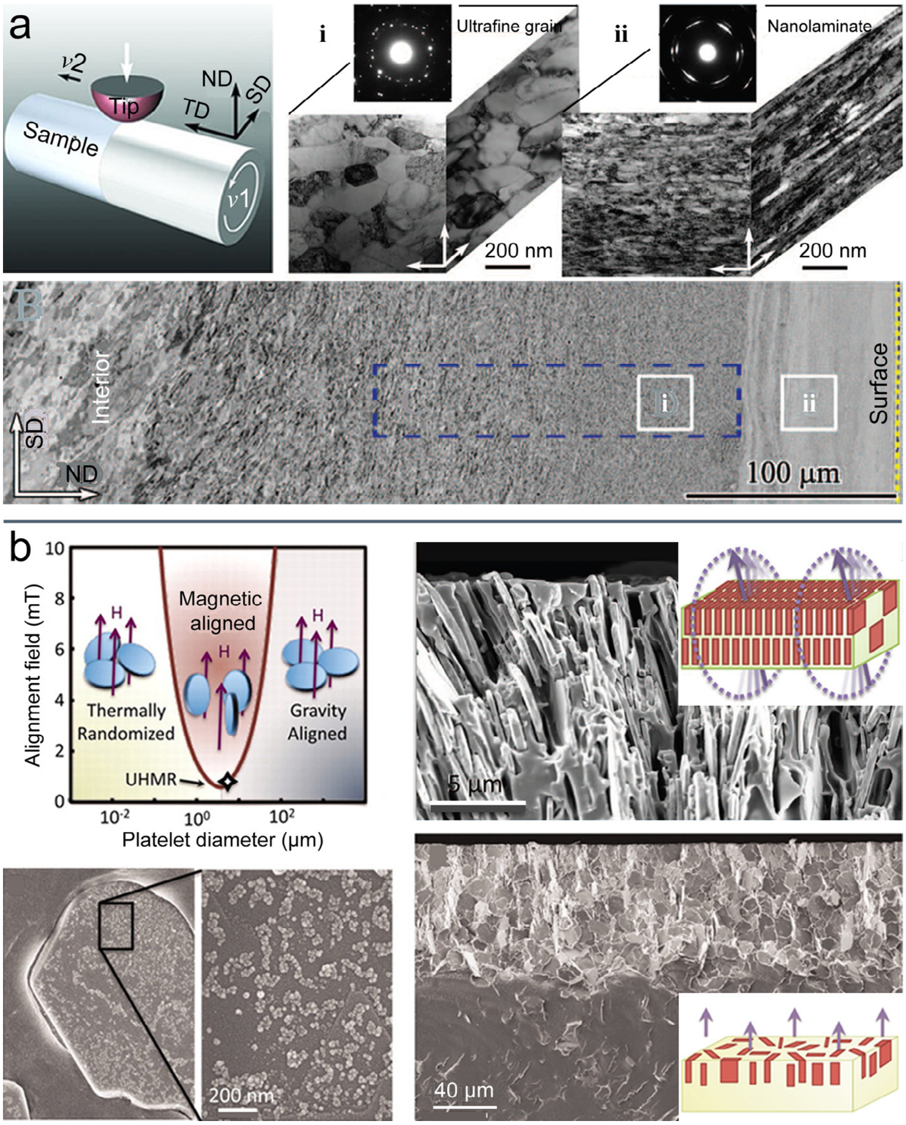

Fig. 18. New processing techniques for bioinspired gradients: (a) Surface mechanical grinding treatment represents an effective means to introduce graded structures at the surface region of metals and alloys [6,187,188]; (b) by coating reinforcing microparticles with superparamagnetic nanoparticles, the spatial distribution and orientations of reinforcements can be readily manipulated through applying low magnetic fields [29]. The reinforcements can also be concentrated towards specific regions by subjected to gradient magnetic fields.

manufacturing in particular has promising advantage for the rapid construction of complex architectures from the "bottom-up" manner (Fig. 19a), and accordingly has attracted considerable interest [30–35,190–198]. A variety of building blocks including polymers, metals and ceramics can be adopted and remarkable structural complexities can be achieved at sub-micrometer to macro length-scales, although the surface finish and the quality of the material, both properties that are critical to structural integrity, are generally still lacking. As a result, the fracture toughness and fatigue properties are usually inferior to those of conventionally-processed materials, but in many applications the advantages can override these shortcomings. In addition, the use of computational thermodynamics, such as CALPHAD, can guide the designer in the tailoring of the phases comprising their interfaces and spatial distributions [192–196]. The local compositions/constituents can also be regulated using several nozzles to produce gradients [192,193,196].

Additive manufacturing by such techniques as 3D printing has essentially unlimited potential to create complex bioinspired gradients. A prime example is the application of a 3D magnetic printing method to fabricate ceramic/polymer composites [190]. The active photopolymer-ceramic blend containing magnetic-responsive reinforcements can be printed into desired architecture, and the orientations of reinforcements can then be tuned by applying rotating magnetic field before being subsequently fixed by ultra-violet polymerization of the polymer matrix. With such a strategy, the local structure

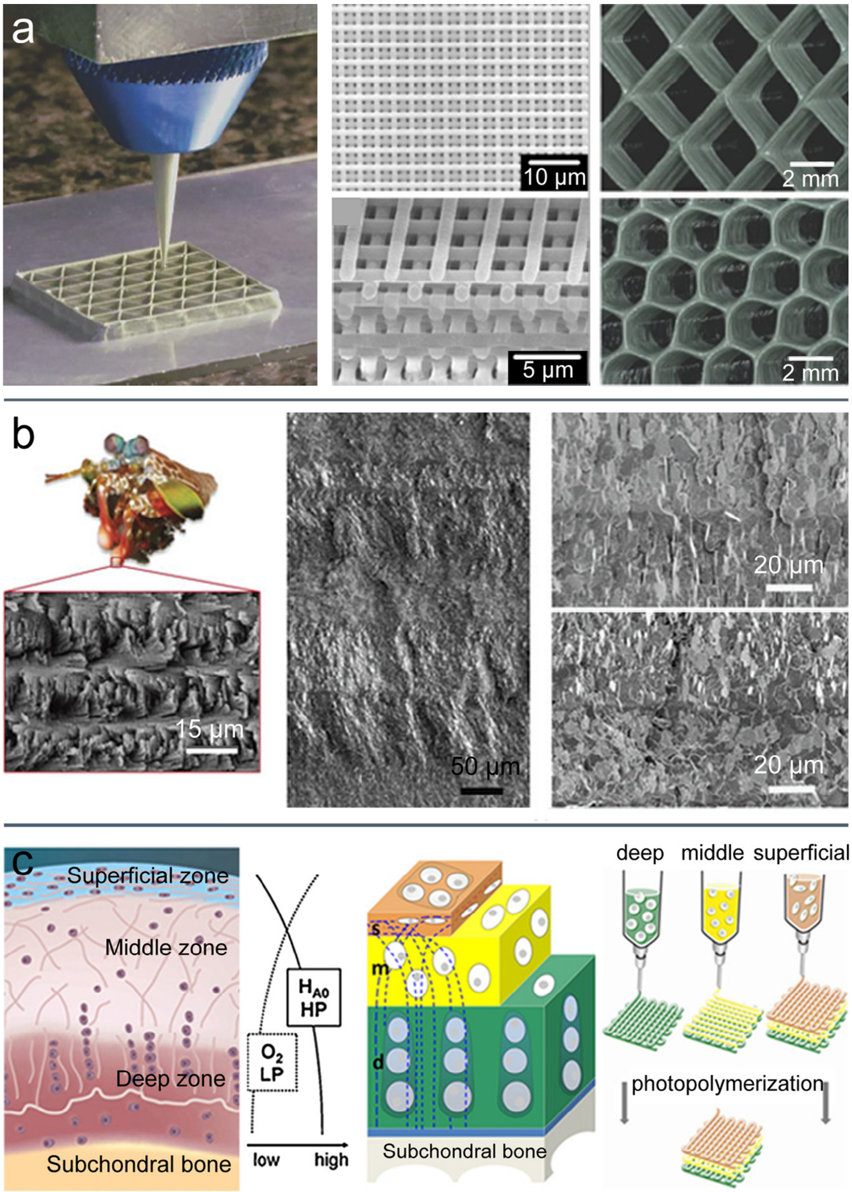

Fig. 19. Additive manufacturing for processing bioinspired gradients: (a) Additive manufacturing can be utilized to achieve structural complexities at multiple length-scales [33–35,198]; (b) the orientations of magnetic-responsive reinforcements within each voxel can be tuned by applying rotating magnetic field during the 3D magnetic printing process, thus enabling the recreation of bioinspired gradients [190]; (c) the gradients of articular cartilage, as represented by the changes in levels of oxygen, collagen cross-links of lysylpyridinoline (LP) and hydroxylysylpyridinoline (HP), and compressive modulus (HAO) through the thickness of tissue, can be mimicked in the cell-material construct fabricated by additive manufacturing [202–205].

within each individual voxel can be finely modulated during the manufacturing process, thus enabling the recreation of various bioinspired gradients, potentially as complex as the twisted plywood structure of the stomatopod dactyl club (Fig. 19b) [113,114,190]. The magnetically-directed assembly has been further introduced into the slip casting process of aqueous-based suspensions [191]; such combinations offer a simple and cost-effective route to produce complex-shaped objects with locally tunable composition and texture and high concentrations of inorganic constituents. Additive manufacturing can also be applied to create graded cell-material constructs mimicking the target native tissues or organs [202–206], as represented by the man-made articular cartilage shown in Fig. 19c. By tuning the material/hydrogel composition, construct architecture and encapsulated cell types during processing and subsequent fixation (*e.g.*, via polymerization), complex compositional and structural gradients can be produced resembling the osteochondral tissue [202,203].

7. Conclusions and outlook

With a limited choice of building blocks, often displaying relatively modest mechanical properties as compared to their synthetic counterparts, living organisms have broadly evolved and flourished by creating high-performance materials which effectively employ hierarchical architectures with functional gradients to address their environmental challenges during long-term evolution and selection. Generally, biological materials are featured by site-specific properties that are generated through spatial regulation, mediated by self-assembly, of chemical compositions/constituents and structural characteristics, including the arrangement, distribution, dimensions and orientations of the building units. Interfaces between dissimilar components in these materials invariably involve localized gradients in the form of continuous transitions of local properties. It is particularly common that a variety of gradients is integrated in parallel and over multiple length-scales within the same material.

Biological materials utilize their gradients to enhance a series of properties and functionalities, including load bearing and support capabilities, contact damage resistance for predation and protection, interfacial strengthening and toughening, as well as non-mechanical functions. Joining the diverse structural design elements used by Nature (Fig. 1b), gradients also perform a vital function to ensure the integrity of each system. In this regard, gradients play an integral role in enabling organisms to survive and thrive in their living environments by endowing the biological materials, from which they are made, with remarkably high performance.

Although Nature has developed a considerable wealth of gradients with distinct functions for its vast diversities of organisms and their living environments, only a comparatively few have gained the attention of researchers and even fewer have been thoroughly studied over multiple length-scales. Indeed, the compositions, structures and resulting mechanical and functional properties under physiological conditions still remain to be explored for a wide range of biological materials. In particular, it is critical to discern the key material parameters that dictate their properties with special focus on how these are affected by the presence of the gradients. There is need for significant efforts to establish the composition/structure-property relationships and to uncover the underlying governing mechanisms through a combination of experimental, theoretical and modeling research to be able to derive the design principles of biological materials. It is equally important to clarify the relevant formation processes of biological materials and their gradients, such as biomineralization, to provide guidance for corresponding artificial processing routes.

Although such naturally-occurring gradients provide a rich source of inspiration for the design of new synthetic materials, to capitalize on this there is an obvious requirement of learning how we can make such bioinspired graded materials. The translation of Nature's design motifs clearly offers new pathways towards improved materials performance, but numerous challenges exist in the actual processing of such new materials that can replicate their biological paradigms over multiple length-scales. Nevertheless, there are examples where this has been achieved to a limited extent. Of note here has been the use of surface mechanical grinding techniques, magnetically-assisted manipulation of constituents and additive manufacturing.

The potential of further developing these techniques, and perhaps other synthesis and processing methods, to achieve the required precise control of composition and structure at the nano- to near macro-scales, coupled with the ability to recreate intricate gradients and graded interfaces in the image of biological materials, is literally unbounded as it offers the promise of new generations of materials with unprecedented combinations of mechanical properties and functionalities.

Acknowledgements

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