Hydration-induced nano- to micro-scale self-recovery of the tooth enamel of the giant panda

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Abstract

The tooth enamel of vertebrates comprises a hyper-mineralized bioceramic, but is distinguished by an exceptional durability to resist impact and wear throughout the lifetime of organisms; however, enamels exhibit a low resistance to the initiation of large-scale cracks comparable to that of geological minerals based on fracture mechanics. Here we reveal that the tooth enamel, specifically from the giant panda, is capable of developing durability through counteracting the early stage of damage by partially recovering its innate geometry and structure at nano- to micro-length-scales autonomously. Such an attribute results essentially from the unique architecture of tooth enamel, specifically the vertical alignment of nano-scale mineral fibers and micro-scale prisms within a water-responsive organic-rich matrix, and can lead to a decrease in the dimension of indent damage in enamel introduced by indentation. Hydration plays an effective role in promoting the recovery process and improving the indentation fracture toughness of enamel (by ~73%), at a minor cost of micro-hardness (by ~5%), as compared to the dehydrated state. The nano-scale mechanisms that are responsible for the recovery deformation, specifically the reorientation and rearrangement of mineral fragments and the inter- and intra-prismatic sliding between constituents that are closely related to the viscoelasticity of organic matrix, are examined and analyzed with respect to the structure of tooth enamel. Our study sheds new light on the strategies underlying Nature’s design of durable ceramics which could be translated into man-made systems.

Statement of Significance

Tooth enamel plays a critical role in the function of teeth by providing a hard surface layer to resist wear/impact throughout the lifetime of organisms; however, such enamel exhibits a remarkably low resistance to the initiation of large-scale cracks, of hundreds of micrometers or more, comparable to that of geological minerals. Here we reveal that tooth enamel, specifically that of the giant panda, is capable of partially recovering its geometry and structure to counteract the early stages of damage at nano- to micro-scale dimensions autonomously. Such an attribute results essentially from the architecture of tooth enamel but is markedly enhanced by hydration. Our work discerns a series of mechanisms that lead to the deformation and recovery of enamel and identifies a unique source of durability in the enamel to accomplish this function. The ingenious design of tooth enamel may inspire the development of new durable ceramic materials in man-made systems.

1. Introduction

Synthetic ceramics develop their properties largely from the chemical compositions and generally exhibit a significant brittleness that restricts their structural applications. Differing markedly
from man-made systems, biological ceramic materials have perfected their performance principally through ingenious structural designs involving multiscale hierarchical architectures often with exquisite gradients and interfaces [1–8]. An excellent case in point is the tooth enamel of vertebrates, which is a hyper-mineralized tissue that is used directly as a weapon or as a means to fulfill a series of mechanically stringent tasks, e.g., fighting and/or breaking down food. Despite being composed almost entirely of mineral (primarily hydroxyapatite (HAP)), tooth enamel exhibits an exceptional durability to resist impact and wear, generally throughout the lifetime of organisms, as most vertebrates cannot replace their adult teeth. Such characteristics make tooth enamel superior to many synthetic ceramic counterparts. It is well established that the mechanical properties of tooth enamel are derived primarily from their hierarchical structure [9–16]. The basic constituents of HAP nanofibers are arranged over several length-scales with the organic matter that accounts for a minimal content located mainly at the interfaces. In particular, the chemical and microstructural characteristics of enamel are finely modulated even down to the atomic level. This is represented by the presence of an intergranular Mg-rich amorphous calcium phosphate phase between the HAP crystallites as revealed by atom probe tomography [17,18].

Tooth enamel is subject to a mixed-mode stress condition in service and may fail in different manners, e.g., caused by longitudinal or transverse cracks or by edge chipping from sharp contacts [13,15,19–21]. The fracture resistance of enamel has been quantitatively accessed based on the fracture mechanics which invariably deals with large-scale cracks of hundreds of micrometers or more [15,22–25]. The primary feature is that the enamel displays an increase of resistance to the extension of cracks as represented by a rising R-curve behavior. Such an attribute results principally from a variety of extrinsic toughening mechanisms, such as crack deflection/twisting and uncracked-ligament bridging, which operate mainly at a coarse length scale of enamel prisms. These mechanisms are closely associated with the architecture of enamel and rely on the interfacial organics [7,15,22–25]. However, enamel still exhibits a remarkably low resistance to the crack initiation, i.e., with a toughness on the order of 1 MPa m$^{1/2}$ as denoted by the onset of the R-curve, which is comparable to that of monolithic HAP mineral [22–24]. The apparent inconsistency of the low crack-initiation toughness of tooth enamel in light of its obvious physiological structural integrity provokes some reconsideration of the origins of its exceptional durability, in particular its resistance to the formation of large-scale cracks, and the precise role of its micro-/nano-scale structure in generating such properties.

In this regard, we specifically examine here the tooth enamel of the giant panda which enables the animal to chew bamboo (the giant panda feeds on both leaves and stems of bamboo) – another biological material that is well-known for outstanding strength and toughness [26,27]. The chemical and microstructural characteristics of such tooth enamel are known to be similar to the human enamel and the enamels of other animals [16]. We investigate the structure of the giant panda’s tooth enamel and its mechanical response at micro- to nano- length-scales using fine-scale approaches of testing and characterization with the aim of illuminating the means by which enamel has developed its durability. We find that tooth enamel, especially in its hydrated state, is capable of autonomously counteracting the early stage of damage by partially recovering its innate structure at the micro-scale and more significantly at nano-scale dimensions. To discern the structural origins of such self-recovery behavior, we examine the nano-scale deformation/recovery mechanisms to identify those realized by different constituents and analyze their contribution to the mechanical response of the material. The design principles that we recognize from tooth enamel shed new light on the strategies created by Nature to develop durable ceramics; they further demonstrate a promising potential that could be translated to synthetic ceramic materials to generate self-recovery/healing capability on the basis of their benchmark properties.

2. Materials and methods

2.1. Sample collection and preparation

A detailed description about the collection of tooth samples from giant panda and the preparation of specimens for microstructural characterization is given in our previous study [16]. For micro- and nano-indentation testing, the occlusal surface of teeth at the cusp was slightly polished to provide a flat profile within tens of micrometers close to the original free surface. Naturally-dried and rehydrated samples were obtained, respectively, by exposing to ambient air and immersing in deionized water for at least 12 h. Such treatment results in a stable hydration level of the teeth and further exposure for even longer time causes no additional changes.

2.2. Field-emission scanning electron microscopy imaging

Field-emission scanning electron microscopy (SEM) analysis was performed on the etched samples for microstructural characterization and on the indented samples before and after hydration-induced self-recovery to examine the morphological changes between them. The samples were prepared and observed following the procedure described in detail elsewhere [16]. Here all the samples, both before and after recovery, were observed in their air-dried state to exclude possible effects of hydration level on the morphologies.

2.3. Transmission electron microscopy imaging

Transmission electron microscopy (TEM) imaging was performed using an FEI Tecnai G2 F20 transmission electron microscope operated at 200 kV. The diameter of the regions probed in selected area electron diffraction (SAED) analysis was ~700 nm. A detailed description about the preparation of TEM samples used for microstructural characterization is given elsewhere [16]. Two samples were additionally prepared from the subsurface regions under the indents created by nano-indentation in dehydrated tooth enamel using an FEI Helios Nanolab 650 Dual Beam. A protective platinum layer was firstly deposited on the top of the desired region. Slabs of thickness ~1.5 μm were then cut through the central axis of the indents by focused ion beam (FIB) and then transferred to a copper grid using an OmniProbe nanomanipulator. The samples were thinned by FIB at a sequentially decreasing ion beam voltage of 30, 5, and 2 kV until the thickness was reduced below 100 nm. Final polishing was conducted at 1 kV to obtain a clean surface. The samples were sputter-coated with carbon to reduce charging effect before imaging. After observation, the samples were allowed recovery by immersing in water for 12 h, and then air-dried and re-examined by TEM so as to compare the morphologies corresponding to the same areas before and after recovery.

2.4. Micro-indentation

Micro-indentation was conducted on both dehydrated and rehydrated samples following the procedure described in detail elsewhere [16]. A peak load of 200 g was applied with loading perpendicular to the occlusal profile of tooth. Such a load has been shown to result in a constant measurement of the mechanical properties of giant panda’s tooth enamel [16]. The fracture tough-
ness, $K_c$, of tooth enamel was accessed using the average length, $c$, of the radial cracks induced by indentation following the expression:

$$K_c = \frac{\sqrt{E/H}}{2c}(\frac{P}{c})^{3/2}$$

where $E$ and $H$ are the Young's modulus and hardness of enamel, $P$ is the indentation load, and $\gamma$ is the geometric constant of indentor that equals 0.016 [16,28,29]. The application of testing methods based on fracture mechanics in tooth enamel is usually restricted by its small size. Although such measurement can be realized, e.g., by embedding the enamel in a resin foundation [23–25], the approaches were excluded in the present study as the size of involved cracks far exceeds the characteristic micro- to nano-scale dimensions at which the recovery occurs. Despite the controversy about indentation in creating mode I cracks of linear-elastic behavior in materials, we believe that it is reasonable to compare the damage resistance of the same materials in different states by employing such indentation toughness methods [29].

### 2.5. Nano-indentation

Nano-indentation testing was performed on the polished occlusal profile near the free surface of tooth using an Agilent G200 Nano Indenter (Keysight, USA) equipped with a Berkovich diamond tip operated in displacement-control mode. A displacement rate of 10 nm/s was used for the loading and unloading processes. The peak displacement and hold time at the peak load were set at 450 nm and 10 s to evaluate the nano-scale mechanical properties. The nanohardness and reduced elastic modulus were calculated following the methods of Oliver and Pharr [30]. For the creep experiment, the samples were loaded to a maximum displacement of 350 nm, followed by holding at the peak load for 900 s before unloading.

### 2.6. Morphological observation of indents

The time-dependent morphological changes of tooth enamel were examined immediately after micro-indentation and recorded by laser scanning confocal microscopy (LSCM) imaging using an Olympus LEXT OLS 4000 confocal laser microscope. The indentation and observation were conducted on both dehydrated and rehydrated samples with their hydration states kept constant during the experiments. Imaging was performed in both optical and laser modes. Tomography images were created and analyzed using the LEXT software. Atomic force microscopy (AFM) imaging was carried out to characterize the morphological changes of tooth enamel with time after nano-indentation. Dehydrated samples were used for indentation with the rehydration performed about two hours after indentation. Originally rehydrated samples were not adopted here because the recovery speed was so high that the process was almost completed before the AFM imaging could be performed. An Innova atomic force microscope (Bruker, USA) equipped with an OTESPA probe tip was used in a tapping mode to obtain tomography images of the indented region. The lateral pixel size was ~9.8 nm with the vertical resolution at the Ångstrom scale. The images were analyzed using the NanoScope Analysis software.

### 2.7. Statistical analysis

The data are expressed as mean ± standard deviation in the Results section and the figures. Possible differences between the data were tested using two-tailed Student’s t-test with values of $P < 0.05$ being considered statistically significant. The original data and detailed results of statistical analysis are provided in the Supplementary Materials.

### 3. Results

#### 3.1. Micro-/nano-scale structure and interfaces

Field-emission SEM imaging of etched samples reveals a columnar architecture of the outer region of the giant panda’s tooth enamel (Fig. 1a). Enamel prisms, 5.4 ± 0.3 μm in diameter, are arranged in parallel and oriented perpendicular to the occlusal profile of tooth. These prisms are surrounded by an organic-rich inter-prismatic matrix (Fig. 1b). The organics appear in a relatively high concentration as a result of the erosion of adjacent minerals. TEM imaging revealed that the enamel prisms are composed of abundant fibers having a diameter of 35 ± 10 nm (Fig. 1c). These nanofibers are aligned preferentially along the long axis of prism and deviate gradually towards the inter-prismatic boundaries by 8–25° at the peripheral zone of the prisms. The boundary region between the prisms is characterized by a low packing density of constituents within the thicknesses of tens of nanometers, forming a sheath that is rich in organic phases (Fig. 1d). Within the enamel prisms, the interfaces between the nanofibers display a bright contrast in bright-field TEM micrographs (Fig. 1e). This, combined with SEM observations (Fig. 1b), suggests the relative enrichment of organic phases around the fibers. The nanofibers were identified, by high-resolution TEM imaging and corresponding fast Fourier transform techniques, to be single-crystalline HAP minerals (Fig. 1f). Neighboring fibers exhibit differing crystallographic orientations with a nano-scale clear boundary between them. Additional TEM micrographs are shown in the Supplementary Materials (Fig. S1).

#### 3.2. Micro-scale mechanical properties and self-recovery

Using micro-indentation to introduce damage in the enamel, the changes in the cross-sectional profiles of the indents, revealed by LSCM, clearly demonstrate the capability of giant panda’s tooth enamel to slightly mitigate micro-scale damage under hydrated conditions (Fig. 2a). The dimension of indent, as represented by its width and depth, displays a decreasing trend with hydration time and is diminished on average by ~15% of depth in nearly one day. In addition to the surface crater, tiny cracks and bumps are created by indentation primarily along the boundaries between prisms, indicating the occurrence of internal structural damages and relative slipping between prisms (Fig. 2b and c). Specifically, the cracks originate near the corners of indents and propagate preferentially along a wavy path but are restricted to the inter-prismatic matrix. Such damage becomes almost indiscernible by LSCM due to self-recovery after one-day of hydration (Fig. 2d and e).

The recovery kinetics of the hydrated enamel, measured using the time dependence of the indent depth and evaluated by LSCM, reveal that the recovery speed, as indicated by the slope, decreases gradually with the hydration time (Fig. 3a). The recovery process is almost completed in one day and further hydration after that leads to little further decrease of the indent depth. A direct comparison of the diagonal profiles of one indent before and after one-day’s hydration is shown in the inset. In contrast to the case of hydrated samples, the tooth enamel in the dehydrated state effectively cannot repair any damage introduced at the micro-scale. This is represented by a minimal decrease in the dimension of indents by less than 1% after exposing to ambient air for one day without the intervention of water (Fig. 3a). Besides accelerating the recovery process, hydration plays a critical role in ensuring the resistance of tooth
enamel to the initiation of large-scale cracks. The fracture toughness of the enamel, as measured by indentation, is significantly enhanced (by ~73%) through hydration, as compared to that of dehydrated samples (Fig. 3b); this is achieved at the low cost of ~5% decrease in micro-hardness (Fig. 3c). Moreover, comparison of the SEM micrographs of an indented region before and after one-day’s hydration clearly reveals another role of water, that of decreasing the crack width, as shown in Fig. 3d (the processing method of this image is described in detail in the Supplementary Materials). Examination of more than ten indent corners indicates an average decrease in the maximum width of the main corner cracks by as much as ~30%. This possibly provides a conservative estimate of the decrease in crack width as the vacuum in the SEM chamber generally leads to the opening of cracks in teeth [31].

3.3. Nano-scale mechanical properties and self-recovery

The load-displacement curves measured by nano-indentation, shown in Fig. 4a, suggest different mechanical properties of the tooth enamel at the nano-scale under dehydrated versus hydrated conditions. The nanohardness and reduced elastic modulus of enamel, as identified by the peak load at given displacement and the slope of curve upon unloading [30], are decreased, respectively, by ~17% and ~4% through hydration (Fig. 4b and Table S3 in the Supplementary Materials). Such a loss in nano-scale properties is unlikely to lead to a critical degradation in the performance of the tooth enamel, akin to the case at the micro-scale described above; both represent a compromise that is acceptable, especially when compared to the motivation of the recovery behavior.

AFM imaging reveals that the hydration-induced self-recovery capability of tooth enamel is markedly enhanced as the dimension of damage, here represented by the indent depth, is reduced from the micrometer to the nanometer scale (Fig. 4c–e). This is evident by an improved proportion of total inelastic deformation that can be recovered by hydration coupled with a shorter time to complete the recovery process. The depth of indent is typically decreased by ~30% in one hour after rehydration; the recovery process essentially saturates at this point with subsequent hydration resulting in little further change (Fig. 4d). As such, the plasticity that cannot be recovered by hydration accounts for about half of the total deformation exerted by nano-indentation, as illustrated in Fig. 4a. The inherent recovery speed is most probably even higher considering the slow water absorption of tooth enamel as restricted by its minimal content of organics. In contrast, the dimension of indent is only diminished by ~10% under dehydrated condition despite a longer recovery time of about two hours (Fig. 4d), demonstrating the critical role of water in dictating the recovery kinetics. A lateral view of the indents reveals an increase in the surface roughness of their inner side faces after hydration-induced recovery (Fig. 4e). This implies that the strain response of tooth enamel during the recovery process is not homogeneous at the nano-scale.

The viscoelastic nature of giant panda’s tooth enamel is corroborated by its creep behavior which represents a time-dependent enlargement of indentation displacement at constant load. Here

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**Fig. 1.** Micro-/nano-scale structure and interfaces of giant panda’s tooth enamel. (a and b) Field-emission SEM micrographs of etched samples showing the columnar architecture of the tooth enamel of the giant panda and the presence of organics between prisms and within them. (c–e) TEM images showing the abundant inter- and intra-prismatic interfaces that are rich in organic phases. The inset in (d) is a magnified view of the dashed square box showing the organics between prisms. (f) High-resolution TEM micrograph and corresponding fast Fourier transform patterns showing the interface and phase structure of the mineral nanofibers.
Fig. 2. Micro-scale self-recovery of giant panda's tooth enamel. (a) Variations of the cross-sectional profiles (along line I) of one indent created by micro-indentation as a function of the hydration time in a hydrated tooth enamel which was kept hydrated after indentation. The indent depth normalized by the original one at different hydration time is indicated. (b–e) Optical- and laser-mode LSCM images showing the morphological changes of the indent (b and c) before and (d and e) after being subject to hydration-induced self-recovery for 1425 min. The arrows indicate the cracks and bumps created by micro-indentation.

Fig. 3. Micro-scale recovery kinetics, mechanical properties, and decrease of crack width. (a) Time-dependent changes of the normalized depth of indents created by micro-indentation in giant panda's tooth enamel under dehydrated and hydrated conditions. The hydration state was kept constant after indentation. The inset shows representative LSCM images of the diagonal profiles of one indent (along line II in Fig. 2e) created in hydrated enamel before and after one-day's hydration. (b) Fracture toughness and (c) microhardness of enamel under dehydrated and hydrated conditions measured by micro-indentation. Asterisks indicate statistically significant difference. (d) Comparison of SEM micrographs of the indented region in hydrated enamel before and after one-day's hydration showing the lessening of cracking extent by self-recovery (Details on the image editing procedure are given in the Supplementary Materials).
the creep behavior of enamel was accessed using the depthsensing nano-indentation technique [32–35]. The application of bulk methods, e.g., uniaxial compression, is strictly limited by the small size of enamel and the large length-scale they measure that far exceeds the characteristic micro- to nano-scale dimensions for enamel recovery. As shown in Fig. 4f, viscoelasticity is more significant in hydrated enamel as illustrated by a marked increase of the creep strain rate. The role of water can be additionally appreciated from the differing sensitivity of the nano-scale mechanical properties of tooth enamel to the strain rate under dehydrated and hydrated conditions. The strain-rate sensitivity can be evaluated quantitatively by the relationship:

\[
\log r = a + m \log \dot{\varepsilon}
\]

where \( r \) is the nominal stress representing the indentation load divided by the projected area of the indent and \( \dot{\varepsilon} \) is the strain rate produced by the creep behavior [33,36]. The strain-rate sensitivity of enamel, as denoted by the parameter \( m \), demonstrates a threefold improvement through hydration as compared to the dehydrated state (Fig. 4g). This suggests a more remarkable hardening/stiffening effect of the hydrated tooth enamel when subjected to high strain rates – an attribute that is highly beneficial for resistance to impact and wear during service.

3.4. Inelastic/recovery deformation at the nano-scale

TEM imaging of the subsurface regions under the indents created by nano-indentation in dehydrated samples reveals a set of inelastic deformation mechanisms in the giant panda’s tooth enamel at the nano-scale. The long HAP nanofibers become bent and reoriented towards primarily the outward periphery away from the indent tip (Fig. 5a). Examination of the region within 1 μm of the indents reveals considerable fragmentation of the HAP mineral crystallites (Fig. 5b). The fragments are rearranged and reoriented with the long axes of newly-formed low-aspect-ratio columns deviating from the vertical direction (examples are indicated by the dashed lines). In addition, the inner surface of the crater shows nano-scale asperities which demonstrate a width comparable to the diameter of the HAP nanofibers, as delineated by the arrows in Fig. 5b. This provides a prima facie evidence of the inherent nano-scale flexibility of the HAP nanofibers in the tooth enamel. There is no sign of
any occurrence of deformation twinning in the enamel, implying a differing mechanism of plasticity distinct from the mechanical behavior of the aragonite and calcite minerals in mollusk shells [5,37,38]. High-resolution TEM imaging reveals the presence of dislocations in the enamel minerals after deformation which was not distinguished in the case of undeformed samples (Fig. S2 in Supplementary Materials). However, it remains unclear whether such dislocations originate from the formation process of enamel, are caused by deformation, or even whether they play a role in the deformation.

The nano-scale mechanisms that are responsible for the recovery deformation of tooth enamel can be visualized by comparing the TEM micrographs corresponding to the same areas of indented samples before and after being subject to one day of hydration.1 As shown in Fig. 6a–c, the HAP nanofibers and their fragmented columns of lower aspect ratio reorient primarily with their long axes rotating back towards the vertical direction (examples are indicated by the dashed lines). The surface asperities become less evident with their height diminished through hydration owing to the relaxation of underlying structure (Fig. 6b). The size of small holes formed by FIB milling is also decreased (Fig. 6c). The similarity between the SAED patterns taken from the areas close to and ~2 μm away from the crater in recovered samples suggest a significant extent of recovery in the alignment of HAP crystallites under the indents (Fig. 6d).

4. Discussion

The fact that enamel must last a lifetime and provide distinct functions under mechanically stringent conditions during use necessitates an exceptional durability to resist failure. However, it is clear that such durability cannot be engendered simply by a tolerance to cracks of hundreds of micrometers or more, as implied by its minimal crack-initiation fracture toughness comparable to that of HAP minerals [15,22–25]. Such apparent brittleness of tooth enamel though is likely the result of the fact that the methods used to access the toughness of enamel invariably introduce levels of damage that are unreasonably large compared to physiological conditions. It is mainly the extrinsic toughening mechanisms, as represented by the crack deflection/twisting along the interfaces and uncracked-ligament bridging, that operate at such length-scales to resist the propagation of these cracks [15,22–25,39–41]. Damage tolerance is additionally generated in the tooth through the attachment of a compliant dentin foundation to the enamel.

1 Random bright spots and domains can be seen in the recovered samples. However, their appearance is presumed to be an artefact caused by the electrical charging in the TEM as a result of the damage to carbon coatings caused by the recovery deformation.
using the graded interface of dentin-enamel junction [31,42,43].

Nevertheless, as damage in the tooth enamel certainly occurs, most commonly from abrasion, erosion and impact with tiny grits during mastication [44,45], the self-recovery process that acts in vivo under mild physiological environments over short timescales in the tens of minutes plays a vital role in inhibiting it from growing more extensive. In this scenario, the hydration-induced self-recovery represents a source of durability that differs markedly from the conventional protocol of fracture mechanics which primarily deals with large-scale cracks.

The unique architecture of tooth enamel, in particular the embedding of nano-scale mineral fibers and micro-scale prisms within an organic-rich matrix, enables a series of inelastic deformation mechanisms, including the plasticity and cracking of HAP crystallites, the rearrangement and reorientation of the fragmented pieces, and the sliding between the nanofibers (intra-prismatic) and between the prisms (inter-prismatic) along their organic-rich interfaces [12–16,32–35,46–50], as illustrated in Fig. 7. More specifically, the mechanisms that are closely associated with the viscoelasticity of the organic matrix are largely reversible under hydrated conditions, bestowing the enamel with remarkable self-recovery capability at nano- to micro-lengthscales. Such recovery behavior is consistent with the constant decrease of the contact depth after removal of load (back-creep response) in the tooth enamel recorded by nano-indentation [32,33,51,52]. The nano-scale responses of the HAP crystallites of enamel to load have been identified as plucking, plastic deformation and fragmentation, among which the former two are essentially associated with the organic matter [49,50]. The rotation of minerals, as a mechanism of plastic deformation, has also been observed in other highly-mineralized biological materials, such as nacre and stomatopod dactyl club [3,5,53].

Although present in a minimal content of less than 1 wt%, the organic phase plays an essential role in the self-recovery of the tooth enamel as the deformation of enamel occurs primarily in the form of sliding of the HAP nanofibers and prisms along their organic-rich interfaces that is viscoelastic and time-dependent [12–16,32–35,46–50]. The organics comprise principally proteins and lipids integrated with minor concentrations of carbohydrates and ions, with the amino acid compositions of the proteins identified by biochemical analysis [54–57]. The presence of abundant hydrophilic residues makes the organics water absorbent and responsive so as to react to hydrated conditions. A series of reversible mechanisms, such as the unfolding and refolding of hidden length, the breakage and reformation of non-covalent sacrificial cross-links, and the water-induced swelling and glass transition, make the organics of many biological materials recoverable [7,58–64], which enables the recovery of tooth enamel. Meanwhile, the flexibility and plasticity of organics can be significantly improved under hydrated conditions [7,32,64–66], leading to the easy sliding and rearrangement of the mineral constituents through the viscous flow of matrix. Specifically, the role of hydration-induced swelling is not significant in the recovery process as recovery still occurs in the originally hydrated enamel after indentation without swelling induced by water-absorption (Fig. S3 in Supplementary Materials).

Although tooth enamel has far lower organic content than other recoverable bio-materials, such as wood, bird feathers, pangolin scales and sheep horn [58,62,63,67], the role of the organics can be markedly amplified in enamel by its micro-/nano-scale architecture. Specifically, the organics are highly concentrated at the inter- and intra-prismatic interfaces of the mineral constituents. Their nano- to micro-scale dimensions result in a considerable area density of interfaces, ~10^2–10^3 mm^-2 for the inter-prismatic interfaces and ~10^2–10^5 mm^-2 for the intra-prismatic interfaces, which enhances their contribution to the global properties of bulk enamel. Moreover, these interfaces are arranged along the direction normal to the occlusal profile of tooth as a result of the vertical alignment of enamel constituents – an orientation that helps optimize the vertical stiffness [68,69]; this is precisely the most realiz-
tic loading direction of the enamel in vivo. Such preferred alignment helps ease the slipping deformation along the interfaces and maximize the generated shear strain in their direction, thereby strengthening the viscoelastic component of the enamel [70].

It is established that the structural hierarchy of tooth enamel has a significant influence on its elastic/inelastic deformation and fracture characteristics [14–16,71–73]. Here the damage recovery behavior of enamel is revealed to rely on the indentation size in a manner that recovery is markedly enhanced as the indentation size decreases from the micro- to nano-scale. Micro-indentation creates severe deformation and damage in a relatively wide range of tens of micrometers that a number of prisms are involved. The recovery process necessitates the coordinated motion of large deformation units (e.g., fractured prisms) over a broad region. In contrast, the damage results largely from the deformation and fragmentation of HAP nanofibers as well as the rearrangement of fragments within the prism for nano-indentation where only one or two prisms are involved. The size of deformation units for recovery (e.g., fragments of HAP nanofibers) is much smaller and their coordinated motion is much easier compared to the case of micro-indentation. Besides, micro-cracking and plasticity account for a reduced proportion of total deformation as the indentation size decreases [14,52]. Therefore, the damage of enamel can be recovered to a larger extent in a shorter time as the indentation size decreases to nano-scale.

In addition to the inherent structure, hydration plays a leading role in promoting the self-recovery of tooth enamel by enlarging the region that can be recovered and accelerating the recovery kinetics. Such effect results primarily from the improved viscoelasticity of the abundant organic-rich interfaces. Hydrated conditions are readily available in vivo in the tooth because of moisture from the tissue fluid and contact with saliva. A wide range of biological materials can be toughened with the presence of water by promoting the intrinsic plasticity of the organic phases and enhancing the extrinsic toughening mechanisms, such as crack deflection, associated with the soft matrix [4,7,64,67]. Despite the apparent toughening effect in the tooth enamel, the low crack-initiation resistance makes the partial recovery of geometry and structure fairly important to ensure the durability of enamel. The control of nano-to micro-scale damage by recovery plays an effective role in mitigating the incipient growth of small cracks at these scales and, as such, inhibits the formation of larger-scale cracks. The concomitant decrease in hardness and stiffness of the enamel caused by hydration is much less significant compared to the enhanced self-recovery. Such an attribute is highly desirable in synthetic ceramic materials because of their fatal sensitivity to flaws and damage.

5. Conclusions

In conclusion, we find that the tooth enamel, specifically that of the giant panda, despite having a limited resistance to the initiation of large-scale cracks, has developed an exceptional durability by autonomously diminishing the dimension of any indent damage, created by indentation, through partially recovering its geometry and structure at nano-to micro-scale length-scales. Such an effect of self-recovery relies principally on the presence of water and becomes more evident as the length-scale of damage decreases. The unique architecture of enamel, involving specifically an arrangement of nano-scale mineral fibers and micro-scale prisms set within an organic-rich matrix in a highly oriented fashion, serves to aid the recovery process by limiting any critical degradation in hardness and stiffness under hydrated conditions. Our study visualizes and discerns a series of mechanisms acting at the nano-scale that lead to the inelastic and recovery deformation of the tooth enamel. In particular, the mechanisms that operate primarily based on the viscoelasticity of the organic matrix are largely reversible under hydrated conditions, thus allowing for the partial recovery behavior of tooth enamel. Our findings shed new light on the principles underlying Nature’s design of durable bioceramics, specifically tooth enamel, which rely principally on the nano-to micro-scale dimensions of their structural characteristics,

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2 Note that this unfavorable effect of reduced stiffness and hardness under hydrated conditions is further diminished, or even eliminated, at increasing loading rates, e.g., under the impact of realistic bites and mastication. This is a result of the improved strain-rate sensitivity of tooth enamel when it is hydrated.
the vertical alignment of highly anisotropic mineral constituents, the incorporation of minimal organic components and their distribution at the interfaces, and the self-recovery mechanisms associated with the organics induced by hydration. By implementing such principles into man-made systems, this study may offer inspiration for the synthetic fabrication of durable ceramic materials with outstanding mechanical properties.

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Competing interests statement
The authors declare no conflict of interest.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.actbio.2018.09.053.

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