While many organisms synthesize robust skeletal composites consisting of spatially discrete organic and mineral (ceramic) phases, the intrinsic mechanical properties of the mineral phases are poorly understood. Using the shell of the marine bivalve *Atrina rigida* as a model system, and through a combination of multiscale structural and mechanical characterization in conjunction with theoretical and computational modeling, we uncover the underlying mechanical roles of a ubiquitous structural motif in biogenic calcite, their nanoscopic intracrystalline defects. These nanoscopic defects not only suppress the soft yielding of pure calcite through the classical precipitation strengthening mechanism, but also enhance energy dissipation through controlled nano- and micro-fracture, where the defects’ size, geometry, orientation, and distribution facilitate and guide crack initialization and propagation. These nano- and micro-scale cracks are further confined by larger scale intercrystalline organic interfaces, enabling further improved damage tolerance.
Skeleton materials in biological systems are typically composites that consist of mineral and organic phases. The biomineralized building blocks are often hierarchically organized across multiple length scales in these composites, which is responsible for their remarkable mechanical performance. In contrast to pure geological or synthetic minerals, those formed through biologically controlled processes usually exhibit nanoscopic structural heterogeneities through the incorporation of intracrystalline macromolecules within their crystaline mineral matrices. These intracrystalline proteins often include a high percentage of acidic amino acids, such as aspartic acid, which can be secondarily modified by sugar residues. Previous studies have shown that the proteins extracted from biogenic minerals can induce changes in crystal morphology during the in vitro formation process of minerals. In particular, it has been suggested that the highly acidic groups preferentially bind to calcium ions or the (0001) planes in calcite, where stereochemical effects may also contribute to this process. Moreover, the incorporation of intracrystalline organic inclusions is proposed to be the main structural origin for the lattice distortions in biogenic crystals, which is widely observed in many biogenic calcium carbonate systems.

Although the importance of the hierarchical designs in biological composites is well recognized in the literature, the intrinsic mechanical properties of individual mineral building blocks are yet to be fully elucidated. The mechanical strengthening effects of the intracrystalline defects have been suggested through the mechanical characterization of biomimetic synthetic minerals with incorporated amino acids, micelles, or other entities. However, the relationship between the morphology of these intracrystalline defects (e.g., size, spacing, geometry, orientation, and distribution) and the deformation behavior of biominerals, especially in the inelastic regime, is yet to be established. For example, the “conchoidal” fracture behavior of biogenic calcite, one of the most abundant biominerals, has long been recognized despite its unclear mechanisms, and represents one of the most intriguing puzzles in the field of biomineralization. Beyond its biological implications, understanding the intrinsic mechanical properties of biominerals is also technically important for the design and synthesis of biomimetic structural materials with improved mechanical performance.

Direct measurement of the intrinsic mechanical properties of biogenic minerals in these skeletal composites, however, is challenging due to their complex hierarchical organization, which often prevents effective isolation of individual units and precise control of the loading and boundary conditions during mechanical characterizations. Here we approach this problem by studying the intrinsic mechanical behavior of biogenic calcite from the bivalve Atrina rigida through quantitative multiscale mechanical characterization, systematic structural analysis before and after deformation, and theoretical and computational modeling. The results reported here elucidate the fundamental mechanical roles of the nanoscopic intracrystalline defects in these calcitic biominerals, including strengthening by suppressing dislocation slips and toughening by promoting fracture along non-cleavage lattice planes. These small-scale toughening mechanisms are further amplified by structural hierarchies at larger length scales for additional damage localization and energy dissipation.

**Results**

**Hierarchical structure of the biogenic calcite in Atrina rigida.**

The pen shell, *A. rigida*, is a bivalve mollusk native to Caribbean Sea, and has shells consisting of two distinct mineral phases, an inner aragonitic nacreous layer and an outer calcitic prismatic layer (Fig. 1a). The inner nacreous layer has the well-known “brick-and-mortar” structure, while the outer prismatic layer consists of prism-like calcite crystals with polygonal cross sections (Fig. 1b). These prismatic calcite crystals typically measure 20–50 μm in diameter and several hundreds of micrometers in length along the shell normal (N direction, Fig. 1b, c). Selected area electron diffraction (SAED) patterns acquired from these calcitic prisms reveal their single crystal nature (Fig. 1d, e), with their c-axes generally aligned with their longitudinal axis. At

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**Fig. 1 Biogenic calcite in the prismatic layer of the mollusk Atrina rigida.**

- a Scanning electron micrograph (SEM) of the shell cross-section showing the prismatic layer (upper region) and the nacreous layer (lower region) in the shell. The normal direction of the shell is indicated by the arrow ("N").
- b Backscattered SEM (BSEM) image of the prismatic layer sectioned and polished in the direction perpendicular to "N", which shows the polygonal cross sections of the prismatic crystals.
- c SEM image of one isolated prismatic crystal.
- d e Selected area electron diffraction (SAED) patterns taken from two sections prepared from two orientations, parallel to the normal direction (zone axis (ZA): [1100]) and perpendicular to the normal direction (ZA: [0001]).
- f Energy dispersive spectroscopy (EDS) elemental mapping of the calcitic prisms and their intercrystalline organic interfaces. From top to the bottom, BSEM image revealing the local differences in electron density, and their corresponding Calcium, Carbon, and Oxygen maps.
the boundaries between adjacent calcite prisms are thin intracrystalline organic layers (thickness: ca. 500 nm) (Fig. 1f)[6,33], which consist of glycine-rich proteins and are believed to provide a scaffold for the templated growth of the calcitic prisms[6].

In addition to the intracrystalline organic interfaces, defect-like nanoscopic inclusions, which were previously demonstrated to contain organic materials[10], are present within the single-crystal calcite prisms. These defects can be visualized with bright-field transmission electron microscopy (TEM) imaging due to their comparatively low electron densities (Fig. 2a–f and Supplementary Fig. 1). While previous studies estimated the presence of ca. 0.4 wt% of intracrystalline organic material within these calcite prisms[15], the distribution of these defects is not homogeneous. Instead, the defects exhibit regions with high and low densities that are clearly visible in low-magnification TEM images (Fig. 2b–d). When viewed in longitudinal cross section, the high- and low-density regions exhibit an alternating zone-like morphology, with a characteristic spacing of several hundreds of nanometers (Fig. 2b, c). Etching experiments performed on the longitudinally polished sections also reveal this pseudo-layered structure within the calcitic prisms (Supplementary Fig. 2). This modulation in the distribution of intracrystalline defects was proposed to be related to their sequential stepwise deposition during the process of shell growth[16]. For comparison, regions with high- and low-densities of intracrystalline defects are randomly distributed in the transverse cross section (Fig. 2d). Moreover, individual defects are elongated along the horizontal direction when viewed in the longitudinal cross section (Fig. 2e), but circular in the transverse cross-sections (Fig. 2f), consistent with previous studies[10]. Quantitative geometrical measurements show that these defects can be represented as equal-axed ellipsoids with a height of ca. 5 nm and a lateral span of ca. 10 nm (Fig. 2g, h and Supplementary Note 1 and Supplementary Fig. 3).

**Uniaxial micro-pillar compression tests.** To probe the intrinsic mechanical properties of the biogenic calcite in *A. rigida*, we performed microscopic uniaxial compression tests on micro-pillars fabricated via focused ion beam (FIB) milling from individual prismatic crystals (Figs. 3a and 4a). Micro-pillars of both biogenic and geological (single crystal Icelandic spar) calcite were prepared with their c-axes in the vertical direction, with typical dimensions of *D* (top diameter) ca. 2 μm, η (aspect ratio) ca. 3, and α (taper angle) ca. 2.5° (Fig. 3b). The as-measured force, *F*, and compression depth, δ, were converted to normalized engineering stress, *σ* and strain, ε, by applying Sneddon’s formula, which accounts for the substrate compliance for a slightly tapered pillar[14]. Typical *σ*-ε data obtained under displacement control conditions (displacement rate: 10 nm/s) for both the biogenic and geological calcite are shown in Fig. 3c (see full data in Supplementary Fig. 5). Both materials exhibited a linear elastic regime before yielding, and the Young’s moduli, *E*, were measured to be 34.4 ± 5.3 (average ± standard deviation, *n* = 25) and 47.9 ± 5.5 GPa (n = 22) for the biogenic and geological calcite, respectively (Fig. 3e). The *A. rigida* biogenic calcite exhibited a slightly higher yield strength, *σ* y, in comparison to its geological counterpart (1.16 ± 0.22 vs. 0.96 ± 0.37 GPa, Fig. 3e), which is consistent with the increase of hardness measured from instrumented nanoindentation (Supplementary Fig. 6). Moreover, the Weibull analysis suggests that the *A. rigida* biogenic calcite has a smaller variation for σ y as indicated by its higher Weibull modulus (*A. rigida* biogenic calcite: 5.74 ± 0.30; geological calcite: 2.24 ± 0.06, Fig. 3d). The *A. rigida* biogenic calcite also yielded at a higher strain level as indicated by its greater elastic limit, ε el, (*A. rigida* biogenic calcite: 0.0379 ± 0.0109; geological calcite: 0.0229 ± 0.0077, Fig. 3e).

The micro-compression tests also revealed dramatically different inelastic deformation behavior between the *A. rigida* biogenic and geological calcite. The *A. rigida* biogenic calcite continued to carry load after yielding as indicated by the gradual increase of σ ε with ε, leading to a failure strain (ε f) of 0.0740 ± 0.0341 until an abrupt fracture (Fig. 3c). This behavior also resulted in an ultimate fracture strength (σ f) of 1.32 ± 0.23 GPa for the *A. rigida* biogenic calcite (Fig. 3e). Inspection of compression test results performed on biogenic calcite, which were stopped before final failure, revealed the presence of micro-cracks running vertically, which were often later deflected horizontally (Fig. 4b, d). Surprisingly, the damaged biogenic
**Fig. 3** Quantitative mechanical properties of biogenic and geological calcite via uniaxial micro-pillar compression tests. a, b Schematic illustrations of the uniaxial compression of micro-pillars with a flat-end diamond tip (bottom diameter, 10 μm). The parameters of the micro-pillars include D1 (top diameter), D2 (bottom diameter), and h (height of the micro-pillars). c Representative normalized engineering stress-strain (σ–ε) curves for uniaxial compression of the biogenic and geological calcite micro-pillars. The calculated mechanical parameters include E (Young’s modulus), σY (yield strength), σm (maximum strength), ε0 (elastic limit), εf (failure strain), and σf (flow strength for geological calcite). d Weibull analysis of σY, where f is the failure percentage. e Measured distribution of E, σY, σm, ε0, and εf as functions of the pillar average diameter, Davg. The histograms on the right show the average and standard deviation of each data set. Asterisks represent statistical significance at a level of 0.05 via two sample t-tests.

calcite could still sustain load and eventually fractured into microscopic pieces (Fig. 4c). Geological calcite, on the other hand, exhibited a large displacement jump accompanied by an appreciable load drop at the yield point (Fig. 3c). After the compressive loading was re-established, it yielded at smaller stresses followed by a steady plastic flow (σp 0.47 ± 0.12 GPa, n = 14). Geological calcite micro-pillars exhibited first permanent deformation in their upper regions during compression tests, as evidenced in the samples where loading was immediately stopped after yielding (Fig. 4d), while those after significant inelastic deformation showed classical slip steps on the surface of the micro-pillars (Fig. 4g, h). Such behavior has been observed in slip events or dislocation avalanches of some metals during displacement-controlled compression, which leads to a sudden increase in the displacement (Δε) at the yield point (Fig. 3c), followed by an “unloading” segment, during which the
Fig. 5 Microscale deformation mechanisms in biogenic and geological calcite. a TEM image of a thin slice taken from a significantly deformed geological calcite micro-pillar after compression testing. Inset, SEM image of the micro-pillar used for TEM sample preparation and the yellow plane shows the orientation of the TEM slice. b High magnification TEM image showing the dislocation arrays developed within the deformed geological calcite micro-pillar. c TEM image showing micro-/nano-scale cracks developed within the A. rigida biogenic calcite after deformation. White and yellow arrows indicate the cracks in the low- and high-density defect regions, respectively. d TEM image taken at the boundary of a fractured zone in biogenic calcite, showing the propagation directions of several cracks (white arrows) in parallel to the long axes of the defects, which is perpendicular to the calcite’s c-axis. e TEM image demonstrating that a crack formed via the coalescence of micro-cracks initiated from defects and followed the long-axis directions of defects. Inset: TEM imaging showing a site of crack initiation through nanoscopic damage coalescence from adjacent defects. f SEM and g atomic force micrograph (AFM) of the fractured surface of biogenic calcite, showing the nanoscale rough surfaces.

Microscale deformation mechanisms. Thin sections prepared from micro-pillars after compression tests were used for TEM imaging to further understand the underlying deformation mechanisms (Fig. 5). For micro-pillars of geological calcite after significant plastic deformation, a large number of dislocations were generated, where individual dislocation lines are highly curved without well-defined geometries, a characteristic of dislocations in calcite (Fig. 5a, b)\(^36\). It has long been known that geological calcite deforms plastically via deformation twinning and slip, particularly at elevated temperatures and confined hydrostatic pressures\(^37\)–\(^39\). The primary plastic deformation mechanisms in geological calcite include twinning on \(e \{1018\} \langle 4041 \rangle^+\) (three systems), slip on \(r \{1014\} \langle 2021 \rangle^\pm\) (three systems), and slip on \(f \{1012\} \langle 2201 \rangle^\pm\) (six systems)\(^36\)–\(^39\). In the present study, uniaxial compression of the calcite micro-pillars along the \(c\) axis led to the highest Schmid factors \((m)\) in the \(r\) systems \((0.49996)\), resulting in the activation of this slip system in the negative sense (Supplementary Note 3), which was also confirmed through the direct comparison of the slip morphology between experimental observations and structural modeling (Supplementary Fig. 8). Gliding on two and sometimes on all three equivalent \(r\) systems led to retention of the circular cross section after compression (Supplementary Figs. 9 and 10 and Supplementary Note 4). In our testing conditions, we did not observe significant slips in \(e\) or \(f\) systems, or \(e\)-twining. TEM analysis of micro-pillars immediately after yielding revealed that dislocations were concentrated in the top portion of micro-pillars, consistent with SEM observations (Fig. 4f and Supplementary Fig. 11).

In stark contrast to geological calcite, TEM imaging of the A. rigida biogenic calcite after deformation revealed that microcracking, instead of dislocation slipping, was the dominating deformation mechanism (Fig. 5c–e). More importantly, these cracks are more tortuous in the regions with higher densities of intracrystalline defects (yellow arrows in Fig. 5c). This behavior was also evident from TEM imaging of a thin section before and after crack formation, revealing that cracks were deflected at the nanoscale by defects, particularly in high-density regions (Supplementary Fig. 12). Additionally, these microcracks tended to propagate along the prisms’ transverse orientation, which is parallel to the intracrystalline defects’ long-axis directions (white arrows in Fig. 5d). This fracture behavior leads to the formation of layered pieces in some fracture surfaces (Supplementary...
The thickness of these layered pieces is approximately several hundred nanometers, matching well with the characteristic length scale in the density modulation of intracrystalline defects along the c-axis.

In addition to regulating crack propagation, the intracrystalline defects also affect the initial formation of micro-cracks. Figure 5e shows a forming crack as it travels through a series of adjacent intracrystalline defects along their long axes over a distance of several hundred nanometers. The inset shows the formation of a crack embryo in a region with high defect density, which just began to bridge adjacent defects (white arrow). Similar crack initiation processes were also observed in our indentation samples (Supplementary Fig. 14).

The cracking pattern facilitated by the intracrystalline defects produced rough fracture surfaces with nanoscopic bumps of typical height and lateral spans on the order of several hundred nanometers (Fig. 5f, g), which is on the same length scale as that of the heterogeneous distribution of intracrystalline defects. Results obtained from in situ SEM mechanical experiments with these fracture surfaces with similar morphologies, thus eliminating the possibility that the observed features were artifacts from the employed sample preparation protocols (Supplementary Fig. 15). Regions with a low-density distribution of defects exhibited a similar fracture pattern to those seen in geological calcite, i.e., cleavage along {1014} planes (white arrows in Fig. 5c and Supplementary Fig. 12).

The increase in strength directly measured from the micro-pillar compression tests is consistent with our results and previous studies that showed higher hardness in various biogenic calcites compared to their geological counterparts. Polishchuk et al. recently showed that the biogenic calcite in the brittle star *Ophiothrix cambria* is strengthened by Mg-rich nanodomains through a compressive stress field similar to Guinier-Preston’s zones in metals. The Mg concentration in the calcite prisms of *A. rigida* (0.36 ± 0.3 at% based on energy dispersive X-ray spectroscopy, consistent with ref. 44), however, is much lower than that from brittle stars (3.03 ± 0.04 at%) and other echinoderms44–46. Previous studies have also shown that the incorporation of Mg is likely to contribute only ca. 20% of the hardness increase in the biogenic calcite of *A. rigida*, suggesting that Mg is unlikely to be the major contributing factor for the increased strength. Moreover, the post-deformation TEM imaging clearly revealed that the dislocation slips observed in geological calcite were effectively suppressed in the *A. rigida* biogenic calcite. These results suggest that the nanoscopic intracrystalline defects strengthen the *A. rigida* biogenic calcite through a strategy similar to the classic precipitation strengthening mechanism, which is governed by the wing crack formation and crack coalescence from intracrystalline defects, which strongly depends on their spacing (L) and orientation (θ), and can be evaluated via

\[
\sigma_y = \left( \frac{2\pi}{11} \right)^{1/4} L^{1/2} \left( \frac{\sigma_{Yc} \sqrt{\pi \alpha}}{20\beta(\theta) a} \right)
\]

where \(\beta(\theta)\) is a function of θ and the frictional coefficient between the defects and the matrix, a is the half length of the defects (ca. 5.5 nm), and \(\sigma_{Yc}\) represents the compressive strength of geological calcite (0.96 GPa based on micro-pillar compression results) (Fig. 6c and Supplementary note 7). As shown in Fig. 6f, as the defect spacing L reduces from the nominal value of ca. 32 nm to 10 nm in the high-density regions, the strength θ reduces by ca. 0.4 GPa; a similar reduction can be achieved when θ increases to 30–50°. Finite element/discrete element-based fracture simulations further confirm that: (1) local damage tends to initiate at regions with a high density of defects through crack coalescence along the long-axis directions of defects (Fig. 6g, and Fig. 7e mechanism ii), which is consistent with TEM observations (Fig. 5e), and (2) regions with higher inclination angles have lower strength by channeling cracks along the alignments of defects (Fig. 7e mechanism iii, Supplementary note 8, and Supplementary Fig. 18). With control of their size, geometry, orientation, spacing, and distribution, these intracrystalline
defects effectively control the initiation and propagation of micro-cracks beyond the intrinsic preference of cleavage-plane fracture of pure calcite. It should be noted that this mechanism differs from the prestressing strategy by the coherent Mg-rich nanodomains reported in the calcitic skeleton of brittle stars, which generate local compressive stress fields in the mineral matrix to impede cracking. Moreover, the controlled fracture process via judicial placement of intracrystalline defects within the continuous A. rigid biogenic calcite reported here is also distinct from the toughening strategy by the nanogranular features, which are often observed in aragonite-based biominal building blocks. Previous experimental investigations revealed that this nanograin morphology in aragonitic nacre tablets enhances toughness by facilitating torturous crack propagation through intergranular organic matrix, grain rotation and deformation.

The capacity of energy dissipation and damage tolerance in the biogenic calcitic composite of A. rigid shells is further enhanced through the combined effect of different levels of its structural hierarchy. For example, while high load indentation (maximum load = 500 mN) induced extensive fracture in the prisms, the damage was well constrained within a localized region due to crack impediment and deflection at the intercrystalline organic interfaces (Fig. 7a, b; see Supplementary Fig. 19 for indentation curves), whereas similar loading on geological calcite induced significant radial cracking (Fig. 7a, inset). Cross-sectional TEM imaging of an indentation site close to an organic interface further confirms that the damage was effectively constrained by the organic interfaces even beneath the surface, and adjacent prisms were undamaged (Fig. 7c). Moreover, the characteristic nanoscale saw-tooth-like structure at the mineral-organic interface, may lead to improved adhesion between the prisms and organic interfaces (Fig. 7d). Similar jagged interfaces with characteristic sizes of several micrometers were previously observed after etching, which was considered as the evidence of cyclic growth of calcitic prisms. Together with the gradual upward change in defect orientation close to the prism boundaries, the A. rigid biogenic calcite is able to increase the density of microcracking and facilitate crack propagation upwards, thus avoiding deep fracture into the shell interior (Fig. 7e mechanism iii). With effective large-scale damage localizing effects provided by the intercrystalline interfaces, the individual calcite prisms often undergo extensive fracture into nano-/microscopic (ca. 200 nm) pieces (Fig. 7f), which further enhances energy dissipation by generating more fracture surfaces as well as promoting rotation and friction between fractured pieces. Such simultaneous restriction in crack propagation via intercrystalline boundaries and extensive fracture within individual prisms can also be observed in macroscopic compression and indentation through synchrotron-based in situ measurements and post-deformation structural analysis (Supplementary Note 9 and Supplementary Figs. 20 and 21).
correlated to the biomineralization process in 3D. The structural characteristics of intracrystalline defects are highly correlated to the biomineralization process in 3D. A. rigida indicates the spatially controlled morphology and distribution of sic strengthening and toughening effects of this common structural organization of biological composites, the internal morphological evolution of which will enable a further in-depth understanding of the hierarchical organization in biological structural materials, which is important to obtain a clean surface and minimize damage. Imaging with standard bright-field, dark-field, and SAED techniques was carried out using a JEOL (Akishima, Japan) TEM operated at 120 kV.

**Discussion**

Nanoscopic intracrystalline defects are ubiquitous in a variety of biominerals from a wide range of taxa, and the work presented here for A. rigida highlights the simultaneous intrinsic strengthening and toughening effects of this common structural motif. Our experiment- and modeling-based analysis indicates the spatially controlled morphology and distribution of intracrystalline defects guide and control the local fracture behavior of the A. rigida biogenic calcite. This deformation mechanism may underlie the primary structural origin for the observed “conchoidal” fracture of biogenic calcites, where the structural characteristics of intracrystalline defects are highly correlated to the biominereralization process in 3D. The intrinsic mechanical properties of biogenic calcite revealed here can be combined with the extensive knowledge of the hierarchical organization in biological structural materials, which can provide new insights into the development of damage-tolerant bio-inspired composites through the rational engineering of the internal microstructure of their ceramic phases.

**Methods**

**Samples.** Live A. rigida shells were purchased from Gulf Specimen Marine Laboratories, Inc. FL, USA. The specimens were frozen upon receiving and maintained under wet conditions prior to the experiments. Samples of single crystal geological calcite (origin, Mexico) were obtained from Pisces Trading Company, LLC.

**Electron microscopy.** Samples were coated with ultra-thin carbon or Au to reduce charging effects prior to imaging with an FEI (Hillsboro, Oregon, USA)Helios Nanolab 600 Dual Beam scanning electron microscope (SEM) at an acceleration voltage of 5 kV and a working distance of ca. 4 mm. Energy dispersive spectroscopy (EDS) measurements were acquired from the same system equipped with an INCA Energy EDS system at an acceleration voltage of 20 kV. Low-magnification SEM images shown in Fig. 7 were acquired with Tescan (Brno, Czech Republic) Vega GMU SEM with an acceleration voltage of 20 kV. Cross-sectional transmission electron microscope (TEM) samples were prepared using ion beam milling. A detailed sample preparation procedure is as follows: (1) A platinum protective layer (ca. 0.5 μm) was first laid down on top of the desired region; (2) Another platinum protective layer (ca. 1.5 μm) was further deposited on top of the region where the TEM slab was to be milled out; (3) Two trenches, one on each side of the platinum protective stripe, were milled by FIB, leaving the specimen slab (thickness: ca. 1.5 μm); (4) The slab was then cut through by FIB and transferred to a copper TEM grid by an Omniprobe and welded securely with platinum deposition; (5) The lift-out lamellar specimen was sequentially thinned by FIB at 30, 16, 5, and 2 kV ion beam voltages. Final cleaning at 2 kV and 28 pA is important to obtain a clean surface and minimize damage. Imaging with standard bright-field, dark-field, and SAED techniques was carried out using a JEOL (Akishima, Japan) 2011 TEM operated at 120 kV.

**In situ fracture experiments** inside the SEM and fracture of TEM samples were conducted with a Helios Nanolab 600 Dual Beam scanning electron microscope equipped with an Omniprobe system. For in situ SEM imaging of fracture surface, a sample with fractured surfaces was first coated with ultra-thin carbon. After an
Atomic force microscopy: Tapping mode AFM (TM AFM) imaging in ambient conditions was carried out using a Veeco Multimode SPM IIIA (Santa Barbara, California, USA) equipped with an AS-130 "JV" scanner. TM AFM imaging was conducted with NANOSENSORS Si TMAFM cantilevers (PPP-NCHR-10). Typical scan speed was 1–5 μm/s; other parameters were optimized upon tuning.

Nanoindentation: The A. rigida shells were first thoroughly cleaned with DI water to remove algae and sand particles on the surface, and then the pristine surface was removed from the shell with a razor blade. The dried pieces of the pristine layers were then cut into small square-shaped plates (ca. 5 mm), and embedded in a room temperature cured epoxy (Spurr low viscosity embedding kit, Polysciences Inc.). Samples with two different orientations were prepared, i.e., parallel and perpendicular to the c-axis (longitudinal direction) of prisms. The embedded samples were then polished on a South Bay Technology (San Clemente, California, USA) Model 920 lapping machine with aluminum oxide pads stepwise (15 μm, 6 μm, 3 μm, and 1 μm), and finally with 30 nm silica colloidal solution on a microcloth. Load-controlled nanoindentation was performed on the polished sample surface by using Berkovich (trigonal pyramid, semi-angle = 65.3°) and conical (taper radius = ca. 1 μm, semi-angle = 30°) diamond probe tips on a Hysitron TriboIndenter (Minneapolis, Minnesota, USA). The piezoelastic transducer was first allowed to equilibrate for 105 s (the last 45 s with digital feedback) and another 40 s for calculation automatically prior to each indent. Typical load functions included loading (10 s), holding (20 s), and unloading (10 s). Maximum loads varied from 1 to 10 mN.

Data analysis for uniaxial micro-pillar compression: The as-measured force, F, and compression depth, δ, were converted to normalized engineering stress, εn, and strain, εn, by applying Sneddon’s formula, which accounts for the substrate compliance for a slightly tapered pillar. The yield strength, σy, and geometrical maximum stress, σmax, were estimated as

\[ \sigma_n = \frac{F}{\pi D^2} \]  
\[ \sigma_{\text{max}} = \frac{F_{\text{n}}}{D^2} \]

where \( F_{\text{n}} \) is the onset load of nonlinear portion, and \( F_{\text{max}} \) is the maximum load. A Weibull distribution model was used to analyze the materials’ strengths, \( \sigma_n \), by fitting the failure percentage, \( f \), as

\[ f = 1 - e^{-\left(\frac{\sigma}{\sigma_0}\right)^m} \]

where \( \sigma_0 \) and \( m \) (Weibull constant) are fitting parameters.

Molecular dynamics simulations: Molecular dynamics (MD) simulations were performed using the large-scale atomic/molecular massively parallel simulator (LAMMPS). The Ovito visualization tool was used for post-processing the results of MD simulations. The calcite pillars were shaped by cutting a cylinder with diameter \( d = 200 \text{ Å} \) out of a periodic cube with the initial size of 450 × 450 × 450 Å. The pillar’s axis was set along the c-axis of the calcite crystal. The total charge of the system was kept neutral in the pillar by cutting the cylinder in a way such that each cross section contained an equal number of calcium and carbonate groups. The pillar with no defects, simulating pure geological calcite, contained a total of 1,102,400 atoms. In the sample with ellipsoid defects (simplified as a void), representing biogenic calcite with nano-inclusions, atoms in an ellipsoid containing 218 CaCO₃ units were deleted inside the cylinder. Interatomic forces in calcite were described by a rigid ion version of the interatomic potential reported by Pavese et al., which has been shown to be capable of accurately predicting various mechanical properties of calcite. The simulation time was set as 1 fs, and thermal equilibrium was applied to the system using time integration on Nose-Hoover style non-Hamiltonian equations of motion in canonical (NVT) ensembles for F = 10 K for 100 ps. The pressure along the longitudinal direction showed that the system was equilibrated and the stress along the axis was zeroed after 100 ps. The pillar was then loaded in compression up to 30%
by continuously changing the box volume along the periodic direction with a constant strain rate of $3 \times 10^8$ s$^{-1}$. During loading the NVT with Nose-Hoover thermostat was applied to maintain the temperature constant.

**Data availability**

The main data supporting the findings of this study are available within the Article and Supplementary Information files. Additional data are available from the corresponding authors on reasonable request.

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Author contributions

L.L. designed and supervised the research. Z.D., T.Y., and L.L. conducted electron backscattered diffraction, nano- and microindentation, atomic force microscopy, and electron microscopy. Z.D., T.Y., and L.L. conducted X-ray in situ mechanical experiments. Z.D., T.Y., and L.L. conducted X-ray in situ mechanical experiments. L.L. designed and supervised the research. Z.D., T.Y., and L.L. conducted electron backscattered diffraction, nano- and microindentation, atomic force microscopy, and electron microscopy. Z.D., T.Y., P.D.S., and F.D.C. performed X-ray in situ mechanical testing. H.C. performed the finite element/discrete element fracture simulation. J.C.W. performed large-area SEM imaging. R.M. conducted atomistic modeling of micro-pillar compression. Z.D., Z.J., and L.L. analyzed the data and wrote the draft manuscript. All authors contributed to data interpretation and manuscript revisions.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to L.L.

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