Microstructure provides insights into evolutionary design and resilience of *Coscinodiscus* sp. frustule

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We conducted in situ three-point bending experiments on beams with roughly square cross-sections, which we fabricated from the frustule of *Coscinodiscus* sp. We observe failure by brittle fracture at an average stress of 1.1 GPa. Analysis of crack propagation and shell morphology reveals a differentiation in the function of the frustule layers with the basal layer pores, which deflect crack propagation. We calculated the relative density of the frustule to be ~30% and show that at this density the frustule has the highest strength-to-density ratio of 1,702 kN/m³/kg, a significant departure from all reported biologic materials. We also performed nanoindentation on both the single basal layer of the frustule as well as the girdle band and show that these components adopt similar mechanical properties that also agree well with bending tests. Transmission electron microscopy analysis reveals that the frustule is made almost entirely of amorphous silica with a nanocrystalline proximal layer. No flaws are observed within the frustule material down to 2 nm. Finite element simulations of the three-point bending experiments show that the basal layer carries most of the applied load whereas stresses within the cribrum and areolae layer are an order of magnitude lower. These results demonstrate the natural development of architecture in live organisms to simultaneously achieve light weight, strength, and exceptional structural integrity and may provide insight into evolutionary design.

Diatoms are single-cell algae that form a hard cell wall made of a silica/organic composite. The ability to produce a functional biosilica shell presents several natural precedents that fascinate and inspire scientists and engineers. One fascinating aspect of such silica glass shells is their intricate, varied, and detailed architecture. Diatoms are generally classified based on the symmetry of their shells: Centric diatoms display radial symmetry whereas pennate diatoms have bilateral symmetry. Fig. 1A shows a schematic of a typical centric diatom and reveals that the shells are composed of two halves, called frustules, that fit together in a Petri-dish configuration. The frustules are attached to each other around the perimeter of the shell by one or several girdle bands. The frustules are usually porous with pore size and density varying between species. The frustule shell can also be composed of multiple layers with a cellular structure within the shell. The proposed evolutionary functions for these intricate shell designs include nutrient acquisition, control of diatom sinking rate, control of turbulent flow around the cell, and protection from grazing and viral attack (1). Evidence in favor of a protective function is that the degree of shell stiffification depends on the environment, with greater amounts of silica found in shells grown in a predatory environment (2). As a deterrent to predation, the frustule makes use of an inherently brittle glass as a structurally protective material while balancing other evolutionary pressures. A denser shell may provide greater protection but will cause the diatom to sink beyond depths suitable for photosynthesis. A solid shell might also prevent exchange of resources and waste between the diatom cell and its environment. This requires adaption through control of the frustule micromorphology or modification of the constituent silica/organic composite material (3). The protective aspects of the frustule shell are clear; what remains an open question is how much the intricate pore structure and cellular design contribute to the amplified structural resilience vs. biological function.

The size of most diatom species ranges from 2 to 200 μm (4, 5), which renders most of the traditional mechanical testing methods inadequate to characterize such complex materials; a few mechanical studies on diatoms have been reported (6–11). The majority of studies perform atomic force microscopy (AFM) indentation (6–9) on a full frustule of centric or pennate diatoms. Reported values of hardness ranged from 0.06–12 GPa and values of elastic modulus from 0.35–22.4 GPa. Differences in local pore structure and the nonplanar geometry of the frustule were often cited for the variance in mechanical properties. Three-point bending tests on beams that were extracted from the diatom frustule reported failure strengths of 336 ± 73 MPa but were complicated by local penetration of the indenter tip and tilting of the frustule during testing (10, 11). This overview demonstrates a wide range in the reported hardness and elastic moduli for biosilica shells. Most of these experiments were performed on full diatom shells, which in some instances contained organic cellular material; it is unclear whether the measured mechanical data represent the deformation of the constituent biosilica or the overall deformation of the shell through bending, local twisting, pivoting, and so on. Indentation using AFM can introduce inaccuracies such as tip sliding, and the resulting uncertainty in compliance within a single set of experiments, as well as among the data obtained with different instruments, makes it challenging to compare mechanical properties of the diatoms across the reported experiments. Within a single species, these mechanical data may provide qualitative trends in the structural response of the diatom shells; it is difficult to make any conclusions on the mechanical properties of the constituent biosilica. The mechanisms of silica...
biogenesis likely varies among the species (12), but it is unclear to what extent these differences reflect the variation in elastic modulus and hardness between species and within an individual frustule.

To investigate the mechanical properties of the diatom frustule and constituent biosilica as decoupled from the full-shell structural response, we conducted in situ three-point bending experiments on beams with roughly 3.5-μm-square cross-sections fabricated from the frustule of *Coscinodiscus* sp. performed in a scanning electron microscope equipped with a nanoindenter, as well as ex situ nanoindentation on an individual basal plate that had been isolated from a frustule and the girdle band. We determined the elastic modulus to be $36.4 \pm 3.5$ GPa and the failure strength to be $1.1 \pm 0.3$ GPa. We discuss these results, as well as deformation and failure mode of the diatoms, in the context of their atomic-level microstructure obtained from transmission electron microscopy (TEM) and finite element method (FEM) simulations of the three-point bending tests.

**Frustule Morphology and Microstructure**

The cross-section of the frustule of a *Coscinodiscus* sp. is shown in Fig. 1B. The cribrum and cribellum constitute the distal surface of the shell, and the basal plate composes the proximal surface. Areolae walls span these two layers and approximate a honeycomb lattice configuration. Fig. 1C shows the hexagonal arrangement of pores of the cribrum and cribellum. The cribrum is composed of clusters of hexagonally arranged, elliptical pores with average dimensions of 364 nm for the major axis and 283 nm for the minor axis, and the clusters are arranged in a hexagonal pattern. The cribellum layer is composed of 50-nm-diameter pores that are hexagonally arranged across the entirety of the surface and is laid atop the cribrum. Fig. 1D shows the basal plate and areolae cells. Each areolae cell has five or six side walls and contains a single foramen with an average inner diameter of 822 nm.

Fig. 2A shows a TEM micrograph of the frustule of *Coscinodiscus* sp. Fig. 2B-D provide site-specific energy dispersive spectroscopy (EDS) data with diffraction patterns in the insets and convey that the material in the areolae wall is made nearly exclusively of silicon. Smaller copper peaks correspond to signal contamination from the copper TEM grid. The inset diffraction pattern indicates that the region is entirely amorphous. Fig. 2B shows nearby regions that correspond to the interior surfaces of the areolae cell; the amorphous/nanocrystalline Pt peaks come from the Pt needle that was used during ion-beam assisted deposition and not from the biological sample. Fig. 2C provides EDS data for a 275-nm-thick region of the basal plate and shows strong Si and Ga peaks, with the latter caused by the Ga$^+$-ion milling during thinning. It seems that Ga was localized within this basal plate region even though the entire sample was exposed to ion milling. We believe that the porosity observed within the nanocrystalline region (Fig. 2C, Inset) facilitated Ga sequestration into it; Ga content is lower in the amorphous regions (Fig. 2B, Inset). The difference in the microstructure between the nanocrystalline and amorphous silica is reflected in the difference in the diffraction contrast between the two regions (Fig. 2A, Inset) and shows that a sharp interface exists between the two microstructures. Fig. S1 shows this contrast difference within the basal plate was also observed in SEM imaging.

The vast majority of the frustule, including the areolae walls, shows a smooth, amorphous microstructure (labeled region A in Fig. 2A). The only place where microstructure deviated from amorphous was within the basal plate, which shows several bands between 50–80 nm in thickness, oriented parallel to the frustule surface, and separated by striated patterns of darker contrast (region B in Fig. 2A). There is a 10-nm-thick band that displays rough contrast that is adjacent to the smooth material in region A. The nanocrystalline diffraction pattern in Fig. 2C confirms the presence of these regions of local order. Region B has a total thickness of 275 nm, about 47% of the thickness of the basal plate, and displays a darker contrast than the material in region A, which suggests that it is either more densely packed or contains a higher silica/organics ratio than material in region A.

Previous work has uncovered that the microstructure of the frustule silica can vary between species and across stages of frustule development. Reported microstructures include compacted spheres, networks of fused spheres, silica microfibrils, and smooth homogeneous silica (13, 14). Schmid et al. (3) and Rogerson et al. (15) investigated wall morphogenesis in centric diatoms *Thalassiosira eccentrica* and *Coscinodiscus asteromphalus* and identified “growing zones” that are loose aggregates of 12-nm-diameter silica spheres, where new frustule growth occurred, and “compacting zones” that display a homogenous morphology where more mature growth had occurred. In the centric diatoms studied by these authors, the frustule growth occurred in a distal direction such that the growing zones were oriented outward relative to the compacting zones. This orientation suggests that the differences in microstructure observed here likely do not correspond to growing and compacting zones. Observations of wall morphogenesis by Schmid and Volcani (16) and Hildebrand et al. (17) reported the presence of clustered spheres in *Coscinodiscus wailesii* and *Thalassiosira pseudonana*. These authors reported that frustule growth began in the center and extended compacted silica sphere strings radially to the margin. Later growth stages of the basal plate occurred by cross extensions and compaction, forming an 85- to 100-nm-thick template for subsequent distal growth. The proximal nanocrystalline microstructures observed in this work may correspond to this initial basal template. It has been suggested that following radial growth, sintering plays an important part in the morphogenesis of the frustule by filling in the spaces of the compacted spheres with monomeric silica, flattening the deposition surface, increasing its radius of curvature, and promoting adherence (14, 18). This type of sintering would result in the amorphous material observed in region A shown in Fig. 2 A and B.
Mechanical Response in Bending and Nanoindentation

Fig. 3B shows the stress strain data for representative three-point bending tests. The data indicate linear elastic loading with no plastic deformation up to failure. Failure stress varied between 850–1,460 MPa and failure strain varied between 2.2–4.0%. The average elastic modulus was calculated to be 36.4 ± 8.3 GPa based on a linear fit to the slope. The experiments were performed in displacement-controlled mode, and if a settling event resulted in a displacement rate greater than the prescribed one the instrument controller adjusted the indenter head to maintain the prescribed rate. This feedback response occasionally manifested itself as a drop in stress when the feedback loop adjustment caused a short local unloading. Failure was catastrophic and occurred faster than the imaging scan rate of the SEM. Upon failure, most samples released the accumulated strain energy by launching from the substrate and could not be recovered. Fig. 4 shows one half of such a bending sample that was recovered and shows that failure occurred by propagation of a crack through the center of the beam.

Fig. 3E gives the calculated modulus against the contact depth acquired during nanoindentation of the basal plate and the girdle band. In both materials, the modulus decreased with contact depth. The modulus calculated from indentation on the basal plate varied from...
Differentiation of Frustule Layers in Mechanical Response

Fig. 4 shows the fractured surface of a representative bending test sample. Fig. 4A and B show the path of crack propagation across the basal plate and the cribrum. Bending induces a compressive stress along the inner edge of the beam, abutting the indenter head, and a tensile stress along the outer edge, which is likely where the crack initiated. It seems that a central pore present in the basal plate (indicated by the blue arrow) and the cribrum (indicated by the red arrow) served as a stress concentrator and the crack nucleation site because it is close to the location of maximum bending moment. Within the cribrum, the crack traveled upward toward a cluster of pores beneath the applied load, and its trajectory continued through a series of these pores upward, tracing a path between stress concentrations (Fig. 4A). The postdeformation crack surface shown in Fig. 4A, Inset illustrates that the pores acted as perforations for the crack propagation. In the basal plate (Fig. 4B) the crack propagated upward, bending slightly to follow the path of the crack within the cribrum. These paths diverge when the crack propagating through the basal plate encounters a foramen near the top edge of the beam. Instead of traveling through the pore, the crack is deflected around the pore. The foramina differ from the cribrum pores in that they have a raised rim around the circumference of the pore. Our experiments revealed that the pores in the cribrum act as stress concentrators and fail by crack propagation whereas the rim reinforcements in the foramina seem to shield them against failure. This may shed light on the differentiation in function between the basal plate and the cribrum: The resilient pores in the basal plate may have a primarily structural function, whereas the pores of the cribrum and cribellum may serve more in the capacity of resource acquisition.

Elastic Properties of Diatom Shell Components

Fig. 3B shows that the stress strain data from bending tests has a signature of linear elastic loading ending in brittle failure. No plasticity or controlled crack nucleation or growth was observed before failure. Any nonlinearity in the data can be correlated to a rigid body movement of the beam captured in the video. We expect some variation in the measured load due to a small misorientation (~2°) of the indenter head relative to the sample surface normal between tests as well as nonideal contact between the beam and the testing platform and indenter head. The measured elastic modulus from bending tests was consistent among all four reported samples, at 36.4 ± 8.3 GPa.

Nanoindentation response of the isolated basal plate and the girdle band shows remarkably similar. Average elastic moduli varied with increasing contact depth from 21.0 ± 7.7 at 28 nm to 39.7 ± 8.3 GPa at 217 nm in the basal plate, and from 19.9 ± 2.1 at 53 nm to 40.2 ± 4.0 GPa at 267 nm in the girdle band. The submicron thickness of the samples and the nanomechanical experiments in general render it challenging to eliminate all sources of experimental error. For shallow contact depths, some measurement error likely stems from the nonideal geometry of the tip, and at greater contact depths we encounter effects from the stiff substrate. Despite the uncertainty, these extreme cases set a range that matches well with the modulus calculated from bending tests.

The elastic moduli obtained in this work are in contrast to previously reported elastic moduli for Coscinodiscus sp. between 0.06–0.53 GPa (9) obtained from AFM indentation as well as results reporting differences in mechanical properties between different components of the diatom shell. Beyond inherent differences between three-point bending, nanoindentation, and AFM indentation, the boundary conditions of the previous AFM indentation tests are the most significant difference from the results reported here. Indentation into the full diatom frustule shell or even into half of the frustule—in either a concave or convex configuration—can result in a deformation response of the entire structure, which would not be indicative of its mechanical properties. This can include pivoting of the shell or localized shell buckling if the shell is immobilized. Such movement and localized deformation has been verified in situ during previous mechanical testing (10, 11). The low values and variance reported in previous studies are most likely due to movement or deflection of the frustule during testing and differences in compliance between loading the diatom axially and radially. By using the in situ bending tests on an extracted lamella representative of the frustule and nanoindentation on an isolated basal plate and girdle band we are able to significantly reduce these possible adverse displacements and to characterize the material more precisely.

The similarity in the elastic properties between the basal plate and the girdle band observed in this study suggests that they are mechanically equivalent composite materials. Swift and Wheeler (19) estimated that 20–40% of the dry weight inside the diatom silica valves is protein and carbohydrate. Kroger et al. (12) reported that the silica precipitated from in vitro studies of silica-depositing long-chain polyamines extracted from a diatom frustule had 1.25 μg of SiO2 per 1 μg of polyamine. Using the density of 2 g/cm³ for silica and 0.8 g/cm³ for the organic material in the diatoms studied here (14), we estimate the volumetric fraction of silica to be between 37.45 and 66.67%.

The rule of mixtures can provide an upper bound for the elastic modulus of the composite material. The expression for the composite modulus is given by

\[ E_c = fE_s + (1-f)E_o, \]

where \( f \) is the volume fraction of silica and \( E_s \) and \( E_o \) are the elastic moduli of the silica and organic component, respectively. It is reasonable to assume that the elastic modulus of the organic component is significantly lower than that of the silica; applying Eq. 1 to the estimated range of volume fractions predicts the composite modulus to be between 26.2–46.7 GPa, consistent with the values reported here and similar to synthetic bi-silica composites such as BioGlass®5S5 (45% SiO2, by weight) at 35 GPa (20).

Strength vs. Relative Density

The average failure stress was 1.1 ± 0.3 GPa at a strain of 3.5 ± 0.7%, with variations up to 250 MPa, which seem to be partly related to the distribution of pores in the beam. The highest stresses occurred in samples that lacked foramen segments along the bottom edge of the testing beam. Samples that failed at lower stresses had foramen segments, which suggests that their role in strengthening the diatom against stress concentration does not prevent them from serving as the weak points for failure along the free edge when the beam is subjected to bending. The average relative density of the beam samples used in this study, as calculated from direct volume measurements taken in
the SEM is 30.1%. A schematic of the frustule and list of measured geometries and relative densities is provided in Fig. S2 and Tables S1 and S2. Using the density of bulk silica at 2,210 kg/m$^3$, which provides the upper bound for the constituent material density, gives the frustule material a specific strength of 1,702 kN·m/kg, a value well above those of other natural cellular, composite and silk materials including bamboo (693 kN·m/kg), mollusk shell (127 kN·m/kg) (21, 22), and spider silk (1,000 kN·m/kg) (23).

Fig. 5 shows an Ashby plot of strength vs. density for several natural biomaterials (22). Diatom frustule samples show specific strengths above other reported cellular materials and are comparable to the strongest natural polymers. The theoretical maximum is determined by extrapolation of the strength and density of diamond.

Fig. 5. Ashby plot of strength vs. density for naturally occurring biological materials (22). Diatom frustule samples show specific strengths above other reported cellular materials and are comparable to the strongest natural polymers. The theoretical maximum is determined by extrapolation of the strength and density of diamond.

Stress Distribution Within Frustule Layers

The presence of an architecture within the diatom design results in a heterogeneous stress distribution within the frustule. Fig. 6 shows the Mises stress distribution at the maximum bending strain of the simulated FEM beam (Fig. 6B and E) along the bottom edge of the cribrum and basal plates (Fig. 6C and F). The Mises stress of a solid beam at similar strain is shown as a black line for comparison. Within the cribrum, some thin sections surrounding the pores show local stress concentrations, manifested as two symmetric peaks $\pm 0.12$ away from the center and one peak $\pm 2.2$ away from the center. In the rest of the sample, the stresses remain below those in the solid beam. This is likely because the multiple pores present along the bottom edge are unable to sustain high stresses along this edge. Within the basal plate, the stress distribution closely follows that in the solid beam and increases toward the center of the beam. Near the center, the presence of a reinforced foramen results in a local reduction of stress. Local fluctuations in the stress distribution within both layers...