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Sea urchins have teeth? A review of their microstructure, biomineralization, development and mechanical properties

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Abstract

Sea urchins possess a set of five teeth which are self-sharpening and which continuously replace material lost through abrasion. The continuous replacement dictates that each tooth consists of the range of developmental states from discrete plates in the plumula, the least mineralized and least mature portion, to plates and needle-prisms separated by cellular syncytia at the beginning of the tooth shaft to a highly dense structure at the incisal end. The microstructures and their development are reviewed prior to a discussion of current understanding of the biomineralization processes operating during tooth formation. For example, the mature portions of each tooth consist of single crystal calcite but the early stages of mineral formation (e.g. solid amorphous calcium carbonate, ions in solution) continue to be investigated. The second stage mineral that cements the disparate plates and prisms together has a much higher Mg content than the first stage prisms and needles and allows the tooth to be self-sharpening. Mechanically, the urchin tooth's calcite performs better than inorganic calcite, and aspects of tooth functionality that are reviewed include the materials properties themselves and the role of the orientations of the plates and prisms relative to the axes of the applied loads. Although the properties and microarchitecture of sea urchin teeth or other mineralized tissues are often described as optimized, this view is inaccurate because these superb solutions to the problem of constructing functional structures are intermediaries not endpoints of evolution.

Keywords

Biomineralization; calcite; mechanical properties

Introduction

Prior to 2000 the author knew little about sea urchins other than poisoning and probably death would result from a spine puncture (which, as it happens, is a huge exaggeration). Prior to a 2001 move to Northwestern University from the engineering faculty at Georgia Tech, the author was reintroduced to Professor Arthur Veis in a hallway of the medical school. Previously, the author met Veis once socially in mid-1970s when the author was an

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undergraduate at Northwestern. In the way of such chance encounters between near strangers, the author asked about the research in Professor Veis's lab. One topic was sea urchin teeth and their minerals. The author's response was: "Sea urchins have teeth?"

Veis explained the very interesting proteins (1) and internal structure of sea urchin teeth, in particular, the stone part (see below) where staining revealed a high concentration of acidic proteins. Now, all that the author knew about proteins was that they contain C, H, O and N, but, if protein were localized within the stone part, then the stone part should have less mineral than elsewhere. Imaging with X-ray Computed Tomography (microCT), which the author had been using for some years, could reveal the extent of the stone part in 3D. The author obtained a *Lytechinus variegatus* tooth from Veis, scanned it with microCT and was so disappointed with the results that he hesitated to show it to Veis and his lab members. The author did so, nonetheless, and Veis and coworkers greeted the results with such enthusiasm that the author decided further assays were warranted. As the cliché goes, the rest is history recorded in a number of papers covering, not only sea urchins but also mammalian dentin (2–18). If Murphy's Law is an important scientific and engineering principle, then serendipity is its converse.

As this article's title indicates, it reviews what has been learned about tooth microstructure, mechanics, development and biomineralization. The regular sea urchins and their oral structures are introduced in the first section, and the second section discusses tooth development. Mineralization in the sea urchin with emphasis of the tooth comprises the third section, and the coverage ends with a discussion of tooth functionality. The proteins involved with mineralization of sea urchin calcite are not discussed except in the most general terms.

Class Echinoidea and the regular sea urchins

Most readers with a background in biology are familiar with sea urchins mainly because they are a traditional model in developmental biology. Sea urchin mineralized tissues are also a very interesting biomineralization model and a model for tough ceramics. Sea urchin mineral is based on magnesium-rich calcium carbonate varying in form from skeletal elements (ossicles) of highly fenestrated calcite crystals, a structure called stereom, to the dense calcite of the teeth. As discussed below, some hold that amorphous calcium carbonate is the initial mineral phase and persists in fully mature ossicles and teeth. Before discussing biomineralization and mechanics of sea urchin teeth, however, it is necessary to introduce the past and extant diversity of the teeth and the structures within and the ontological development of teeth.

The earliest fossils of sea urchins are found in the upper Ordovician (~450 MYA), and Archaeocidarids, precursors of all modern forms, developed in the Devonian (~420–360 MYA). The Jurassic (200–145 MYA) saw differentiation of most major lines of echinoid including the split between cidaroids and euechinoids (19,20).

The fossil record reveals a myriad of different mineralized structures existed in class Echinoidea, and the considerable diversity among ossicles such as the test plates and spines provides much of the basis for placing extant and fossil urchins in their orders, families and

genera. More recently, techniques such as gene sequencing have been employed to estimate when different sea urchin lines diverged (21), but this approach is unavailable for fossils. As noted by Jackson (22) one century ago, differences in morphology of the urchin's oral apparatus are also very valuable in systematics of this group, and these differences, particularly those in tooth macro- and microstructure, are especially germane to the present article.

The jaw structure or Aristotle's lantern has five-fold symmetry, with five jaw sections or pyramids each containing a single tooth (Figure 1). The teeth attach with collagenous tissue (23) to the pyramid at a structure called the dental slide, are supported over only a fraction of their length and extend beyond the dental slide as curved cantilevers (Figure 1b), the consequences of which are discussed in the section "Tooth Functionality" below. The teeth grow continuously, meaning that a single tooth contains all stages of mineralization, and advance over time to replace material lost to wear, something which is also important in functionality.

Sea urchin teeth are quite long and slender with the exception of those in the sand dollars. Transverse tooth cross-sections can be classified into four types of shape: grooved or U-shaped (often termed aulodonts in the literature), T-shaped (divided into two groups, stirodons and camarodonts), prism-shaped and wedge-shaped teeth (see Figure 2 of (24) for schematics and (25) for a recent discussion). Microstructural studies of sea urchin teeth date back to Salter (26) and Giesbrecht (27) in the 1800s, and availability of scanning electron microscopy (SEM) led to renewed emphasis on how tooth microstructures (plates, needle-prisms, lamellae, see Figure 1c) differed between orders and families (24,28–36). Much of this work focused on stages before mineralization was complete and in which the individual microstructural elements could be separated, but some was on polished cross-sections. More recently, microComputed Tomography (microCT) was employed to study pyramid and tooth microstructure in 3D, e.g. (5,8) and (2,7,25,37), respectively, with (8) and (37) presenting quantification of stereom and tooth plate geometry, respectively, which in a sense follows the quantification of different microstructural fabrics of stereom by Smith (38).

Figure 1(c) shows a schematic transverse cross-section of a camarodont tooth; coverage will be limited to this tooth type in order to keep length manageable. Although the schematic shows disconnected primary and secondary plates, each primary plate is connected to a single secondary plate in the lateral portion of the flange (Figure 1d). Likewise, secondary plates are integral with the carinar process plates lying along the margins of the keel. In both cases, the plates are shown as disconnected from simplicity. The secondary plates (in the flange) gradually curve into a very different orientation in the keel; unlike the primary–secondary plate system, there is no well-defined point where the secondary plate ends and the carinar process plate begins. In addition to plates, the tooth also contains a lamella–needle–prism system. Lamellae attach to the primary plates near the midline of the flange and extend as needles which thicken over time into what are then termed prisms; note that the connection between needles and prisms is not shown in Figure 1(d) nor are the lamellae.

Tooth development

Following Märkel (39), the tooth consists of three stages. The plumula forms first, is the least mineralized and extends to the point where the keel begins to form. The shaft begins where the keel has reached its fullest extent and is reckoned to end where the tooth protrudes past the dental slide. The cantilevered portion of the tooth is termed the incisal or oral end, and little, if any, additional mineralization is occurring in this part of the tooth.

Within the aboral plumula, two rows of tooth elements form, and the initially triangular plates grow larger and interdigitate along the tooth's midplane ((27) and see the 3D microCT rendering in Figure 1(c) of (16)). After these primary plates extend laterally and aborally, secondary plates develop and grow from the lateral edges of the primary plates and make an acute angle with the primary plates, (microCT slice in Figure 2a). It is important to note that while the tooth elements are growing larger, they are simultaneously moving adorally toward the incisal end. When more developed, the series of successive primary–secondary plate complexes forms a cone-in-cone structure (more precisely, two symmetric half cone arrays) with an adorally oriented apex (Figure 1d). The slice in Figure 2(b) shows a stage just before the keel has developed and contains prominent secondary plates and needle-prisms. The keel is partially developed 4 mm later (Figure 2c), is mostly complete 2 mm after that (Figure 2d) and has reached its maximum extent 4 mm beyond Figure 2(c) (image not shown). In Figure 2(e), 8 mm beyond Figure 2(d), the tooth has nearly reached its maximum level of mineralization (compare to the amount of open space within the tooth in Figure 2d) and changes little over the remaining 3 mm imaged. A second mineralization stage, described below, fills the space between needle-prisms and primary, secondary and carinar process plates.

Pillar bridges (Figure 2e) are a porous structure to which collagenous tissue attaches and connects the tooth to the dental slide; these first appear within a millimeter or so aborally from this slice. The slices in Figure 2 show prisms are larger the farther they are away from the flange; this is a direct consequence of the angle the needles make with the tooth axis (e.g. Figure 1 in (39)) and growing in width as they elongate. In the slice in Figure 2(d), the edges of the carinar process (i.e. where the top and bottom surfaces of the plates intersect the slice) are quite wavy, and this indicates the plates undulate from a simply curved structure. This is not the case in all species of camarodonts (9,37).

The descriptions above are quite simplified, many details are omitted and variations among the camarodonts and between camarodonts, stirodons and other sea urchins are not covered. The complex 3D structure is elucidated in SEM micrographs and schematics in much greater detail elsewhere (24,26–34,40).

Sea urchin mineralization

As mentioned above, sea urchin teeth are mineralized in two stages. The first forms the primary, secondary and carinar process plates and the lamella-needle-prism complexes. The second stage cements the first stage material together. Both stages are magnesian calcite

$\text{Ca}_{1-x}\text{Mg}_x\text{CO}_3$, have the same crystallographic orientation (4,28,41,42) but have different compositions ($x \sim 0.13$ and 0.32 for *L. variegatus* first and second stages, respectively (4)). In the same species, Schroeder et al. (43) obtained similar maximum and minimum Mg compositions ($x \sim 0.08$ and 0.41), but Weber's survey (44) of powdered teeth from many orders gave $x \sim 0.1$, presumably a biased result because there is much more first than second stage mineral. There are also small amounts (less than 0.5 wt. %) of organic material intercalated within the mineral of sea urchin teeth (45,46).

Within the different regions of the tooth, the stone part has long been established as having the greatest Mg content (36,39,43). The first stage mineral of the stone part has the finest scale structure within the tooth but also the greatest volume fraction of soft tissue in the immature state. After replacement of the soft tissue with the cementing phase, the volume fraction of second stage mineral reaches the highest levels in this region (38) and accordingly the highest regional Mg content.

In common with ossicle stereom and larval spicules, the first stage mineral of teeth forms in spaces between cellular syncytia (7,29,40,42,47–49,50) formed by odontoblasts in the plumula. The undemineralized thin section of Figure 3(a) shows cellular syncytia in the plumula of a *L. variegatus* tooth. Using autoradiography, Holland (51) showed the temporal distribution of labeled syncytial and epithelial nuclei changed in a complex fashion. Later the same odontoblast syncytia form the second stage mineral while simultaneously shrinking in size. What causes the second stage mineral to begin to form and why the Mg composition also changes are questions that remain to be answered.

Once second stage mineralization has begun, it proceeds very rapidly. Figure 3(b) and (c) show synchrotron microCT slices $360 \mu\text{m}$ apart (corresponding to about 1.5 days growth (29,51), see below). There is little second stage mineral in Figure 3(b) and substantial amounts in Figure 3(c). Because the plate periodicity is $\sim 6 \mu\text{m}$, the plate thickness $\sim 4 \mu\text{m}$ and the voxel size is $1.8 \mu\text{m}$, these data allow only approximate values to be obtained for the rate of second stage mineral addition. The non-mineral volume fraction (between plates) is between 0.22 and 0.31, depending on the threshold used for binary segmentation of mineral from non-mineral voxels, in Figure 3(b) and decreases to between 0.07 and 0.16 for the matching thresholds in Figure 3(c). In the $360 \mu\text{m}$ between these two stage, roughly one-half to two-thirds of the channel volume has been filled with second stage mineral. Note that this mineral is not preferentially distributed near the open ends of the plates. In the ~ 1.7 days between stages in Figure 2(b) and (c) and within the volume pictured, the amount of second stage mineral added corresponds to $\sim 1.2 \times 10^4 \mu\text{m}^3$. As Cavey and Märkel noted (40), the ions needed to form the second stage mineral must travel in the channels between the impervious plates. In order to reach the most axial portion of the channels between the plates, these ions must travel $\sim 300 \mu\text{m}$, and this, together with the amount of material formed and the restricted width of the channels, demonstrates that the fluxes of Ca and Mg ions must be very large.

Even as early as in Figure 3(c), the channels between plates are beginning to be blocked (abaxial surface of the flange). As the paths between plates become more constricted, second stage mineral formation slows considerably and never completely fills the volume. The

medial portion of the flange in Figure 3(d), a position within a millimeter or two from the incisal tip, contains 0.9–0.95 volume fraction mineral, depending on the threshold used, and thin sections of the demineralized incisal end contain cell nuclei which occupied the small pores in the living tooth (52). The internal porosity is linked to the external surface of the tooth with micrometer-sized channels, some of which are visible in the slice of Figure 3(d). The 3D rendering of the void space in Figure 3(e) shows the channels and their spatial distribution clearly. The white solid at the bottom of the image is the air outside the specimen, the channels are arranged in rows, reflecting the presence of the primary plates between the rows, and, within a row, the channels appear regularly spaced.

The shapes of the syncytial spaces (and of their defining syncytia) dictate the morphology of the calcite skeletal elements much like an ensemble of crystals grown from solution takes its macrostructure from its container. At several stages in the mineralization process, the syncytia of odontoblasts abruptly change the geometry of the calcite phase formed. These include the growth of the secondary plates from the lateral edges of the primary plates; nucleation and growth of lamellae at the axial edge of the primary plates and their transformation into needle-prisms; the onset of second stage mineral formation. In the case of the secondary plates, syncytia are in place long before calcite begins to form within these syncytial spaces (e.g. Figure 6 of (7), upper left corner of section T401), but the secondary plates do not begin to form until a well-defined time point. In this and the other two cases, control of the changes may be due to cellular signaling, some sort of geometrical control of shape (limit to the extensibility of the epithelial layer enveloping the tooth and or the $> 90^\circ$ curvature of the plumula) or changes within the syncytia such as stress from being isolated from the external environment and its source of oxygen and ions.

Most reports describe the second stage mineral as polycrystalline disks (e.g. (39,40)) despite much birefringence data showing that both stages of mineral have the same crystallographic orientation (29). Exceptions to the previous statement are polarized light observations of a range of crystal orientations in diadematooids and clypeasteroids (30,53). Recent position-resolved X-ray diffraction studies also show that the first and second stage mineral have the same crystallographic orientation (2,41), i.e. are co-oriented (Figure 4a) and perhaps even epitaxially related. The distinction between co-orientation and epitaxy is not pedantic because the former implies that each disk nucleates independently and the latter implies that each disk (the author prefers to call this a column to distinguish it from the putative polycrystalline entities) grows from the underlying plate or prism. It is certainly true that the second stage mineral nucleates and grows simultaneously at many different sites in a given channel between plates or prisms, but, if epitaxy is present and given the higher Mg content of second stage mineral, then the resulting complex-shaped structure is a compositionally modulated crystal not dissimilar to heteroepitaxy in optoelectronic crystals like $\text{Al}_y\text{Ga}_{1-y}\text{As}/\text{GaAs}$ or superlattices $(\text{Al}_y\text{Ga}_{1-y}\text{As}/\text{GaAs})_n/\text{GaAs}$ with large y and $n > 20$. Note that epitaxy can exist regardless of whether the interface is incoherent or coherent (i.e. whether or not interface dislocations accommodate atomic mismatch) but requires physical continuity between the two layers. If the second stage mineral co-orient through nucleation events independent of the first stage mineral, one is left with the question of what determines the crystal orientation, a question that applies as well to the first mineral formed in the plumula.

The consensus view is that single crystal calcite exists in the mature tooth, but the author believes questions remain that are not so clearly decided that they warrant no further investigation. For example, does a transient amorphous calcium carbonate (ACC) phase precede the formation of calcite? Do some regions of mature plates and prisms or second stage mineral contain ACC? Does crystal material coexist with ACC in an intimate mixture, the so-called mesocrystals (54)?

Early work on sea urchin mineralization explicitly assumed calcite grew from the liquid (47–49), but recent thinking centers on the calcite of spicules, stereom and tooth elements forming from a solid precursor of ACC that may or may not completely transformed into calcite (39,41,55–60). Differential etching behavior of the central portions of primary plates and prisms has been cited as evidence for the presence of ACC, either as a transient precursor or even as a long-lived constituent of the teeth, but this interpretation is not the only possibility. For example, Figure 4(b) shows polished and etched prisms with the highly etched center that often has been interpreted as showing ACC, but the step structure (“G” in Figure 4b) is not explained. The core and step structure are also seen in transmission optical micrographs (12), including the early report of Giesbrecht (27), and the author and coworkers suggested that the central core and sharp radial step are produced by a screw super-dislocation (micropipe) running up the center of the prism (12), i.e. an analog of the screw dislocation structure in metal single crystal whiskers (61). Hollow core super-dislocations are common in the other molecular crystals such as SiC (62).

The layered structure of etched prisms can be attributed to dispersion of the intercalated macromolecules, and the rough surface of etched cross-sections of plates and prisms is widely interpreted as showing the presence of mesocrystals, but both can be explained by more prosaic structures. Layered structures could result from growth spiraling around a central screw dislocation with atomic impurity decoration. The high density of dislocations in the highly imperfect calcite crystal skeletal elements of the sea urchin teeth can also produce roughness during etching: etch pits formed at the intersection of dislocations with the surface were the basis of pre-transmission electron microscopy (TEM) studies of dislocations. Submicrometer voids (50–150 nm diameter) documented in sea urchin mineral with TEM (10,63), see Figure 4(c), could produce roughness where they intersect the polished surface; the latter study reported rapid decomposition of material within the voids, consistent with decomposition of macromolecules under electron beam irradiation.

Recent synchrotron X-ray diffraction provides a picture of structure consistent with highly imperfect crystals or possibly mesocrystals. Diffraction peaks of spicules and of teeth are quite wide, and, if broadening were interpreted as only due to small crystallite sizes, i.e. small coherent scattering domain dimensions, Scherrer’s equation (64) gives widths ~ 200 nm in spicules (65). Microstrains (also known as rms strains) also produce peak broadening through gradients of lattice parameter, and crystallite size and microstrain can be separated by applying the Williamson-Hall approximation to analyze widths of multiple reflections (64). In *L. variegatus* teeth, crystallite size and microstrain are 200 nm and 0.35%, respectively (18), and the spicule data mentioned above (65) yields similar values if analyzed this way. High resolution TEM of pulverized teeth reported matrix nanocrystals 10–20 nm (56) but one wonders whether sample preparation allows for valid size

measurements. Further, sea urchin calcite is extremely sensitive to electron beam damage (10). The spicule scattering studies (65) attributed the crystallite size to decoration by macromolecules, but the size and microstrain values are consistent with the presence of subgrains and low angle, dislocation-defined boundaries. The presence of nanovoids also would contribute to producing small coherent scattering domains regardless of whether macromolecules directly disrupted the crystal lattice. Small angle X-ray scattering (SAXS) and high resolution TEM have been interpreted as showing mesocrystal structure in sea urchin spines (54).

Secondary ion mass spectroscopy (SIMS) of polished cross-sections of *L. variegatus* teeth (12) showed low total ion, high Ser and low Asp counts between first and second stage mineral (Figure 4d–f). In the middle of the plates, SIMS also showed a central zone bordered by high total ion counts and containing elevated Asp. Organic sheaths were observed around the prisms in partially demineralized teeth (39,66), but other studies suggest that the organic layers may not be continuous (12,41). In model calcite crystallization studies, Asp-rich proteins (derived from the prismatic layer of *Atrina rigida* shells) were occluded and disordered the mineral (60). *In vitro* studies of magnesium calcium carbonate mineralization showed magnesium content and crystal morphology were affected by the presence/absence of soluble organic matrix macromolecules extracted from sea urchin tests and spines (67). Inorganic calcite overgrown on sea urchin teeth has the same orientation as the underlying biomineral (55).

Data exist that show ACC transforming into calcite in sea urchins. In one experiment, needles from teeth produced no birefringence initially (i.e. no calcite was present) and after hours in water calcite was observed (55). In other work, newly formed embryonic spicules and newly regenerated areas of spines did not initially diffract synchrotron X-radiation and only later produced diffraction patterns (58,59). Substantial ACC also was observed in spicules using Fourier Transform Infra-Red (FTIR) spectroscopy (57). No other groups appear to have tried to replicate any of these results.

Killian et al. (68) suggested first stage mineral elements remained ACC until adorally lying and previously transformed plates transferred their orientations to the aboral plates through the pillar bridges on the abaxial surface of the flange. Diffraction from living teeth in their native milieu (coelomic fluid), however, revealed calcite at early stages of the plumula formation (16), well aboral of pillar bridge formation, and pillar bridges cannot co-orient the primary plates. The author notes the former study (68) examined teeth missing their plumulae, and the absence of pillar bridges prior to the formation of the keel has been known since Salter's work in the 1860s (26).

Tooth functionality

Teeth function in concert with Aristotle's lantern and its muscles, and teeth would be "overdesigned" if their fracture toughness and strength greatly exceeded values encountered when lantern muscles and ligaments were overloaded. Such an "overdesign" would also work against the self-sharpening design of the teeth, see below. There are only limited data in any case. Andrietti et al. investigated the mechanics of the muscle system of lanterns and

specifically measured lantern lateral and axial displacement as a function of applied force (69), and Ellers and Telford measured the biting force in sand dollars (70).

Teeth of all echinoids but the sand dollars are loaded in bending. The U- or T-shaped cross-sections can be analyzed as girders, and engineering statics shows such girders of homogeneous material are equally resistant to positive or negative bending moments, i.e. to tension in the girder's web (keel, Figure 1) or compression in the girder's flange (flange, Figure 1) or vice versa (71). Ultimate bending moment and ultimate shear force of camarodont teeth were measured by applying force in the abaxial direction (i.e. the "natural" loading geometry) and in the reversed loading direction (keel in compression and flange in tension) (36). This study found the keel resists 1.8 times the tensile stress that the flange can and demonstrates that microstructure (see Figure 1) has evolved to improve fracture resistance in camarodont teeth. Specifically, the fiber "reinforcements" (prisms) are located in the keel where they experience tensile loading (much like engineered fiber-reinforced composites) and the plate-like reinforcements in the flange where compressive loads are encountered (36,71).

Stock et al. (7) suggested that the carinar process plates provide additional fracture resistance in the keeled teeth by resisting lateral bending, i.e. bending induced when one side of the flange but not the other scrapes across a substrate. The absence of the carinar process plate "buttresses" along the lateral surfaces of the keel (as in some stirodons) might allow shear forces to more easily separate the prisms along their interfaces, much like axial shear would act on a bundle of pencils held together by an elastic band. Carinar process plate geometry differs among camarodonts (9) and may or may not affect the mechanical functionality of their teeth, a subject requiring further quantitative microstructural quantification and modeling (37).

Indentation hardness provides another measure of resistance to fracture, and the hardness of the sea urchin teeth may be larger than twice that of inorganic calcite (66) and that of the incisal end of teeth was constant for urchins from four different orders (72). Hardness in camarodont teeth varies by up to 2.6 times from the stone part (maximum) to the small prism zone of the adaxial keel (minimum) (39,71), and Figure 5(a) shows hardness as a function of distance from the abaxial surface of a mature *L. variegatus* tooth. Note that the stone part is where the largest volume fraction of second stage mineral is found, the finest structure and also the highest Mg composition. Nano-indentation shows higher hardness in second than in first stage mineral (54) and produces no cracks in former and cracks in the latter, mineral indicating the former has higher toughness (66).

The primary-secondary plate half cones are oriented with these plates parallel to the sides of the incisal tip. Fracture parallel to the surface of the plates, either by contact with hard substrates or with the other teeth, renews the sharpness of the cutting edge. The actual cutting surface is made up by the needles in the stone part, supported on the sides by the plates (Figure 1c). In camarodonts, approximately 1.5 mm of the tooth length is replaced weekly (29,51) which corresponds to about 7 pairs of tooth elements daily (40).

In order to self-sharpen, sea urchin teeth must “direct” the fracture path to follow locus of the plates although some crack deflection into adjacent plates may occur because of the multi-axial nature of loading. Biogenic (sea urchin) calcite does not cleave, and this means that entire teeth will not fail due to one cracking event. Instead, plates and prisms fracture conchoidally (39), and this deflection, both within individual plates or second stage mineral layers and across them, increases energy absorption during crack propagation. Figure 5(b)–(d) shows examples of different fracture paths in primary plates in *L. variegatus* (12), but most of the fracture is along the interface between first and second stage minerals. In Figure 5(b), there is a mixture of fracture along the first-second stage mineral interface and across plates and their attached columns (left of “C”); except for the midlines, the primary plate fracture surfaces are smoothly curving with some sharp reorientations. Interestingly, primary plate thicknesses vary within the volume shown in Figure 5(b) as do the thicknesses of column layer; this indicates that the syncytial spaces (defining the plate thicknesses) and the syncytia (determining the column thicknesses) are somewhat plastic dimensionally. Just to the right of “ic” in Figure 5(c), a primary plate has fractured in a series of closely spaced undulations spanning the plate’s surface. In Figure 5(d), the fracture surface extends directly across the neighboring plates and columns, the crack does not deflect and the column layers (white arrowheads) are much thinner than the neighboring primary plates. In all of the micrographs of Figure 5, a small amount of material adheres to primary plates midlines. Every couple of micrometers, a larger “hanging chad” decorates the plates’ fracture surfaces; these are particularly prominent to the left of “PP” in Figure 5(c).

When fracture exposes intact columns, the depth-of-focus of SEM allows one to peer between adjacent undisturbed columns and observe the shape of the growth surface of the columns. At the stage where the columns nearly fill the volume between primary plates, the very high Mg columns have slightly concave growth surfaces (e.g. column “C” in Figure 5c), indicating the mineral phase prefers to grow along the interface with the first stage mineral.

Closing thoughts

Fundamentally, sea urchin teeth are highly evolved structures, for a specific mechanical function, obtaining food. These teeth employ many materials reinforcement or strengthening strategies, and many of these were explicitly or implicitly encountered above. The mechanisms range from strengthening of the base material (solution hardening via Mg incorporation, dispersion strengthening via macromolecules within the crystal lattice, inclusion toughening via voids) to crystallographic alignment of structures (like in aircraft single crystal turbine blades) to interface tailoring to functional gradients (in morphology, composition, dimensions) to site-specific microarchitecture. The only thing apparently missing from the bag of tricks humans have stumbled upon, are the use of knots in structures.

Many authors write that various biomineral structures including sea urchin teeth are “optimized” for this or that function, but these structures are part of an evolutionary continuum, and such a view can be quite misleading. Past and present teeth represent samplings of the “materials design space” for functional biocalcite structures under the

constraint that structures must evolve from earlier forms. Current “design” compromises may well function better than earlier “designs” but may also have evolved coincidentally in tandem with another more important change; instead of optimized “solutions” current forms should be viewed as intermediate steps in a continuing process.

The literature reports detailed examination of single species and teeth from a limited number of mature individuals. This is understandable because such detailed microstructural and functional studies require significant time and resources and use of multiple techniques. These studies may have captured the functionality of camarodont teeth in broad brush terms, but the variation of carinar process plate morphology across taxa (9,37) suggests details are important and exemplars from many more families need to be studied.

To date, interpretation of the role of different microstructural elements of teeth have been qualitative. Quantitative analysis is needed that incorporates realistic boundary conditions and might be done with methods such as finite elements. Models incorporating isotropic material could be compared to idealized microstructure. Loading-induced strains and their spatial distribution need to be measured and compared to inferences drawn from microstructure or from numerical modeling; this might be done with position resolved synchrotron X-ray diffraction and/or diffraction tomography. It may be that current interpretations may need to be revised.

Much remains to be established in terms of sea urchin tooth biomineralization. First, what nucleates first stage mineral with same orientation? Test plates, for example, do not always have the same c-axis orientation across taxa (73), so why is this orientation invariant for each spatially separate primary plate? Are composition gradients within the same plate or prism a common feature? Why does composition change between first and second stage mineral? The author speculates this is because cation (and metabolic necessities) access is greatly reduced in the syncytia once the plates form. The development of second stage columns/mineral needs to be studied in much more detail, particularly in the early stages where the columns have not grown across the channel. Rates of second stage mineral formation need to be quantified and related to cellular processes and ion fluxes. Do teeth advance at the same rates for small versus large sea urchins of the same species? What controls the transition of syncytial spaces from primary plate morphology to the lamellae-needle-prism morphology? What controls formation of keel in camarodonts?

There are examples of 3D geometry used as phylogenetic characters for classification of different groups of sea urchins. One study used tooth transverse cross-section in the analysis (25). A second examined the distribution of local angle of the carinar process plates with the tooth axis for members of different camarodont families (37). Similar analyses may shed new light on the evolution of echinoids.

New synchrotron X-ray techniques and techniques such as cryoTEM have much to offer to investigators studying sea urchin teeth. One legacy of Professor Veis is the aggressive, early application of new techniques to fundamental problems in biomineralization, one of which is the sea urchin tooth. Throughout the 50+ years of continuous funding of his NIH grant on matrix mineralization, this emphasis on new approaches was balanced by fundamental

biochemistry and physical chemistry. Going forward, studies of sea urchin teeth need a similar balance between new approaches and fundamentals, be they chemistry or the pre-existing literature, which in the case of sea urchin teeth is not so large that it cannot inform all current research.

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Figure 1.

(a) Geometry of Aristotle's lantern of a regular sea urchin. (left) Side view of the sea urchin test showing spines "s" and lantern "L". A typical adult test diameter might be 35 mm along the adoral–aboral axis and 65 mm perpendicular to this. (right) Structure of the camarodont lantern along with the test opening through which the lantern protrudes during feeding. A pair of complementary demipyramids "dp" and "dp'" are labeled on the face of the lantern closest to the viewer, and all five teeth "t" of the lantern are labeled. The adoral ends of lantern and teeth point down, and teeth in large adults can exceed 25 mm in length. Modified from ref. (5). (b) MicroCT-based 3D rendering of a *Lytechinus variegatus* (pyramid numerically sectioned to expose the tooth. The least mature portion of the tooth is the plumula "P". The tooth shaft begins below the arrow, and the keel and flange are labeled "K" and "F", respectively. The portion of the tooth cantilevered from the dental slide is indicated by the solid bar just to the right of the tooth. (c) Geometry and architectural elements of the camarodont tooth. The left-hand schematic shows a portion of the tooth and the orientation of the thick section. The central schematic shows the different structures within the thick section, and the right-hand diagrams enlarge the flange constituents. The structures of the tooth are labeled in normal type face, and the tooth macrostructures (keel and flange) and directions in italic face. Adapted from ref. (9). (d) Schematic of the cone-in-cone structure of the primary and secondary plates. Adapted from (74).

Figure 2.

Series of synchrotron microCT slices from a *Lytechinus variegatus* tooth. Several millimeters of tooth length are not shown on either side of the first and last slices, i.e. at the aboral plumula and incisal ends. The number in the lower left or right corners of (b–e) indicates the adoral distance of the respective slice from (a). All slices of Figure 2 at the same scale (bar in upper left corner of (c)). Note that the tooth curves and, therefore, (a) is slightly tilted with respect to (c) and the axial width of (d) appears greater than that in (e). Data collected at station 2-BM, APS, 1.8 μm voxels, 20.9 keV.

Figure 3.

The primary plate regions of *L. variegatus* teeth. (a) Thin section of a tooth in the early stages of mineralization (slightly aboral from that in Figure 2a), stained with Toluidine blue and imaged with optical microscopy. The arrowheads show two pairs of syncytial membranes between which the first stage mineral forms and “n” a stained nucleus. (b, c) Synchrotron microCT slices of the lateral portions of the flange with (c) being 360 μm adoral from (b). The slices are from the same specimen shown in Figure 2 and imaged under the same conditions. (d) Adaxial medial portion of the flange approximately 2–3 mm from the incisal tip. (e) Tooth porosity (solid, mineral transparent) in 3D rendering of the volume centered on the middle of the area shown in (d). The large solid area at the bottom of the image is the air outside the tooth. The horizontal field of view is 23 μm in (a); 270 μm in (b, c) and 800 μm in (d). In (e), there are 100 μm from front to back in the rendering. In (d), the voxel size and X-ray energy are 1.45 μm and 21.6 keV.

Figure 4.

Lytechinus variegatus teeth. (a) Calcite 208 diffraction spot. The arrows indicate the three compositions present in this portion of the tooth (4). (b) Polished and etched prisms showing the central core and steps “G” (12). (c) TEM of voids in calcite plates. The black arrowheads identify voids which developed facets during electron irradiation. Data from the study published in ref. (10). (d–f) SIMS maps of primary plates and columns: Total ions (left), Ser (serine, center), Asp (asparagine/aspartic acid, right). Columns (very high Mg) are labeled “vh” and plates (high Mg) “h”. The sharp pair of lines running along the center of the plates in the total ion image (asterisks) appear in maps for many masses, none of which could be unambiguously attributed to a particular amino acid fragment. The Asp signal is low at column/plate interface (dashes) with weak broad maximum at the plate center (asterisks). The middle panel shows the Ser signal is high at column/plate interfaces (positions where the total ion and Asp signals are low), i.e. it is anti-correlated with the latter two signals in plate structure. The lighter the shade (online shade of orange/yellow), the greater the signal. The scale bars are 10 μm (12).

Figure 5.

(a) Vickers microhardness versus distance from the abaxial surface of the flange. The micro-indentations were recorded in a line along the axis of symmetry of the *Lytechinus variegatus* tooth. (b–d) Fracture surfaces of primary plates plus columns in the mature tooth. The labels “hc”, “ic” and “ip” refer to a column hanging from a primary plate “PP” over empty space, to the interface of a column (with the primary plate, revealed through fracture) and to the interface of a primary plate (with a column), respectively. Scale bars equal 10 µm. (b) Oblique fracture path. (c) Nominal fracture surface oblique to the primary plates in some areas and normal to the plates in others. (d) Fracture largely planar and perpendicular to the plates. (b–d) reproduced from ref. (12).