Mechanical Design of Selected Natural Ceramic Cellular Solids

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ABSTRACT

While the structure and mechanical properties of natural cellular solids such as wood and trabecular bone have been extensively studied in the past, the structural design and underlying deformation mechanisms of natural cellular solids with very high mineral contents (> 90 wt%), which we term as natural ceramic cellular solids, are largely unexplored. Many of these natural ceramic cellular solids, despite their inherent brittle constituent biominerals (e.g., calcite or aragonite), exhibit remarkable mechanical properties, such as high stiffness and damage tolerance. In this thesis, by carefully selecting three biomineralized skeletal models with distinctly different cellular morphologies, including the honeycomb-like structure in cuttlefish bone (or cuttlebone), the stochastic open-cell structure in sea urchin spines, and the periodic open-cell structure in starfish ossicles, I systematically investigate the mechanical design strategies of these natural ceramic cellular solids. The three model systems are cuttlefish *Sepia officinalis*, sea urchin *Heterocentrotus mammillatus*, and starfish *Protoreaster nodosus*, respectively. By investigating the relationship between their mechanical properties and structural characteristics, this thesis reveals some novel structural design strategies for developing lightweight, tough, strong, and stiff ceramic cellular solids.

The internal skeleton of *S. officinalis*, also known as cuttlebone, has a porosity of 93 vol% (constituent material: 90 wt% aragonite), which is a multichambered structure consisting of horizontal septa and thin vertical walls with corrugated cross-sectional profiles. Through systematic ex-situ and synchrotron-based in-situ mechanical measurements and collaborative computational modeling, we reveal that the vertical walls in the cuttlebone exhibit an optimal
waviness gradient, which leads to compression-dominant deformation and asymmetric wall fracture, accomplishing both high stiffness (8.4 MN∙m/kg) and high energy absorption (4.4 kJ/kg). Moreover, the distribution of walls reduces stress concentrations within the horizontal septa, facilitating a larger chamber crushing stress and more significant densification.

For the stochastic open-cell foam-like structure, also known as stereom (porosity: 60-80 vol%, constituent material: 99 wt% calcite) in *H. mammillatus*, we first developed a computer vision-based algorithm that allows for quantitative analysis of the cellular network of these structures at both local individual branch and node level as well as the global network level. This open-source algorithm could be used for analyzing both biological and engineering open-cell foams. I further show that the smooth, highly curved branch morphology with near-constant surface curvature in stereom results in low-stress concentration, which further leads to dispersed crack formation upon loading. Combined synchrotron *in-situ* analysis, electron microscopic analysis, and computational modeling further reveal that the fractured branches are efficiently jammed by the small throat openings within the cellular structure. This further leads to the formation of damage bands with densely packed fracture pieces. The continuous widening of the damage bands through progressive microfracture of branches at the boundaries contributes to the observed high plateau stress during compression, thereby contributing to its high energy absorption (17.7 kJ/kg), which is comparable and even greater than many synthetic metal- and polymer-based foams.

Lastly, this thesis leads to the discovery of a unique dual-scale single-crystalline porous lattice structure (porosity: 50 vol%, constituent material: 99 wt% calcite) in the ossicles of *P. nodosus*. At the atomic level, the ossicle is composed of single-crystal biogenic calcite. At the lattice level, the ossicle’s microstructure organizes as a diamond-triply periodic minimal surface (TPMS) structure. Moreover, the crystallographic axes at atomic and lattice levels are aligned, i.e., the *c*-axis of calcite is aligned with the [111] direction of the diamond-TPMS lattice. This single
crystallinity co-alignment at two levels mitigates the compliance of calcite in the c-axis direction by utilizing the stiff <111> direction of the diamond-TPMS lattice. Furthermore, 3D in-situ mechanical characterizations reveal that the presence of crystal defects such as 60° and screw dislocations at the lattice level suppresses slip-like fracture along the {111} planes of the calcitic diamond-TPMS lattice upon loading, achieving an enhanced energy absorption capability. Even though the skeleton of echinoderm is made up of single-crystal calcite, the structure fractures in a conchoidal manner rather than along the clipping plane, which can continuously fracture the fragments into small pieces and enhance energy dissipation.
Mechanical Design of Selected Natural Ceramic Cellular Solids

Ting Yang

GENERAL AUDIENCE ABSTRACT

The application of engineering ceramic cellular solids as structural components is limited by their brittleness and flaw sensitivity. In contrast, nature has evolved ceramic cellular materials such as sea sponge, sea urchin spine, and diatom shells that are simultaneously lightweight, strong, and damage-tolerant. These properties are thought to be achieved by the structure design of the component of those materials. Learning design strategies from these natural ceramic cellular solids is significant for developing lightweight bio-inspired ceramic materials with improved mechanical performance.

In this thesis, I investigated mechanical design strategies from natural ceramic cellular solids in three model systems, i.e., cuttlebone from cuttlefish *Sepia officinalis*, spines from sea urchin *Heterocentrotus mammillatus*, ossicles from starfish *Protoreaster nodosus*. These three natural ceramic porous solids have high mineral content in the constituent materials (> 90 wt%) and have a highly porous structure (porosity: 50 vol%-93 vol%). These three model systems are selected to represent the analogs of three typical structure forms of synthetic cellular solids, i.e., honeycomb-like structures, stochastic and periodic open-cell structures, respectively. This thesis aims to establish a quantitative relationship between the 3D multiscale structure and deformation/toughening behavior for these selected natural ceramic cellular solids via a combination of different experimental and computational approaches.
To my grandparents
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Chapter 1 Introduction

Cellular solids, or foams, are an important class of structural materials for packaging, transportation, and infrastructure due to their mechanical efficiency. Current foams are primarily made of metallic or polymeric materials, while ceramics could potentially serve this goal better, as they have higher specific stiffness and strength, and are chemically more stable. What limits the application of ceramic cellular solids as structural components are their brittleness and flaw sensitivity. Overcoming the fragile nature of ceramic foams, making them lighter while reaching higher stiffness, strength, and energy absorption is challenging but critical toward many applications.

Nature has evolved ceramic cellular materials such as sea sponge\(^1\), sea urchin spine\(^2\), and diatom shells\(^3\) that are simultaneously lightweight, strong, and damage-tolerant. These properties are thought to be achieved by the structure design of the component of those materials. Learning design strategies from these natural ceramic cellular solids is significant for developing lightweight bio-inspired ceramic materials with improved mechanical performance.

In this thesis, I investigated mechanical design strategies from natural ceramic cellular solids in three model systems, i.e., cuttlefish \textit{Sepia officinalis}, sea urchin \textit{Heterocentrotus mammillatus}, starfish \textit{Protoreaster nodosus}. These three model systems are selected to represent the analogs of three typical structure forms of synthetic cellular solids, i.e., honeycomb-like structures, stochastic and periodic open-cell structures, respectively. This thesis aims to establish a quantitative relationship between the 3D multiscale structure and deformation/toughening behavior for these
selected natural ceramic cellular solids via a combination of different experimental and computational approaches.

Chapter 2 provides relevant background information for this thesis. Firstly, current knowledge on the structure and mechanical properties of cellular solids, particularly micromechanics-based theoretical modeling based on Ashby and Gibson’s work, are briefly summarized. Secondly, the typical manufacturing processes and mechanical performance of engineering ceramic cellular solids are presented. This is followed by a brief summary of recently developed architected ceramic cellular solids. Thirdly, the structure and mechanical properties of natural ceramic cellular solids are reviewed, followed by an introduction of three model systems used in this thesis. Fourthly, I provide a brief review of in-situ mechanical tests and micro-tomography ($\mu$-CT) method used in biomaterials research, as these two approaches were used extensively in this thesis. Finally, I discuss the motivation of this research in the context of the development of novel structural materials, particularly cellular solids. The comparison between the bioinspired approach and the standard engineering approach in this regard is discussed.

Chapter 3 investigates the mechanical designs of cuttlebone, which is the internal biomineralized shell of cuttlefish. The cuttlebone functions as structural support and buoyancy tank in the cuttlefish\(^4\) and is an ultra-lightweight honeycomb-like cellular structure (porosity, ca. 93 vol%). In this chapter, the 3D structure of the cuttlebone from the cuttlefish \textit{S. officinalis} is firstly characterized through high-resolution $\mu$-CT. By further incorporating the high-resolution 3D tomography with mechanical testing, the detailed damage initiation and propagation process of cuttlebone is visualized and quantified. Finally, the 3D structure quantification parametrically establishes biomimetic models to investigate the mechanical performance and design trade-offs across different length scales. The results show that the vertical walls in the cuttlebone structure
have evolved an optimal waviness gradient that enables compression-dominant deformation and leads to asymmetric wall fracture, which contributes to the high stiffness and high energy absorption.

Chapter 4 presents a method to analyze the structure of the second model system, the random open-cell foam structure in echinoderms’ stereom structure, such as sea urchin spines. This method quantitatively describes and analyzes the 3D structure of the highly porous structure based on high-resolution 3D tomography data of the sea urchin *H. mammillatus* as a model system. The framework includes five steps: synchrotron-based tomography and hierarchical convolutional network-based reconstruction, machine learning-based segmentation, cellular network registration, feature extraction, and data representation and analysis. This method characterizes the stochastic open-cell stereom structure at multiscale levels: individual node and branch level (~10 µm), the local cellular level (~100 µm), and the global network level (~1 mm). It can be tailored to analyze other natural or engineering open-cell materials for a comprehensive characterization of the 3D structure. Furthermore, this method has been used to analyze large volume structure variations, and the relationship between the structure characteristics and the mechanical properties, e.g., stiffness, have been revealed.

Chapter 5 moves on to the deformation mechanism of the stereom in the spines of *H. mammillatus*. Firstly, in addition to the cellular network analysis enabled by the method developed in Chapter 4, the pores and surface curvature of the 3D structure are further characterized and directly compared with synthetic foams, and a 3D printed octet truss. The geometric measurements are further coupled with finite element analysis to reveal the structure-stress concentration relationship. The detailed failure process of the stereom is visualized and quantified for the first time using the *in-situ* mechanical tests. Our results reveal that the stereom’s microstructure reduces
the stress concentration on the branch and node surface and the thickness distribution in space that
could jam the fragments and localize the damage after fracture, thereby achieving high strength
and energy absorption simultaneously.

Chapter 6 explores the structural and mechanical characteristics of the third system, ossicles
of the starfish *P. nodosus*, exhibits a unique single-crystalline design at both atomic and lattice
levels that have not been discovered in nature or realized synthetically before. Each millimeter-
sized porous ossicle possesses a diamond triply periodic minimal surface (diamond-TPMS)
structure with a typical lattice constant of ca. 30 µm. Remarkably, the [111] direction of the
diamond-TPMS lattice is co-aligned with the *c*-axis of the underlying single-crystalline calcite at
the atomic scale, exhibiting the dual-scale single-crystalline nature. This crystallographic co-
alignment mitigates the compliance of calcite along the *c*-axis by utilizing the stiff [111] direction
of the diamond-TPMS lattice. 3D *in-situ* mechanical characterizations further reveal that the
presence of crystal defects such as 60° and screw dislocations at the lattice level, which follow the
dislocation types observed in atomic diamond crystals, suppresses slip-like fracture along the
{111} planes of the diamond-TPMS lattice upon loading and hence enhances the energy absorption
capability.
Chapter 2 Background

This chapter introduces the background information of the thesis. The strength and modulus of ceramic cellular solids can be significantly improved by incorporating stretching-dominated architecture and smaller feature sizes. However, ceramic cellular solids often exhibit catastrophic failure modes upon loading, corresponding to low energy absorption. As a comparison, natural ceramic cellular solids achieve light weight, high strength, high stiffness, and high energy absorption simultaneously. Understanding the mechanical design principles of natural ceramic cellular solids is significant for developing bioinspired lightweight engineering ceramic cellular solids with enhanced mechanical performance. An in-depth study of the mechanisms of damage initiation and propagation on natural ceramic cellular solids is necessary to extract the mechanical design principles. However, the ceramic cellular solids, especially natural cellular solids, exhibit complicated mechanisms with 3D microscale deformation mechanisms\textsuperscript{5,6}. These mechanisms are difficult to characterize by conventional optical and scanning electron microscope methods\textsuperscript{7}. By incorporating synchrotron-based micro-tomography (\(\mu\)-CT) with \textit{in-situ} mechanical testing, the failure process of natural ceramic cellular solids can be visualized and quantified. In this thesis, I seek to establish the structure-mechanical property relationship of natural ceramic cellular solids utilizing complementary three-dimensional (3D) and four-dimensional (4D) mechanical analysis combined with parametric mechanical modeling.

Section 2.1 summarizes the general structure types and the current knowledge on the mechanical performance of cellular solids, particularly micromechanics-based theoretical modeling. In Section 2.2, the mechanical performance of the engineering ceramic cellular solids, including synthetic and additively manufactured ceramic cellular solids upon loading, is
demonstrated. Section 2.3 introduces the structure and mechanical properties of general natural ceramic cellular solids. In Section 2.4, the previous works that combine in-situ mechanical tests and μ-CT are briefly reviewed. In Section 2.5, the motivation of this thesis is summarized. In Section 2.6, the model systems used in this thesis are introduced. Detailed reviews of work related to each model system are given in each chapter, respectively.

2.1 Cellular solids

2.1.1 Structure types

Cellular solids are assemblies of cells with solid edges or faces packed together to fill space. The relative density $\frac{\rho}{\rho_s}$ is used to describe this important feature. Here, $\rho$ is the density of cellular materials, $\rho_s$ is the density of the solid materials. The mechanical properties of the engineering cellular solids can be tailored by their architecture.

Two-dimensional honeycomb structures and three-dimensional foam structures are the most typical structures of cellular solids. Honeycomb structures are two-dimensional cellular designs extruded in the 3rd dimension (Figure 2-1a). The two-dimensional cellular design can be arranged as triangular, square, hexagonal, or related shapes. The hexagonal cells of the beehives are examples of honeycomb structures. The foam structures are three-dimensional assemblies of polyhedral cells and could be differentiated by the cells. If the solid of the foam is contained in the cell edges only, the foam is an edge-based foam, also called open-cell foam (Figure 2-1b). If the solid of the foam is contained both in the faces, the foam is a face-based foam, also called closed-cell foam (Figure 2-1c). For foam materials, it is common that some foams are partly open and partly closed. If the solid of the foam is composed of a single, continuous, smooth-curved shell, the foam is a shell-based foam (Figure 2-1d).
In nature, the architecture of foams can be either as stochastic as cancellous bone and periodic microstructure of wing scales of beetles\textsuperscript{11} and butterflies\textsuperscript{12}. Numerous techniques have been developed for synthesizing stochastic closed-cell\textsuperscript{13,14} and open-cell foams\textsuperscript{15,16}. Recently, the development of additive manufacturing allows the fabrication of materials with sophisticated cellular architecture\textsuperscript{17}, such as the micro-lattice with graded density\textsuperscript{18} and multiscale lattice\textsuperscript{19}. Except for these structures, there are some multifunctional metamaterials with a controlled shape, such as the gold helix photonic metamaterial\textsuperscript{20} and three-dimensional labyrinthine acoustic metamaterial\textsuperscript{21}. Another example is the triply periodic minimal surface (TPMS)\textsuperscript{22}, such as Schwarz’s D-surfaces. TPMS structures include the shell-based structure and strut-based structure. Some cellular structures, such as the gyroid-based honeycomb structure, are combinations of these honeycomb structure and gyroid structures\textsuperscript{23}.
2.1.2 Mechanical performance of cellular solids

Scaling law

In the context of cellular solids design, the most universal parameter is the relative density \( \rho^*/\rho_s \). Many mechanical properties of cellular solids (e.g., stiffness, strength) are directly related to the relative density with a scaling law dependence. According to the analytical models for cellular structure using beams model, Young’s modulus and compressive strength follow the scaling laws:

\[
\frac{E}{E_s} = A (\rho^*/\rho_s)^\alpha \\
\sigma_y = B \sigma_{ys} \tilde{\rho}^\beta
\]

Equation 2-1

Equation 2-2

For honeycomb structure under compression (Figure 2-2a), it is stiffer and stronger when loaded in an out-of-plane direction.

\[
\frac{E^*}{E_s} \sim \rho^*/\rho_s \\
\sigma_{cr}^*/\sigma_{fs} \sim \rho^*/\rho_s
\]

Equation 2-3

Equation 2-4

\( E^* \) and \( \sigma_{cr}^* \) are Young’s modulus and compressive strength of cellular solids, respectively. \( E_s \) is the modulus of the solid materials. \( \sigma_{fs} \) is the failure strength of the solids in tension.

For foam structure, Young’s modulus for open-cell foam (Figure 2-2b) and closed-cell foam (Figure 2-2c) are

\[
\frac{E^*}{E_s} \approx \left( \frac{\rho^*}{\rho_s} \right)^2 \quad (open \ cells)
\]

Equation 2-5
\[
\frac{E^*}{E_s} \approx \phi^2 \left( \frac{\rho^*}{\rho_s} \right)^2 + (1 - \phi) \frac{\rho^*}{\rho_s} + \frac{1/3 \rho_0}{E_s (1 - \rho^*/\rho_s)} \quad \text{(closed cells)} \]

Equation 2-6

Here, \( \phi \) is the fraction of solids in the cell faces.

For brittle foam structure, the compressive strength for open-cell foam and closed-cell foams are:

\[
\frac{\sigma_{cr}^*}{\sigma_{fs}} = 0.2 \left( \frac{\rho^*}{\rho_s} \right)^{3/2} \quad \text{(open cells)} \]

Equation 2-7

\[
\frac{\sigma_{cr}^*}{\sigma_{fs}} = 0.2 \left( \phi \frac{\rho^*}{\rho_s} \right)^{3/2} + (1 - \phi) \frac{\rho^*}{\rho_s} \quad \text{(closed cells)} \]

Equation 2-8

For closed-cell foams, as the fraction increases, the dependence of the strength on density reduces from a power of 3/2 to a linear one.

**Figure 2-2** | Schematic diagram of cellular solids under compression. a, Honeycomb structure, b, Open-cell foams, c, Closed-cell foams.

**Bending vs. stretching dominated behavior**

According to Gibson and Ashby model, the stiffness and strength of the open-cell foam are governed by the cell wall bending mechanism\(^8\). The stiffness scales as \((\rho^*/\rho_s)^2\) and the strength
scales as \((\rho^*/\rho_s)^{3/2}\). For a rigid open-cell foam with all the struts under compressive or tensile stress, the stiffness and the strength scales linearly about the relative density.

Open-cell foam can be treated as a connected set of pin-jointed struts. According to Maxwell’s criterion\(^\text{24}\), for the frame with no self-equilibrated tension on the strut, the criteria for a rigid frame in 2 and 3 dimensions are:

\[
\begin{align*}
    b &= 2j - 3 \quad \text{Equation 2-9} \\
    b &= 3j - 6 \quad \text{Equation 2-10}
\end{align*}
\]

Here, \(b\) is the number of struts in the frame, and \(j\) is the number of joints in the frame. For nonrigid structure, the struts rotate around the joint in the frame under compression for nonrigid structure (Figure 2-3a). For rigid structure, some struts are only under tensile stress while the others are under compressive stress (Figure 2-3b).

Consider a large pin-jointed framework with \(j\) joints and average connectivity (number of struts at a node) \(Z\). The total number of struts \(b\) in the framework is:

\[
b \approx jZ/2 \quad \text{Equation 2-11}
\]

Hence, the necessary (but not sufficient) criteria for a rigid frame is \(Z = 4\) and \(Z = 6\) in 2 and 3 dimensions, respectively\(^\text{25}\).

Moreover, for a special class of frameworks with nodes that are all similarly situated\(^\text{26}\), the necessary and sufficient condition for rigidity of 2D and 3D frameworks is that the connectivity \(Z\) = 6 and \(Z = 12\), respectively. If \(Z\) exceeds these values, the framework is very rigid. Octet truss structure is a typical rigid structure in 3D\(^\text{27}\) (Figure 2-3c).
Figure 2-3 | Topological criteria of bending and stretching dominated deformation. a, Structure with struts rotating about joints under loading. b, Structure with tensile stress on some and compressive on others. c, Octet truss foam.

Theoretical limit

Hashin-Shtrikman (HS)\(^2\) and Suquet upper bounds\(^3\) set the theoretical topological stiffness and strength for isotropic cellular solids. The effective Young’s modulus for Hashin-Shtrikman (HS) bound (\(\frac{E_{HSU}}{E_s}\)):

\[
\frac{E_{HSU}}{E_s} = \frac{2\bar{\rho}(5v - 7)}{13\bar{\rho} + 12v - 2\bar{\rho}v - 15\bar{\rho}v^2 + 15v^2 - 27}
\]

Equation 2-12

The yield strength of a material at theoretical limits, \(\sigma_{y,SU}\), is:

\[
\frac{\sigma_{y,SU}}{\sigma_{y,s}} = \frac{2\bar{\rho}}{\sqrt{4 + \frac{11}{3}(1 - \bar{\rho})}}
\]

Equation 2-13

Here \(E_s\), \(\sigma_{y,s}\) and \(v\) represent Young’s modulus, yield strength, and Poisson’s ratio of the solid material. \(\bar{\rho} = \rho/\rho_s\) is the relative density of the cellular solids.
Size effects

Regarding the design principle of size effects, the "smaller is the stronger" design strategy has been proposed, especially for ceramic cellular solids\(^3^0\). Most ceramic cellular solids fail by fracturing due to crack propagation. However, due to the existence of defects and imperfections, the strength of bulk materials is usually several orders of magnitude lower than the theoretical strength. For brittle materials, the strength for cleavage fracture is given by\(^3^1\)

\[
\sigma_f = Y \frac{K_{IC}}{\sqrt{\pi a_c}}
\]

Equation 2-14

\(a_c\) is the minimum characteristic size of defects (e.g., flaws, voids, and cracks). For 3D-micro-/nanolattices, the characteristic length \(a_c\) is the cross-sectional size of a solid beam or the wall thickness of a hollow tube. \(Y\) is a dimensionless parameter, and \(K_{IC}\) is the fracture toughness. As the size \(a_c\) decreases to the order of nanometers, the fracture strength increases to the theoretical limit\(^3^1\). As the characteristic size decreases, the strength of a single strut increases significantly, facilitating the strength enhancement in cellular solids.

2.2 Engineering ceramic cellular solids

2.2.1 Manufacturing processes

Traditional ceramic cellular solids

The traditional synthetic ceramic cellular solids can be manufactured by replica, sacrificial template, and direct foaming methods\(^3^2\). The composition of synthetic ceramic cellular solids can be \(\text{Al}_2\text{O}_3\,^{3^3-3^5}, \text{ZrO}_2\,^3^6, \text{SiC}\,^{3^7,3^8}, \text{Si}_3\text{N}_4\,^3^9, \text{SiO}_2\,^{4^0}, \text{TiO}_2\,^{4^1}\).
The replica method applies a ceramic slurry that is made by firstly drying the ceramic slurry on the polymer/carbon/nature material template and then burning the template and sintering the ceramic. This manufacturing process results in open-cell reticulated foams. The sacrificial templates method can either be used to fabricate the open-cell foam and closed-cell foam. Foaming a ceramic slurry by mechanical agitation on in-situ evolution of gases leads to predominantly closed-cell foam. The synthetic ceramic cellular solids with various relative densities, pore sizes, and part sizes can be manufactured via different manufacturing methods (Table 2-1). However, these fabrication processes bring structure defects, such as hollow struts and damaged surfaces, resulting in a low relative strength of the synthetic ceramic cellular solids.

Table 2-1 | Summary of the ceramic cellular solids manufactured by synthetic methods

<table>
<thead>
<tr>
<th>Synthetic method</th>
<th>Relative density</th>
<th>Pore size</th>
<th>Relative Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replica Polymer template</td>
<td>0.05 - 0.6</td>
<td>200 $\mu$m – 3 mm</td>
<td>0.002-0.05</td>
</tr>
<tr>
<td>Replica Wood template</td>
<td>0.05 – 0.75</td>
<td>10 $\mu$m – 300 $\mu$m</td>
<td>0.0005-0.03</td>
</tr>
<tr>
<td>Sacrificial template</td>
<td>0.1 - 0.8</td>
<td>1 $\mu$m – 700 $\mu$m</td>
<td>0.002-0.3</td>
</tr>
<tr>
<td>Direct foaming</td>
<td>0.03 – 0.6</td>
<td>35 $\mu$m – 1.2 mm</td>
<td>0.0002-0.25</td>
</tr>
</tbody>
</table>

Additively manufactured (architected) ceramic cellular solids

The recent developments in additive manufacturing techniques have prompted the emergence of architected ceramic cellular solids with complex topology. The additively manufacturing techniques can be categorized as slurry-based, powder-based, and solid-based methods depending on their feedstock systems. For instance, the Slurry-based fabrication process involves liquid or semi-liquid systems dispersed with fine ceramic particles as feedstock. The compositions of ceramic particles in the architected ceramic cellular solids can be SiO$_2$, Al$_2$O$_3$, ZrO$_2$, and SiC, etc. Table 2-2 summarizes different additive manufacturing methods, the
corresponding printed part size, resolution, and surface quality of the printed samples. In parallel, the characteristic size of the architected ceramic cellular solids ranging from several micrometers to hundreds of nanometers.

### Table 2-2 Summary of the ceramic cellular solids manufactured by additively manufactured methods. Adapted from

<table>
<thead>
<tr>
<th>Feedstock form</th>
<th>3D printing techniques</th>
<th>Abbreviation</th>
<th>Part size</th>
<th>Resolution</th>
<th>Surface quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slurry-based</td>
<td>Stereolitography</td>
<td>SL</td>
<td>100 μm – 100 cm</td>
<td>μm</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Digital light processing</td>
<td>DLP</td>
<td>100 μm – 100 cm</td>
<td>μm</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Two-photon polymerization</td>
<td>TPP</td>
<td>1 μm – 1 mm</td>
<td>nm - μm</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Inkjet printing</td>
<td>IJP</td>
<td>100 μm – 10 mm</td>
<td>mm</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>Direct ink writing</td>
<td>DIW</td>
<td>100 μm – 10 cm</td>
<td>μm-mm</td>
<td>Low</td>
</tr>
<tr>
<td>Powder-based</td>
<td>Three-dimensional printing</td>
<td>3DP</td>
<td>10 mm – 10 cm</td>
<td>μm-mm</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>Selective laser sintering</td>
<td>SLS</td>
<td>10 mm – 10 cm</td>
<td>μm-mm</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>Selective laser melting</td>
<td>SLM</td>
<td>10 mm – 10 cm</td>
<td>μm-mm</td>
<td>Low</td>
</tr>
<tr>
<td>Bulk solid-based</td>
<td>Laminated object manufacturing</td>
<td>LOM</td>
<td>100 mm – 10 cm</td>
<td>μm-mm</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>Fused deposition modeling</td>
<td>FDM</td>
<td>100 mm – 10 cm</td>
<td>mm</td>
<td>Medium</td>
</tr>
</tbody>
</table>

Compared with synthetic ceramic cellular solids, the architected ceramic cellular solids exhibit a combination of remarkable mechanical properties, such as low density, high strength, and high stiffness. The current knowledge of design strategies in the additively manufactured ceramic cellular solids, such as topology design and size strengthening, are summarized (Figure 2-4).

The topology design strategy is commonly found in 3D truss lattices, hierarchical structure, structure with the local disorder, shellular structure, etc. For instance, an optimized isotropic
isotruss structure that comprises two kinds of struts with a constant diameter ratio achieves maximum stiffness\(^{48,49}\). Recent work combining finite-element models, analytical methods, and a heuristic optimization scheme demonstrates that the plate lattices (cubic + octet foam) attain the Hashin–Shtrikman upper bounds on isotropic elastic stiffness\(^{50}\). The structure hierarchy and local structure disorder are often adopted from biological materials. For instance, theoretical and computational analyses of the mechanical properties of hierarchical structures indicate that for a given compressive strength, architectures with hierarchical structures are much lighter than those with one-level structures\(^ {51}\). Another category of architecture is shell lattices, which are free of discontinuities, thus promoting a reduced stress concentration upon loading\(^{48,52-54}\). Most studies on shellular structure focused on the triply periodic minimal surface (TPMS)\(^ {55}\). A TPMS is a surface with constant mean curvature and is a locally minimized area for a given boundary. Examples are Schwarz P, Schwarz D, and gyroid surfaces. Finite element modeling reveals that most of these shell lattices possess higher stiffness, strength, and energy absorption capabilities than the truss lattices\(^ {48}\). However, the ceramic shell-based architecture has not been fabricated before. By mimicking the microscale structure of crystalline materials—such as grain boundaries, precipitates and phases, the design of materials with desired properties such as high damage tolerance are enabled despite their brittle constituent material\(^ {56}\).

For the size strengthening design, the characteristic feature is controlled within the range of critical size for flaw sensitivity. Recently, this design strategy is enabled by pyrolyzing 3D polymer templates printed with stereolithography or two-photon lithography. Another hollow alumina nanolattice with 10-nm-thick walls composed of two different densities (top: 0.87%; bottom: 0.43%) shows high damage tolerance\(^ {57}\). A SiOC honeycomb with a wall thickness of 500 nm outperforms commercial SiOC/SiC foams with similar density by ten times in terms of the
compressive strength\textsuperscript{58}. Ultra-strong glassy carbon nanolattices fabricated by pyrolysis of polymeric microlattices, with single struts shorter than 1 µm and diameters as small as 200nm, exhibit material strengths of up to 3 GPa\textsuperscript{59}. Pyrolytic carbon nanolattices derived from IP-Dip photoresist templates have beam diameters from 261 nm to 679 nm and achieve a maximum compressive strength close to the theoretical strength of pyrolytic carbon at a density of (1.0 g/cm\textsuperscript{3})\textsuperscript{60}. The nanostructure as silica inverse opals with layer thickness ~28 nm achieves better stiffness and compressive strength-to-weight ratios compared with truss-based structures\textsuperscript{61}. By combining the benefits of topology optimization and size-dependent strengthening effects, polymeric microlattices coated with ceramic nanofilms exhibit high strength close to the theoretical strength because the high-stiffness ceramic coating constrains beam bending\textsuperscript{62}. Even though the damage tolerance of some additively manufactured lattice material has been greatly enhanced, most of these structures require the incorporation of nanoscale characteristic features. The catastrophic failure of the engineering ceramic cellular solids is still one of the grand challenges.
2.2.2 Damage tolerance: challenges of current synthetic ceramic cellular solids

Although the stiffness and strength of the ceramic cellular solids have been greatly enhanced due to the development of additive manufacturing. Nevertheless, due to the inherent brittleness of ceramic, one of the largest challenges in the synthetic and additively manufactured ceramic cellular solids is the catastrophic failure. The catastrophic failure in the ceramic cellular solids is discussed in detail in this part through different examples.

For the synthetic ceramic cellular solids, the failure of the synthetic ceramic cellular solids originates from preexisting cracks and defects in the structure. The further accumulation and eventual linking together of these small cracks result in catastrophic macroscopic failure. A
A typical example of the catastrophic failure in a synthetic ceramic foam is shown in Figure 2-5. Figure 2-5 shows the deformation behavior of alumina mullite (AM) foam under compression. This synthetic ceramic foam is fabricated through replication method. The failure of the AM foam starts with cracks initiating at multiple weak points and collapse through a damage accumulation process. In this case, no band perpendicular to the loading direction is formed. Another example is the Alumina foam manufactured through replication method under compression. The stress rapidly decreases after the peak stress (Figure 2-5a). The corresponding deformation behavior shows that the deformation of foams starts with a partial fracture at weaker points and continues with increasing breakage of structure with the increasing strain (Figure 2-5b). Finally, the specimen fails macroscopically at strain ~0.25. The weaker points are the high degree of cracking (Figure 2-5c) on the strut resulted from the manufacturing process. Moreover, these preexisting defects in the synthetic ceramic cellular solids degrade the strength predicted by Gibson and Ashby model.
**Figure 2-5** The deformation behavior of alumina foam under compression. *a,b,* The stress-strain curve (*a*) and the corresponding failure sequence (*b*) of the Alumina foam under compression. *c,* The scanning electron micrograph of the Alumina foam in (*b*), the arrows show cracks on the strut. Reproduced from\(^{64}\).

Architected materials are usually composed of identical ‘unit cells’ arranged and have the same orientation. The difference between the periodic open-cell cellular structure and the stochastic open-cell cellular structure is that high stress concentrates at joints of the periodic lattice\(^ {65,66}\) (*Figure 2-6a*). The brittle periodic lattice materials exhibit brittle crushing and hence catastrophic failure under compression (*Figure 2-6b-c*). Moreover, as the relative density of the architected material increases, the deformation mechanism transfers from strut deformation-dominated behavior to node deformation-dominated behavior. In low relative density, the struts in the lattice fail by buckling. If any one of the struts along the highly stressed (110) plane was fractured and lost, the load would propagate to the struts along the (110) plane, and the corresponding struts fail progressively. In high relative density, the strut is much thicker, and the critical buckling stress required to fracture the struts would be much higher than the maximum tensile stress on the struts—the struts in the structure fracture simultaneously.
Figure 2-6 | Catastrophic failure of the alumina Octet Truss lattice with different relative densities under compression. The stress-strain curve and the still frames from the high-speed video capture of the compression testing of alumina Octet Truss lattice with $\rho/\rho_s = 0.15$ (a, b) and $\rho/\rho_s = 0.15$ (c, d).

2.3 Natural ceramic cellular solids

With the intrinsically brittle biomineral as composition, natural ceramic cellular solids achieve a combination of superior mechanical properties, such as high stiffness, high strength, and damage tolerance. The design strategies extracted from natural ceramic cellular solids could be applied to the bioinspired design of ceramic cellular solids with significantly improved mechanical performance. A comparison of mechanical properties between the natural cellular solids and engineering solids is presented in Section 2.3.1. The constituent biominerals are briefly reviewed in Section 2.3.2. The structure and mechanical performance of natural ceramic cellular solids are illustrated with several examples in Section 2.3.3.

2.3.1 Natural cellular solids
Cellular materials are widespread in nature\textsuperscript{67}. The microstructure of natural cellular solids is diverse. Examples are two-dimensional honeycomb-like structures such as wood\textsuperscript{68} and cuttlebone\textsuperscript{4}, three-dimensional open-cell foam structures including trabecular bone\textsuperscript{69} and sponges\textsuperscript{70}, and three-dimensional closed-cell foam porcupine quills\textsuperscript{71}.

Natural cellular solids often exhibit multiple functions such as mechanical, biological, optical functions, etc. Their structure is optimized for their environments and constraints. Figure 2-7 separates the mechanical properties from other properties for natural cellular solids and compares them with the engineering cellular solids\textsuperscript{72,73}. The strength and modulus are normalized by density to eliminate the effect of size and weight. The natural cellular solids show similar mechanical performance and even outperform many engineering cellular solids. However, quite unlike most engineering cellular solids, the basic building blocks for natural cellular solids are proteins, polysaccharides, and minerals\textsuperscript{67}, of which properties are often meager. Natural cellular solids are formed at ambient temperatures with little energy requirements and are typically arranged in a hierarchical architecture structure. Among all the natural cellular solids, natural ceramic cellular solids exhibit higher specific strength and specific stiffness. Moreover, natural ceramic cellular solids are simultaneously lightweight and tough\textsuperscript{67}. They can self-heal and self-repair themselves when damaged\textsuperscript{74,75}. 
2.3.2 Biomineralization

In contrast to engineering ceramic cellular solids, natural ceramic cellular solids, similar to other biomineralized tissues, are assembled from the bottom-up. Organisms can combine different biomineralization processes, e.g., space delineation, formation of preformed organic matrices, mineral nucleation, modulation, and cessation of mineral formation, at the molecular level to produce the final mineralized products with unique complex structure\textsuperscript{76}. The complex mineralized structure with hierarchies can be formed by combining controlled mineral growth and particle self-assembly following external stimuli\textsuperscript{77,78}. 

Figure 2-7| Ashby plot showing the comparison of specific modulus (E/\rho) and specific strength (\sigma_f/\rho) between natural materials (red) and engineering materials (blue). Adapted from\textsuperscript{72,73}. 

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More than 60 different minerals are known to be formed biologically, but a small number of them are common components of endo- and exo-skeletons\textsuperscript{79,80}. The main groups include two polymorphs of calcium carbonate, calcite and aragonite, and silica. Among them, biogenic calcite and aragonite are the most abundant forms in nature\textsuperscript{81}.

The skeletal parts of the echinoderm, such as sea urchin spines and tests, are usually composed of large single-crystal calcite\textsuperscript{82,83}. Some of the mollusk shells are made up of aragonite\textsuperscript{84}. The amorphous silica is isotropic and stable. It is used by a wide range of organisms, from the complex sculpted diatoms to siliceous sponge spicules and plant phytoliths\textsuperscript{85}. The crystal structures of aragonite and calcite are very similar\textsuperscript{86}. Aragonite grows along the $c$-axis and forms as thin needles, while calcite grows as almost isotropic rhombohedra delimited by a set of oblique \{104\} faces\textsuperscript{81}. The stability of \{104\} faces in calcite cause the easy cleavage along \{104\} plane in calcite crystals, where the crack can propagate with minimum energy dispersion\textsuperscript{87}. However, for the skeletal parts of echinoderms, such as the sea urchin spine, glycoproteins are contained inside the spine in amounts of ca. 0.02\% by mass of mineral\textsuperscript{88}, which results in a set of unstable surfaces slightly inclined to the c axis\textsuperscript{89}. They cleave with a conchoidal-type fracture.

2.3.3 Structure and mechanical performance of natural ceramic cellular solids

Numerous microscopic, mineralized structures (natural ceramic cellular solids) can be widely found in invertebrates. Examples are commonly found in the Porifera, Cnidaria, Platyhelminthes, Mollusca, Brachiopoda, Echinodermata, and Asciidae\textsuperscript{90}. The microstructures of natural ceramic cellular solids are significantly different. Among them, natural ceramic cellular solids with three types of structures, including the two-dimensional honeycomb-like structure, stochastic open-cell foam structure, and periodic open-cell foam structure are considered here. Table 2-3 summarizes
the structure type, mineral content, composition, relative density, part size, and characteristic length of natural ceramic cellular solids with these three structures.

The biomineralized cellular solids with the honeycomb-like structure are not common in nature. One example is the columnar, plate-like structures in the cancellous bone. It follows a linear density dependence of longitudinal stiffness as predicted by the theoretical model. Other examples include the chambered structure in cuttlebone. As opposed to the catastrophic failure in the engineering ceramic cellular solids, the chambered structure in cuttlebone exhibits a graceful failure upon compression, accomplishing a high energy dissipation. Moreover, the frustule of diatom *Coscinodiscus sp.* possesses micro-honeycomb structure that achieves the highest specific strength of any known biological materials. Among these honeycomb-like structures, the mineral content in the chambered structure of cuttlebone is the highest ~90 wt%, followed by the columnar structure in the cancellous bone and the frustule in the diatom *Coscinodiscus sp.*

In nature, most species in the echinoderm, diatom, sponge, cancellous bone have open-cell foam structures. For instance, the skeleton of echinoderm, called stereom, is composed of a three-dimensional mesh of trabeculae. The structure design in echinoderm is various among different species ranging from the fully stochastic structure to the rather periodic structure. Even though it’s claimed that the echinoderm stereom with rectilinear mesh type is possibly the periodic structure, the inner periodicity has not been confirmed. The natural ceramic cellular solids with periodic open-cell structures can be rarely found in nature. Echinoderm stereom is single-crystal biogenic calcite with the mineral content as high 99 wt%. The crushing strength of echinoid stereom, spine as an example, have a very high strength to weight ratios and high resistance to crack propagation. The second example of the natural ceramic cellular solids with stochastic open-cell structure is cancellous bone. There are different types of structure for cancellous bone
such as the stochastic open-cell structure and the honeycomb-like structure. The stress-strain curve of cancellous bone upon compression is composed an elastic regime, a plateau regime, and a final densification region.

**Table 2-3** | Summary of the natural ceramic cellular solids

<table>
<thead>
<tr>
<th>Biomineral</th>
<th>Structure type</th>
<th>Composition</th>
<th>Mineral content</th>
<th>Relative density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatom frustule</td>
<td>Honeycomb-like(^{92})</td>
<td>Biosilica</td>
<td>37-67 wt%</td>
<td>0.24-0.26</td>
</tr>
<tr>
<td></td>
<td>Stochastic open-cell(^{94})</td>
<td></td>
<td></td>
<td>0.07-0.37</td>
</tr>
<tr>
<td>Echinoderm stereom</td>
<td>Stochastic open-cell(^{93})</td>
<td>Biogenic</td>
<td>99 wt%(^{103})</td>
<td>0.1-1</td>
</tr>
<tr>
<td></td>
<td>Periodic open-cell(^{95})</td>
<td>Calcite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sponge</td>
<td>Stochastic open-cell(^{95})</td>
<td>Biosilica</td>
<td>99 wt%</td>
<td></td>
</tr>
<tr>
<td>Cancellous bone</td>
<td>Stochastic open-cell(^{96})</td>
<td>Apatite</td>
<td>65 wt%</td>
<td>0.13-0.70</td>
</tr>
<tr>
<td></td>
<td>Honeycomb-like(^{91})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dentin tubules</td>
<td>Honeycomb-like(^{104})</td>
<td>Apatite</td>
<td>70 wt%</td>
<td></td>
</tr>
<tr>
<td>Cuttlebone</td>
<td>Honeycomb-like(^{4})</td>
<td>Aragonite</td>
<td>90 wt%</td>
<td>0.1</td>
</tr>
</tbody>
</table>

2.4 X-ray tomography-based *in-situ* mechanical characterizations on biological materials

The ceramic cellular solids, primarily natural cellular solids, display complex failure modes with multiple interacting 3D microscale damage mechanisms. For instance, bone is a hierarchical structure of five length scales, where each hierarchical structural level contributes to its strength, ductility, and toughness. These 3D microscale damage mechanisms are difficult to understand using conventional 2D inspection methods such as optical microscope and scanning electron microscope. In the meantime, X-ray tomography allows three-dimensional and non-destructive visualization of both internal and external structures. By combining 3D X-ray tomography with *in-situ* mechanical tests, the microstructural changes of the volume could be visualized, tracked, and quantified as a function of time (4D X-ray tomography). In-*situ* X-
ray microtomography has been extensively used to quantify the deformation process of natural ceramic cellular solids in this thesis.

The general experiment set-up for *in-situ* X-ray tomography is shown in Figure 2-8a,b. The current *in-situ* X-ray tomography is an “interrupted in-situ testing,” which comprises more than ten scans per sample, and the load was held during each scan. *In-situ* mechanical testing, including uniaxial compression\textsuperscript{109}, tension\textsuperscript{110–112}, cyclic loading\textsuperscript{113–116}, corrosion-fatigue\textsuperscript{117}, and creep\textsuperscript{118,119} have been conducted. The *in-situ* mechanical testing can be performed in hostile conditions, including the high-temperature\textsuperscript{120}, corrosion, and moisture\textsuperscript{121} with the developments of *in-situ* devices.

![Figure 2-8](image)

*Figure 2-8*| Schematic showing the experimental set-up of the *in-situ* test (a) and the *in-situ* device (b).

This method can be applied to a wide range of materials, including trabecular bone\textsuperscript{122}, wood\textsuperscript{123}, gypsum\textsuperscript{124}, graphite\textsuperscript{125}, ceramic matrix composites\textsuperscript{126–128}. The crack behavior, including crack initiation, crack path, crack bridging, and crack growth, could be visualized and
quantified\textsuperscript{129}. For instance, Figure 2-9\textit{a} shows the crack morphologies of dentin upon indentation observed by 4D X-ray nano-computed tomography with a resolution of 150 nm/pixel\textsuperscript{130}. During the indentation tests, a nano-indenter with a conical tip has been used to incrementally indent three test pieces oriented at different angles (0°, 45°, 70°) to the long axis of the tubules (blue: 3D rendering of the tubules). The morphologies of cracks (yellow: crack intercepting the long tubule axis, cyan: crack along the long axis of the tubules) are different in three cases (Figure 2-9\textit{b}). Unlike the significant crack formation in the 0° sample, the anisotropic crack propagation was observed in the 45° test piece. Moreover, cracks do not propagate significantly in the 70° test-piece, which enables the test piece to bear the greatest loads.

\textbf{Figure 2-9} Crack morphologies in dentin upon indentation tests. \textit{a}, X-ray phase-contrast projections (radiographs) for the 0°, 45°, and 70° oriented test pieces. \textit{b}, 3D visualization of the crack morphology for the cases where the indentation direction is at 0°, 45°, and 70° to the tubule direction. The yellow and cyan color indicate the crack intercepting (transverse) and (longitudinal) the tubules' long axis. Blue: 3D rendering of the tubules. Adapted from\textsuperscript{130}. 
Despite the 3D crack characterization, the strain field can be quantified through \textit{in-situ} X-ray tomography combined with digital volume correlation (DVC)\textsuperscript{131}. The measured DVC results are further desirable for validation of finite element (FE) models\textsuperscript{132,133,134,135,136}. For instance, axial displacement, strain fields, and microdamage on the bone-cement interface due to monotonic and cyclic compression were assessed using DVC and show a higher load transferred to the cement when the cortical bone is mainly to interdigitate with cement, which is consistent with FE predictions\textsuperscript{137}.

\textbf{2.5 Motivation of this dissertation research}

In the past decades, the mechanical properties of ceramic cellular solids, including the strength and modulus, have been significantly improved as the additive manufacturing process allows complex topological design and smaller feature size\textsuperscript{42}. However, due to the brittle and flaw sensitivity of the ceramic material, both the synthetic and additively manufactured ceramic cellular solids suffer from catastrophic failure under compression. For synthetic stochastic ceramic cellular solids, cracks originate from structural defects. Later crack linking of small cracks in the structure result in structural collapse. For additively manufactured periodic ceramic cellular solids, brittle crushing happens when the high stress concentrates on the joints.

Correspondingly, ceramic cellular solids with different topologies such as the honeycomb, stochastic and periodic open-cell structure could be found in nature. Despite their mineral contents as high as 99 wt\% in the constituent materials, natural ceramic cellular solids, including stereom of echinoderm, sponge, diatom, \textit{etc.}, achieve high strength, high stiffness, and high energy dissipation simultaneously. By incorporating the recently developed \textit{in-situ} X-ray tomography with 3D structure characterization and finite element modeling, this thesis seeks to establish the relationship between structure and mechanical properties using three natural ceramic cellular
solids as model systems. These model systems possess honeycomb-like, stochastic open-cell and periodic open-cell cellular structures, respectively.

This thesis aims to extract the design strategies from natural ceramic cellular solids and develop bioinspired ceramic cellular solids with remarkable performance. In this regard, “mechanical design” in the title of this dissertation represents the mechanical design strategies. The main contribution of this bio-inspired approach is that it can potentially help us reduce the vast design space by offering a good starting point, such as a particular structural morphology or some long-range gradient design. For the engineering approach in designing structural materials, including porous or lattice materials, the typical process is still in general trial-and-error based: select a base structure, systematically vary one or more design parameters (such as unit cell size, strut thickness, etc.), conduct corresponding computational or theoretical or experimental evaluations of the mechanical properties, and obtain the optimized design by considering other constraints, such as manufacturing constraints. Although this process can now be significantly expedited due to the advances in rapid prototyping and computing algorithms such as parametric and generative modeling, the structural design space is huge, especially when considering both local (strut thickness, length, profile, node connectivity, etc.) and global (gradients, alignment, order/disorder, etc.) structure. Moreover, as mechanical performance is always multi-faceted: while stiffness is usually easy to model, it is hard to quantify large deformation, fracture, and energy absorption computationally. This becomes a particular issue for ceramic cellular solids, where the energy absorption is primarily originated from the fracture process. The investigation of the mechanical design of natural ceramic cellular solids provides us a new perspective in addressing this challenge. For example, to the best of our knowledge, we haven’t seen that ceramic-based honeycomb structures are used for structural applications, which is primarily due to their
low damage tolerance. The multi-chambered structure with gradient curved walls as revealed in cuttlebone in this thesis offers new structural design insights to address this issue. Similar structures have not been reported in the engineering structural design literature. Other examples include the gradient design strategy observed in the sea urchin spines and the dual-scale lattice structure in starfish ossicles.

2.6 Model systems

In this thesis, the mechanical design strategies are extracted from three model systems. The systems include the cuttlefish *Sepia officinalis* (honeycomb-like structure), the sea urchin *Heterocentrotus mammillatus* (stochastic open-cell structure), and the starfish *Protoreaster nodosus* (periodic open-cell structure), of which exhibits a combination of high stiffness and high energy absorption (hypothesized), a combination of high strength and high energy absorption (hypothesized), a combination of high stiffness and high energy absorption (hypothesized), respectively (Figure 2-10). The three model systems' basic information, including the taxonomy, structure type, mineral content, relative density, etc., is briefly summarized in Table 2-4.
Figure 2-10: Biomineralized structure in natural ceramic cellular solids. a-c, SEM images of honeycomb-like structure in the cuttlebone of *S. officinalis* (a), stochastic open-cell structure in the spine of *H. mammillatus* (b), and periodic open-cell structure in the ossicles of *P. nodosus* (c), respectively.

**Honeycomb-like structure: *Sepia officinalis***

The cuttlebone from *Sepia officinalis* is a chambered structure composed of horizontal septa and vertical walls (Figure 2-10a). *S. officinalis* lives on sandy and muddy bottoms from the coastline (2-3 m depth) to approximately 200 m depth at the Mediterranean Sea, Eastern Atlantic from Southern Norway and Northern England to the northwestern coast of Africa\(^{138}\). The cuttlebone functions as the buoyancy tank, displaying a superior combination of high porosity (93%), high permeability\(^{139,140}\). Moreover, although the mineral content in the cuttlebone is as high as 90 wt\%\(^{141}\), previous studies show that some cuttlefish can survive with partially damaged cuttlebone\(^{142}\). This is due to the progressive, localized damage and self-repair in the cuttlebone\(^{4,141}\). Although the functional requirement of cuttlebone is clearly recognized in the biology field, the
underlying material design strategies for achieving such remarkable mechanical performance with extreme lightweight are yet to be extracted\textsuperscript{4,143}.

**Stochastic open-cell foam: *Heterocentrotus mammillatus***

Stereom is a biomineralized porous structure in echinoderms. The microstructure of stereom varied from the labyrinthic pattern (stochastic open-cell foam) to fully periodic foam\textsuperscript{93}. The stereom in the spine of *H. mammillatus* exhibits a stochastic open-cell structure with relative density ranging 0.2-0.4\textsuperscript{2} (Figure 2-10b). *H. mammillatus* lives at the intertidal zone with depth range 0 - 25 m\textsuperscript{144} in Indo-Pacific, except Arabian Gulf, Pakistan, West India, and Bay of Bengal\textsuperscript{144}. The spines of *H. mammillatus* are subjected to different types of external forces, including hydrodynamic forces generated by waves, attacks from predators, and forces that wedge themselves into small holes for protection, resulting in complex loading modes such as axial compression, axial torsion, and bending\textsuperscript{145–148}. Despite made up of biogenic calcite with mineral content \textasciitilde 99 wt\%\textsuperscript{97–100}, the spines of *H. mammillatus* achieve high strength and high energy absorption to ensure the safe operation of these biological functions\textsuperscript{149}. Moreover, the spine exhibits a graceful failure damage behavior rather than catastrophic failure\textsuperscript{145}. These unique features make sea urchin spines a great model system to inspire the design of engineering ceramic cellular solids. The stereom structure in *H. mammillatus* spine has been examined using 2D microscopy\textsuperscript{150}. Previous studies attribute the graceful failure behavior to the hierarchical structural design in the *H. mammillatus* spine\textsuperscript{2,149}. The 3D characterization of the stereom with stochastic open-cell foam structure has not been conducted. The relationship between the remarkable mechanical performance and the stereom microstructures has not been established.

**Periodic open-cell foam: *Protoreaster nodosus***
The third model system is focused on the mechanical design of periodic open-cell structure on the ossicles in the starfish *P. nodosus* (Figure 2-10c). However, no biomineralized periodic structure has been validated before. *P. nodosus* lives at shallow sandy and muddy bottoms and seagrass beds in the lagoon in the Indo-Pacific Ocean to Eastern Africa\textsuperscript{151}. Previous studies focus on the distribution of this species. No structure characterization or mechanical tests have been conducted on the ossicles of *P. nodosus*. 
Table 2-4 | Comparison of three model systems studied in this thesis, *Sepia officinalis*, *Heterocentrotus mammillatus*, *Protoreaster nodosus*.

<table>
<thead>
<tr>
<th></th>
<th><em>Sepia officinalis</em></th>
<th><em>Heterocentrotus mammillatus</em></th>
<th><em>Protoreaster nodosus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Common names</strong></td>
<td>Common cuttlefish</td>
<td>Slate pencil urchin</td>
<td>Knobby sea star</td>
</tr>
<tr>
<td><strong>Kingdom</strong></td>
<td>Animalia</td>
<td>Echinodermata</td>
<td></td>
</tr>
<tr>
<td><strong>Phylum</strong></td>
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<td>Echinoidea</td>
<td>Valvatida</td>
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<td><strong>Class</strong></td>
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<td>Echinoidea</td>
<td>Asteroidea</td>
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<tr>
<td><strong>Order</strong></td>
<td>Sepiida</td>
<td>Camarodonta</td>
<td>Oreasterida</td>
</tr>
<tr>
<td><strong>Family</strong></td>
<td>Sepiidae</td>
<td>Echinometridae</td>
<td></td>
</tr>
<tr>
<td><strong>Genus</strong></td>
<td>Sepia</td>
<td>Heterocentrotus</td>
<td>Protoreaster</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td><em>S. officinalis</em></td>
<td><em>H. mammillatus</em></td>
<td><em>P. nodosus</em></td>
</tr>
<tr>
<td><strong>Origin</strong></td>
<td>Mediterranean Sea, Eastern Atlantic from Southern Norway and Northern England to the northwestern coast of Africa(^{138})</td>
<td>Indo-Pacific, except Arabian Gulf, Pakistan, West India, and Bay of Bengal(^{144})</td>
<td>Indo-Pacific Ocean to Eastern Africa(^{151})</td>
</tr>
<tr>
<td><strong>Habitat</strong></td>
<td>Sandy and muddy bottoms, 2-3 m depth to 200 m depth(^{138})</td>
<td>Intertidal zone, depth range 0 - 25 m(^{144})</td>
<td>Shallow sandy and muddy bottoms, and seagrass beds in lagoon(^{151})</td>
</tr>
<tr>
<td><strong>Biomineral structure</strong></td>
<td>Cuttlebone</td>
<td>Stereom</td>
<td>Ossicle</td>
</tr>
<tr>
<td><strong>Structure type</strong></td>
<td>Honeycomb-like</td>
<td>Open-cell foam, stochastic</td>
<td>Open-cell foam, periodic</td>
</tr>
<tr>
<td><strong>Composition</strong></td>
<td>Aragonite</td>
<td>Biogenic calcite</td>
<td>Biogenic calcite</td>
</tr>
<tr>
<td><strong>Mineral content</strong></td>
<td>90 wt(^{%})(^{141})</td>
<td>99 wt(^{%})(^{97-100})</td>
<td>99 wt(^{%})(^{97-100})</td>
</tr>
<tr>
<td><strong>Relative density</strong></td>
<td>7 vol(^{%})(^{\text{max}})</td>
<td>20-40 vol(^{%})(^{2})</td>
<td>50 vol(^{%}) (^{\text{max}})</td>
</tr>
</tbody>
</table>
Chapter 3 Mechanical designs in the cuttlebone of the *S. officinalis*


Cuttlefish, a unique group of marine mollusks, produces an internal biomineralized shell, known as cuttlebone, which is an ultra-lightweight cellular structure (porosity, $\sim 93$ vol\%) used as the animal’s hard buoyancy tank. Although cuttlebone is primarily composed of a brittle mineral, aragonite, the structure is highly damage tolerant and can withstand water pressure of about 20 atmospheres (atm) for the species *Sepia officinalis*. Currently, our knowledge on the structural origins for cuttlebone’s remarkable mechanical performance is limited. Combining quantitative three-dimensional (3D) structural characterization, four-dimensional (4D) mechanical analysis, digital image correlation, and parametric simulations, here we reveal that the characteristic chambered “wall–septa” microstructure of cuttlebone, drastically distinct from other natural or engineering cellular solids, allows for simultaneous high specific stiffness (8.4 MN·m/kg) and energy absorption (4.4 kJ/kg) upon loading. We demonstrate that the vertical walls in the chambered cuttlebone microstructure have evolved an optimal waviness gradient, which leads to compression-dominant deformation and asymmetric wall fracture, accomplishing both high stiffness and high energy absorption. Moreover, the distribution of walls is found to reduce stress concentrations within the horizontal septa, facilitating a larger chamber crushing stress and a more
significant densification. The design strategies revealed here can provide important lessons for the development of low-density, stiff, and damage-tolerant cellular ceramics.
3.1 Introduction

The earliest interest in studying cuttlefish dates back to the Greek philosopher Aristotle almost 2500 years ago, who was intrigued by the unique anatomy of cuttlefish\textsuperscript{153}. Although named as a fish, cuttlefish is, in fact, a mollusk of the order Sepiida with over 120 different species. Together with squid, octopuses, and nautiluses, cuttlefish belongs to the class Cephalopoda, which represents a special group of mollusks capable of swimming. Unlike most mollusks with hard shells covering their body exterior for protection, the “shell” of cuttlefish is an internal and highly porous structure (porosity ~93 vol\%)\textsuperscript{4}, commonly known as cuttlebone. This allows cuttlefish to adjust their buoyancy by regulating the gas-to-liquid ratio within the shell, similar to nautiluses\textsuperscript{154,155}. Unlike the soft swim bladders for buoyancy-regulating purposes in fishes, cuttlebone is a stiff honeycomb-like structure composed of > 90wt\% aragonite\textsuperscript{141}. It has been demonstrated that cuttlefish controls the concentration of salt ions of the fluid inside cuttlebone and hence the fluid’s osmotic pressure, which then regulates the charge and discharge of the fluid for buoyancy adjustment\textsuperscript{156}.

Although most cuttlefishes live in shallow waters, some species are known to inhabit as deep as 600 m\textsuperscript{157,158}. This requires the cuttlebone to sustain extreme external water pressure as high as 60 atmospheres and to avoid catastrophic failure\textsuperscript{140,156,159,160}, which entails the cuttlebone to be both stiff and damage tolerant. Previous studies show that some cuttlefish can survive with partially damaged cuttlebone because the damage is progressive and localized, which can be repaired subsequently\textsuperscript{4,142,154}. This is remarkable given the fact that the cuttlebone is primarily composed of intrinsically brittle aragonite\textsuperscript{141}. Moreover, the active fluid exchange for buoyancy control also requires the structure to be open and bi-continuous, whereas the closed-cell design of many natural cellular structures, such as bird feathers, porcupine quills, and cork, cannot achieve...
such function. Although the functional requirement of cuttlebone is clearly recognized in the biology field, the underlying material design strategies for achieving such remarkable mechanical performance with extreme lightweight are yet to be established.

Some researchers proposed predictive models that linked the failure criteria to walls buckling in the chamber, which could be applied to the strength measurements of some implosion tests. By assuming the equally strong septa and walls, the maximum stress occurred, and hence fracture initiated in the center or the edge of the septa. Recently the failure behavior of cuttlebone chambers under compression has been visualized using \( \mu \)-CT. However, it remains unclear how specific the morphological configuration of microstructure determines the local and global mechanical properties, for instance, how the deformation propagate from one chamber to the adjacent chamber, where a wall buckles first. Moreover, researchers have tried to mimic the geometry of cuttlebone, but tough ceramic lattices comparable to cuttlebone have not been accomplished, limited by the incomplete understanding of the structural design and deformation mechanism in cuttlebone.

In this chapter, I seek to establish and quantify the structural designs of cuttlebone utilizing complementary 3D structural and 4D mechanical analysis combined with parametric mechanical modeling. With \textit{in-situ} synchrotron-based micro-computed tomography (\( \mu \)-CT) coupled with mechanical testing, we, for the first time, visualize and quantify the detailed failure process of cuttlebone. The 3D structure quantification further allows us to parametrically establish biomimetic models to investigate the mechanical performance and design trade-offs across different length scales, which is correlated with the \textit{in-situ} results and digital image correlation. Our results reveal that the cuttlebone has evolved an optimal chambered “wall-septa” microstructure that achieves high stiffness as well as graceful failure.
3.2 Methods

Samples: Frozen *S. officinalis* specimens were purchased from the Great Wall Supermarket in Boston and Atlanta.

Microscopy (optical microscopy, scanning electron microscopy): Optical images were taken with a Nikon ECLIPSE LV100 microscope (Tokyo, Japan). The cuttlebone samples were dissected from frozen adult cuttlefish *S. officinalis*. The samples were subsequently air-dried at room temperature before analysis. Sections fractured from the dried cuttlebone were first mounted on SEM stubs and then coated with ~8 nm Pt/Pd with a sputter coater (Leica ACE600 Sputter). Care was taken to induce fracture along specific orientations, including both transverse and horizontal directions. The samples were imaged with a field-emission scanning electron microscope (Zeiss, LEO 1550) with an accelerating voltage of 5kV and at a working distance of ~10 mm.

Compression tests and digital image correlation: Cube-shaped samples (edge length, ~10 mm) were first cut from the dissected cuttlebone by using a low-speed diamond saw. The sample surfaces were further carefully trimmed parallel to septa using a razor blade. The samples were then tested in a quasi-static compression mode by applying load perpendicular to the septum plane at a rate of 0.5 mm/min with a universal testing machine (Instron, Model 5948, Norwood, USA). The videos of the compression tests were recorded with an Amscope camera with a frame rate of 500 f/min, which was used for DIC analysis. The microstructure of the cuttlebone enabled direct image correlation without the introduction of extra speckle patterns. The strain fields were
calculated using VIC-2D (Correlated Solution Inc, Irmo, USA) with an incremental algorithm and a subset size of 35 pixels.

**Synchrotron-based in-situ mechanical tests:** Cube-shaped samples (edge length, ~2 mm) consisting of three to four chambers were isolated from the mid-dorsal region of cuttlebone and then carefully trimmed. The synchrotron-based *in-situ* mechanical tests based on *μ-CT* were conducted at the beamline 2BM from the Advanced Photon Source, Argonne National Laboratory, utilizing a monochromatic X-ray beam with an energy of 27.4 keV. A customized *in-situ* mechanical loading device was used for both synchrotron-based compression and indentation tests, through which the samples can be mechanically tested while allowing for X-ray imaging through its X-ray transparent window. For the *in-situ* compression tests, load was applied by a stepper motor, and the compressive force was measured by the load cell. For the *in-situ* indentation tests, a tungsten rod with a flat punch end (diameter, 0.5 mm) was used to induce localized deformation. Tomography data were collected as the displacements were applied in steps of 0.1 mm monotonically. The beamline employs a single-crystal LuAG:Ce scintillator to convert X-ray into visible light, which was further magnified with a 5× or 10× long-working distance objective lens. Each scan was collected at 0.12° angular increment over a 180° rotation with an exposure time of 0.2 s (corresponding to the total scan time for a single tomography scan of 5 min). The projection images were collected by using a PCO-Edge high-speed CMOS detector (2560×1300 pixels), which resulted in voxel sizes of 0.65 or 1.3 µm depending on the objective lens. The reconstruction and segmentation of the obtained *μ-CT* data were conducted with the open source software *Tomopy*\(^{166}\) and *Ilastik*\(^{167}\), respectively. The reconstructed data was used for 3D volume rendering and quantitative analysis, such as cross-sectional length and surface curvature, via a combination
of methods, including *Avizo* (Thermo Fisher Scientific, USA), Fiji\textsuperscript{168}, Blender (www.blender.org)\textsuperscript{169}, and customized Matlab routines.

*Geometric modeling*: The geometries of the vertical walls were built based on reconstructed tomography data (**Figure 3-1, Figure 3-2**). The collected data was first binarized, skeletonized, and filtered to remove the noise introduced during voxelization. Then, wall profiles at varying heights were extracted using a customized Matlab program, which was further imported into Solidworks (Dassault, Waltham, MA) to generate 3D models. Moreover, a general mathematical description of the wall was developed to investigate the wall shape parametrically (**Figure 3-3**). To do this, the top profile of a wall \( \mathbf{P}_{h=h_0} \) was modeled as the addition of a vector \( A \cdot \mathbf{V} \) to the bottom profile \( \mathbf{P}_{h=0} \), i.e., \( \mathbf{P}_{h=h_0} = \mathbf{P}_{h=0} + A \cdot \mathbf{V} \), where \( h_0 \) is the height of the wall and \( \mathbf{P} = [x, y]^T \) represents points on the wall. Note that \( A \) controls the waviness amplitude, which is varied between 0.1~3 to generate walls with different waviness gradients. Since the wall profile evolves exponentially in the height direction (Eq. 1), the general description of the wavy wall is \( \mathbf{P}(\tilde{h}) = [1 - \alpha(\tilde{h})] \mathbf{P}_{\tilde{h}=0} + \alpha(\tilde{h}) \mathbf{P}_{\tilde{h}=1}, \) with \( \alpha(\tilde{h}) = \frac{e^{\kappa\tilde{h}-1}}{e^{\kappa-1}} \) and \( \tilde{h} = h/h_0 \), where \( \kappa \) is a parameter fitted to the \( \mu\text{-CT} \) data. The geometric modeling is conducted by Zian Jia.
Figure 3-1| Geometric modeling of (a) walls and (b) chambers based on reconstructed slices from \( \mu\text{-CT} \) scans. The reconstructed slices are binarized, skeletonized, and then filtered to remove noise introduced during voxelization. The resultant geometric center-lines of the walls at varying heights are shown in the second column. These center-lines are then swept in Solidworks to form the wavy walls. Thickness is then assigned to produce solid models. (Figure credit: Zian Jia)
Figure 3-2 | The construction of chambered models. a, single-chamber model and (b) multiple chamber model based on reconstructed slices from μ-CT scans. (Figure credit: Zian Jia)
Figure 3-3 | The mathematical model used to describe the shape of the walls. Walls with different waviness are constructed with the same bottom profile but varying top profiles. The cross-section length of each wall is assumed to follow an exponential equation in the form of Eq. 1 in the main text. 

\( h_0 \) is height and \( h \) is height and \( h_0 \) is the total height of the wall. The top profile of a wall \((P_{h=h_0})\) is modeled as the addition of a vector \((A \cdot V)\) to the bottom profile \((P_{h=0})\). 

b, Walls with different top profiles, the amplitudes are \( A = 0.5, 1, \) and \( 2 \), respectively. 

(c) The wall is modeled by function \( P(\bar{h}) = [1 - \alpha(\bar{h})]P_{h=0} + \alpha(\bar{h})P_{h=1} \), with \( \bar{h} \) defined as \( \bar{h} = h/h_0 \). 

d) A resultant wall. 

(e) Walls with varying waviness amplitudes, the wall thickness is adjusted to maintain the same amount of solid material for different walls. (Figure credit: Zian Jia)
Finite element simulation. ABAQUS was utilized to simulate the mechanical response of cuttlebone under compression. The walls and septa were discretized with shell elements and tetrahedral elements, respectively. The walls and septa have Young’s modulus of 51 GPa and 29.6 GPa, respectively, based on literature values\textsuperscript{143}. The elastic performance was calculated with general statics, and the fracture process was simulated utilizing dynamic explicit. In the explicit simulations, the strain rate of loading was set as 0.05 s\textsuperscript{-1}. A smeared brittle cracking model and element deletion was implemented to capture the brittle fracture of the biogenic aragonite-based walls. Crack initiated when the maximum principal tensile stress exceeded the tensile strength of aragonite, $\sigma_f$, which was 102 MPa\textsuperscript{170}. The fracture energy of forming a unit area of the crack surface in Mode I, $G_{II}$, was used as the criteria of element deletion to avoid unreasonable mesh sensitivity\textsuperscript{171}. A material point failed when the critical fracture energy of this point was reached, and an element was deleted when all the corresponding material points failed. A linear shear retention model was also included to consider the reduction of post-cracked shear modulus once the crack opened. The finite element modeling is conducted by Zian Jia.

3.3 Results

3.3.1 Structure characteristics

As illustrated schematically in Figure 3-4a, the cuttlebone is located towards the dorsal side of the cuttlefish body. In this study, the species *Sepia officinalis* was used as a model system, which lives up to a depth of 200 m under sea water, corresponding to an external water pressure of ~20 atm\textsuperscript{142}. The dorsal side of the rigid cuttlebone is covered with a thick and tough layer (~0.5 mm) known as the dorsal shield\textsuperscript{4}, under which a porous chambered structure is placed ventrally (Figure 3-4b). The posterior end of the chambers forms the siphuncular zone, through which the
fluid can flow in and out for buoyancy control (Figure 3-4a,b). In the transverse view along the N direction (defined as the direction pointing from the ventral to the dorsal side, Figure 3-4a), individual chambers can be seen clearly, and their heights gradually decrease from the center towards the ventral side (Figure 3-4c,d). Note that new chambers are added at the ventral side. Therefore, the growth direction (“G”) is opposite to the normal direction (“N”) of cuttlebone.

The chambered cuttlebone structure is based on a characteristic honeycomb-like “wall-septa” design, as shown in the scanning electron microscopy (SEM) (Figure 3-4e) and 3D μ-CT reconstruction (Figure 3-4f). The horizontal septa (thickness, 7-15 μm) separate the cuttlebone into individual chambers, which are supported by numerous vertical walls. The wall thickness is measured as 4-7 μm, where variations may exist among individuals. This honeycomb-like “wall-septa” design results in an extremely high porosity of cuttlebone (ca. 93 vol%).

Figure 3-4| The honeycomb-like chambered “wall-septa” structure of cuttlebone. a, A cuttlefish with the cuttlebone highlighted in yellow. “N” direction points from the ventral to the dorsal side. “G
growth direction” denotes the growth direction. The Siphuncular zone is the striated area on the posterior ventral part of the shell. b, Dorsal and ventral views of the cuttlebone. c, A transverse view of the cuttlebone and (d) corresponding chamber height map. e, A scanning electron microscopy (SEM) image of the chambers with walls and septa indicated by white arrows. The top and bottom of the walls are highlighted by green and red dashed lines, respectively. f, A 3D µ-CT reconstructed “wall-septa” structure. The walls have wavier top profiles (green) compared to the bottom profiles (red).

The walls have corrugated morphologies and become wavier from the bottom to the top of the chamber (along the growth direction), which is also consistent with the increase in the absolute mean curvature values (Figure 3-5a,b). Due to this gradual morphological variation (Figure 3-5c), based on the measurements of 11 walls, the cross-sectional profile of an individual wall is found to follow this relationship,

\[
L/L_0 = 0.998 + 0.002e^{5.2(h/h_0-0.11)} \tag{3-1}
\]

where \( h_0 \) represents the chamber height, \( L_0 \) is the cross-sectional length close to the bottom, and \( L \) is the cross-sectional length at a height \( h \) (Figure 3-5d). Note that in the region with \( h < 50 \) μm \( (h/h_0 < 0.11) \), some walls break into multiple segments, which are not included in the model for simplicity. This mathematical description of the wall profile allows parametric investigations of the wall mechanics later. Moreover, the vertical walls within one chamber are organized in a labyrinthic pattern (Figure 3-5e,f) \(^{172} \). The spacing between adjacent walls is roughly constant at specific heights: \( 88.7 \pm 15.6 \) μm and \( 67.0 \pm 15.7 \) μm at the wall bottom and top, respectively (Appendix A, Figure A-1). Moreover, the gradual increase in waviness from the wall bottom to top is clearly visualized by overlaying the cross-sectional profiles from the same projected region in the chamber, which also reveals the splitting of some walls (white boxes in Figure 3-5g).
density of wall splitting is estimated to be 14/mm² (46% of the walls split). Approximately half of the walls possess one to two triple junctions, and walls with triple junctions tend to be longer compared to those without such junctions (Figure 3-5f and Figure 3-6a,b). Overlay of the patterns of wall bottoms from adjacent chambers shows that the vertical walls from adjacent chambers are not aligned exactly in the same position (Figure 3-5h). On a larger scale close to the inlet of the chamber (9.4 mm x 2.6mm), the density of triple junctions increases from the inlet ~85/mm² to the interior ~212/mm² part (Figure 3-7a,b). The wall spacing is relatively constant 80.1 ± 22.3 μm (Figure 3-7c).

Figure 3-5| The morphology and arrangement of walls in the chamber. a, 3D µ-CT reconstructed individual walls. The contour represents the corresponding mean curvature distribution. b, Scatter plot of the principal curvature distributions (K₁ and K₂), where each data point is colored with the
corresponding value of the mean curvature ($K_{\text{mean}}$). c,d, A 3D reconstructed wall, and normalized wall length $L/L_0$ plotted against normalized height $h/h_0$ of 11 walls. Eq.1 is plotted as the solid black line. The labyrinthic pattern of walls near the (e) bottom and (f) top of the chamber. The white boxes in (f) indicate triple junctions. g, Overlay of (e) and (f), where the white boxes indicate the breaking points. h, Overlay of wall bottoms from two adjacent chambers.

**Figure 3-6** | Analysis of the triple junctions of the walls. a, A representative labyrinthine pattern of the walls, where their triple junctions are indicated by white boxes. b, Profiles of the walls with two triple junctions (1-6), one triple junction (7-22), and without triple junctions (23-46) in (a).
Figure 3-7 | The morphology and arrangement of walls on a large scale. a, The labyrinthic pattern of walls near the bottom with each wall traced by red lines. b, Triple junctions on the labyrinthic pattern. c, The distribution of wall spacings on the labyrinthic pattern.

It is known that cuttlebone is composed of aragonite (one polymorph of calcium carbonate) as the only mineral phase and a small amount of organic material (~9.8 wt% for the whole cuttlebone and ~5 wt% for the chambered structure) consisting of $\beta$-chitin and proteins. Previous studies have shown that the walls have a higher mineral content than the septa. A careful examination of the microstructure revealed that each septum consists of two sub-layers. The upper sub-layer and the wall share the same vertically aligned aragonite crystallites along their
[001] directions (upper part in Figure 3-8a)\textsuperscript{141}, whereas the nanorod-like crystallites in the lower sub-layer rotate their orientations gradually, forming a rotating plywood structure (lower part in Figure 3-8b–e)\textsuperscript{143,177}.

Figure 3-8] The rotating plywood structure in the septum of a cuttlebone. a, Magnified SEM image showing the double-layered structure of the septum. b, Enlarged image of the white box marked in (a) where periodic variation in the morphology of the fractured fibers is observed. c, A 3D model of the rotating plywood structure. d, Cross-section view of the rotating plywood structure cut along the plane highlighted in (c). e, Magnified SEM image showing the gradual change of the fiber orientation (arrows) in the vertical direction. The numbers 1-6 mark the different layers of fibers in the N direction.

3.3.2 Quantitative mechanical properties

Graceful failure for high energy absorption

Compression tests performed on cubic samples (size 5-10 mm, corresponding to 10-30 chambers) reveal the cuttlebone’s remarkable graceful failure behavior, despite its high porosity and mineral density (Figure 3-9a). Due to its graceful failure behavior, cuttlebone achieves an extremely high energy absorption capacity ($W$) of 0.6-1.5 $MJ/m^3$ at a density ($\rho$) of only 180-
260 kg/m$^3$. The resultant specific energy absorption ($W/\rho$) is $4.4 \pm 1.1$ kJ/kg, which is superior or comparable to many advanced foams based on metals, polymers, and carbon (Figure 3-9b)$^{178,179,188,189,180–187}$. More specifically, the stress-strain curves of cuttlebone, unlike engineering ceramic cellular solids, show three stages, including an elastic regime, a serrated stress plateau regime ($\varepsilon$ up to 0.85), and a densification regime, which is a classic behavior observed in foams composed of ductile materials such as metals and polymers (Figure 3-9c)$^8$. Within the plateau regime, the stress-strain curves exhibit periodic fluctuations, where the total number of periods corresponds to the number of chambers ($n$) in the tested sample (Figure 3-10a). This results from the sequential failure of individual chambers. The peak stress of each period, $\sigma_p$, is relatively constant ($1.58 \pm 0.32$ MPa, $N = 212$) despite the variations in chamber heights (Figure 3-10b). The normalized peak-to-peak strain ($n\varepsilon_{pp} = 0.85 \pm 0.13$) and valley-to-peak strain ($n\varepsilon_{vp} = 0.42 \pm 0.16$) also show no significant dependence on chamber heights (Figure 3-10c).
Figure 3-9 | *Ex-situ* mechanical performance of the chambered structure under compression. a, Stress-strain curves of 11 compression tests. b, Energy absorption capacity ($W$) versus density ($\rho$) for cuttlebone in comparison with foams reported in the literature, including AlSi$_{10}$Mg open-cell foams$^{178,179}$, titanium foams$^{180,181}$, sintered fiber stainless steel foams$^{182}$, carbon nanotube reinforced nanocomposites$^{183}$, stainless steel foam$^{184,185}$, carbon nanotube reinforced aluminum foams$^{186}$, nickel foams$^{187}$, Zn/Al/Cu alloy foam$^{188}$ and epoxy foam$^{189}$. The dashed lines represent different $W/\rho$ values, and the shaded area highlights the standard deviation of $W/\rho$ for cuttlebone. c, Snapshots of deformation stages corresponding to the black curve in (a).
The mechanical behavior of the chambered structure in one period. 

The figure shows the mechanical behavior of a chambered structure in a stress-strain cycle. The stress-strain curve has three stages: local penetration (LP), expansion (EXP), and densification (DENS). The red curve represents the relative density ($\rho_d/\rho_s$) of the damaged chamber during deformation. $\varepsilon_{pp}$, $\varepsilon_{vp}$: peak-to-peak strain and valley-to-peak strain in a stress-strain period. $\rho_s$ is the density of the constituent material. 

**b.** Distributions of peak stress, $\sigma_p$, versus chamber height, $h_0$. 

**c.** Distributions of normalized peak-to-peak strain $n\varepsilon_{pp}$ and normalized valley-to-peak strain $n\varepsilon_{vp}$ versus chamber height, $h_0$. 

**d.** Digital image correlation (DIC) results corresponding to points marked in (a). The deformation is mapped with the Hencky strain. The picture is credited to Zian Jia.

### 3.3.3 Deformation mechanism

To gain a deeper understanding of the cuttlebone’s chamber-by-chamber damage process, we first utilized digital image correlation (DIC) to correlate the stress-strain response and the evolution of local strain fields during individual stress periods (Figure 3-10a,d). It is found that the failure
of a single chamber does not occur at once instantaneously but progressively. In particular, we identify three important stages during each period: 1) local penetrations (LP) within the deforming chamber (stage iv, Figure 3-10d), which is manifested by some minor stress drops in the stress-strain curve (Figure 3-10a). This process leads to the formation of multiple high strain regions (yellow arrows, stage iv, Figure 3-10d). 2) Expansions (EXP) of the failure within the damaging chamber (green arrows, stage v, Figure 3-10d), where the stress decreases significantly. 3) Densification (DENS), where the fractured walls in the damaged chamber are gradually compacted, leading to stress increase (stage vi, Figure 3-10d). Evaluating the area under 16 periods in one stress-strain curve further reveals that a significant amount of the energy (55 ± 19%) in a stress period is dissipated by continuous fracture and contact of wall fragments during the densification process.

To further visualize and quantify the failure process in 3D, we conducted in-situ mechanical tests coupled with synchrotron-based high-resolution μ-CT measurements (see methods). As shown in the serial X-ray projection images in Figure 3-11, the morphological evolution of the vertical walls and horizontal septa can be clearly observed during a compression test. Corresponding 3D reconstructions further illustrate the detailed structural evolution in 3D (Figure 3-12). As one chamber is undergoing wall fracture and subsequent densification, the adjacent chamber remains intact until the local penetration of the septum by the fractured pieces (red arrows, Figure 3-12a,b). Continuing densification of the fractured chamber and local penetrations of the septum leads to the propagation of failure to the adjacent chamber (Figure 3-12), consistent with the DIC results. The failure of the adjacent chamber initiates when the damaged chamber has been compressed to a normalized height $h_d/h_0 = 0.23 \pm 0.03$ (N = 8), corresponding to a relative
density \((\rho_d/\rho_s)\) of 30.5 ± 4%. Here the relative density is the density of the damaged chamber \((\rho_d)\) divided by the density of the constituent material \((\rho_s)\).

Figure 3-11| The stress-strain curve and corresponding X-ray projection images of a cuttlebone sample during an in-situ synchrotron-based \(\mu\)-CT compression test.
**Figure 3-12** Synchrotron X-ray μ-CT based *in-situ* compression analysis. a, Progressive failure process of two adjacent chambers under compression. Red arrows and red boxes indicate the local penetration of a septum and expansion of wall fracture, respectively. $h_t$ and $h_b$ denotes the heights of fractured wall attached to the septum’s top and bottom sides, respectively. b, A 3D rendering image highlighting a septum penetrated by fractured walls at stage iii in (a). c, Two adjacent chambers with the septum breaking path highlighted in black. The green and red wall profiles represent the adjacent wall patterns connected to the fractured septum.

The *in-situ* analysis also allows us to directly examine the fracture process and characteristics of individual walls. As shown in **Figure 3-13a**, the walls fracture in a crack cascading manner under compression similar to other brittle slender structures\textsuperscript{190,191}. That is, walls typically fail by first forming cracks in the middle which generates a burst of flexural waves that propagate from the newly formed crack surfaces, further breaking the wall into multiple pieces (**Figure 3-13b**).
Since the top portion of the wall is wavier than the bottom, it possesses more material to resist fracture, as evidenced by its larger remaining height \( h_t \) in comparison to the piece at the bottom \( h_b \) shown in Figure 3-12a (stage ii), Figure 3-13a,b. Quantitative measurements of the fractured wall tops and bottoms yield \( h_t/h_0 = 0.21 \pm 0.15 \) and \( h_b/h_0 = 0.16 \pm 0.11 \) (N = 190, Figure 3-13c). Such asymmetric fracture contributes to more intact wall-septa connections on the top than that at the bottom (Figure 3-13d,e). Therefore, the bottom septum is more prone to local penetration. Moreover, the septum breaks along a tortuous crack path guided by the top wall-septum connections (the green pattern in Figure 3-12c), facilitating energy dissipation.

Similar localized and asymmetric fracture is also salient in the indentation tests, where damage is localized below the indenter, and the septum remains intact until significant densification crushes the walls into pieces (Figure 3-14). The observation that the septa remain undamaged until the chambers are compressed to ~0.15 of the original height reveals the superior penetration resistance of the septa compared to the crushing resistance of the walls. This is related to the rotating plywood structure found in the septa (Figure 3-8), which has been demonstrated as a microstructure to enhance strength, damage tolerance, and toughness considerably \(^{192-194}\). Moreover, the richer organic contents in the septa \(^{141}\) may also improve the toughness \(^{195}\). These tough septa are important to the observed layer-by-layer damage.
Figure 3-13 | Asymmetric fracture behavior in the chamber. a, A vertical reconstruction slice showing the fracture of vertical walls, where the green and red arrows indicate the top and bottom parts of the fractured walls, respectively. b, A 3D rendering image of a representative fractured wall. c, The distribution of normalized height $h_t/h_0$ and $h_b/h_0$ (N = 190). 3D renderings of a chamber at different fracture stages on (d) the wavy side (wall top) and (e) the straight side (wall bottom). Note that the wall-septa connections on the wavy side are relatively more intact in comparison to the straight side at the same stage. The yellow arrows indicate the locations where the wall-septa connections are less damaged.
**Figure 3-14 | Synchrotron X-ray μ-CT based in-situ indentation analysis.** a, Vertical reconstruction slices at five deformation stages during an X-ray in-situ indentation experiment with a flat-punch tungsten tip, where the flat-punch tip was shown as a white cylinder. b, A 3D rendering image of the fractured chambers.

### 3.3.4 Computational modeling

*The computational modeling is conducted by Zian Jia.*

**Optimum wall waviness: balance of stiffness and energy absorption**

Both the in-situ and conventional mechanical experiments have demonstrated the importance of the wall shape in maintaining structural integrity and achieving high energy absorption. Finite element simulations are further conducted to gain deeper insights by providing a direct comparison of the mechanical performance between the wavy walls and straight walls (Figure 3-2, Figure 3-15a), where the straight walls were extruded based on the wall profiles at the top and bottom of the wavy wall. The resultant stress distributions are summarized in Figure 3-15b, which reveals that, compared to the straight walls, the wavy wall exhibits significantly reduced stress (up to 50%)
at the wavy end. Nonetheless, by plotting the scaling relation between stiffness and normalized thickness in Figure 3-15c, we surprisingly found that the wavy wall exhibits a linear scaling relation and possesses over 95% stiffness of the straight walls. This linear scaling relation is direct evidence showing that the wavy walls utilize a compression-dominant deformation mechanism to maintain their high stiffness\textsuperscript{25,196}.

**Figure 3-15** Compression-dominant behavior at the wall level. a, The wall is modeled by function $P(\bar{h}) = [1 - \alpha(\bar{h})]P_{\bar{h}=0} + \alpha(\bar{h})P_{\bar{h}=1}$, with $\bar{h}$ defined as $\bar{h} = h/h_0$. B, A comparison of stress distribution between a wavy wall and straight walls at compressive strain $\varepsilon = 0.001$. The wavy wall (black point) is based on $\mu$-CT reconstruction. The straight walls marked by the hollow circle and solid green circle are generated by extruding the bottom and the top profile of the wavy wall, respectively. c, The stiffness (defined as the slope of the force-strain curve) of wavy wall scales linearly ($n = 1.06$) with the normalized thickness (normalized by the thickness of the $\mu$-CT model), demonstrating a compression-dominant deformation mechanism. (Figure credit: Zian Jia)

This linear scaling relation, however, will degrade if the wall waviness becomes too large, suggesting that nature may have evolved an optimal wall waviness for cuttlebone. To confirm this,
we conducted systematic simulations on walls with varying waviness. Note that in the simulation, wall fracture is implemented by a brittle fracture model. All walls are set to have the same bottom profile $P_{h=0}$ and their top profile ($P_{h=h_0}$) varies to form different waviness. In particular, the top profile is modeled as the addition of an amplitude vector ($AV$) to the bottom profile (Figure 3-16a),

$$P_{h=h_0} = P_{h=0} + A \cdot V$$

Equation 3-2

where $V$ is a set of vectors describing the normal directions of the bottom wall profile and $A$ is the amplitude parameter, with $A = 1$ denoting the original wall profile of cuttlebone. The stress-strain curves of walls with $A = 0.1$~3 are summarized in Figure 3-16b, which show that less wavy walls ($A = 0.1$) have a higher stiffness but tend to break catastrophically, while excessively wavy walls ($A = 3$) have significantly degraded stiffness and strength due to prominent bending deformations. The calculated stiffness, strength, failure displacement, and energy absorption capacity are plotted versus the waviness amplitude $A$ in Figure 3-16c. Optimal failure displacement and optimal energy absorption are found at $A = 1$, which is proved to avoid catastrophic damages as well as bending-induced premature failure. Specifically, as $A$ increases from 0.1 to 1, the reductions in stiffness and strength are 4.0% and 11.6%, respectively, which are much less significant compared to the 49.4% increase of failure displacement and 60.0% increase of energy absorption. Moreover, the walls of the cuttlebone have a stochastic feature, and $A$ for some walls can be slightly off the optimum. Simulating different wall geometries reveals that the optimal $A$ is in the range of 0.5~1.5 (Figure 3-3), indicating that the waviness of the cuttlebone is optimized statistically.
Figure 3-16] Balance of stiffness, strength, and progressive failure at the wall level. a, Mathematical description of walls with different waviness amplitudes, \( P_{h=h_0} = P_{h=0} + A \cdot V \), where \( h \) is height and \( h_0 \) is the total height of the wall. The top profile of a wall \( (P_{h=h_0}) \) is modeled as the addition of a vector \( (A \cdot V) \) to the bottom profile \( (P_{h=0}) \). b, Force-strain curves of wavy walls with varying waviness amplitudes, \( A = 0.1, 0.5, 1, 1.5, 2, \) and \( 3 \). \( A = 1 \) corresponds to the \( \mu \)-CT model. c, Stiffness, strength, failure displacement, and energy (work of fracture) plotted versus the waviness amplitude, \( A \). (Figure credit: Zian Jia)

Our simulations also reveal that the stress of failure decreases at increasing wall waviness, revealing that the walls become weaker although their moment of inertia increases. This result opposes previous assumptions that the walls fail by buckling\(^4\). Instead, it suggests that the walls fail by strength-controlled fracture (the breaking strength of biogenic aragonite is reached before the walls buckle). In particular, the wavy geometry develops uneven stress distribution where regions with higher stress tend to fracture earlier, resulting in more progressive damages than the straight wall (Figure 3-17a,b,c). The fractured patterns of the simulated walls are consistent with \( \mu \)-CT reconstructions (Figure 3-17d, Figure 3-18), confirming the validity of our simulation approach.
Figure 3-17 | Progressive failure at the wall level. a,b, Simulation results of wall fracture (i-v) (a) compared to µ-CT reconstructions (b). The numbers mark the fracture sequence. c,d, The straight wall fractures into small pieces catastrophically (c), while the wavy walls fracture more progressively into larger pieces (i, ii) (d). The 2nd column in each case is from simulation and the 3rd column is from µ-CT reconstruction. (Figure credit: Zian Jia)
Beneficial stress shifts at the chamber level

To further demonstrate the exceptional performance of cuttlebone at the chamber level, we compare the stiffness of cuttlebone chambers with two broadly acknowledged ultra-stiff materials including an octet lattice\textsuperscript{197} and a cubic metafoam\textsuperscript{198} (structure #1 and 2, respectively), at a fixed volume fraction $V_f = 0.07$ (Figure 3-2). To fully reproduce the design of cuttlebone, septa are assumed to be softer but thicker than the vertical walls in structure #3~8, referring to the previous literature\textsuperscript{141,143}. Simulations show that the cuttlebone chambers are over three times stiffer than the octet lattice\textsuperscript{197} and are comparable to that of the cubic foam\textsuperscript{196} (Figure 3-19a). This ultra-high stiffness at the chamber level is essential to the cuttlebone to withstand varying water pressures.

In Figure 3-19b, the stress-strain responses of the cuttlebone chambers (three chambers marked by black lines are simulated to present the statistical behavior) are further compared to
straight wall-based chambers (red and green lines). Consistent with individual wall results, the wavy chambers yield slightly smaller stiffness and strength but more progressive failure (shade areas) and improved energy absorption. The complete failure sequence of the walls within one chamber is depicted in Figure 3-20a, where fractures take place progressively and are distributed throughout the whole chamber. This observed progressive failure is contributed by the varying profiles and waviness gradient at the wall level. As we have demonstrated earlier, the statistic feature of single walls contributes to statistical mechanical response. Moreover, the post-fracture morphology reproduces the asymmetric wall profile observed in experiments with $h_t/h_0 = 0.183$ and $h_b/h_0 = 0.126$, broadly consistent with the trend observed in experiments (Figure 3-20b,c). It should be pointed out that the simulations (Figure 3-19b,c) overestimate the stress compared to the experimental measurements (Figure 3-9a) because the properties of pure aragonite instead of biogenic aragonite are assumed in the simulations. While the absolute value from the simulations may not be directly utilized as the strength of the cuttlebone, if we assume the strength of pure aragonite as the maximum strength possible for the vertical walls, the simulated strength (~6.8 MPa) could be considered as an upper bound strength of the chambered cuttlebone structure.
Figure 3-19 | Mechanical behavior at the chamber level. a, A comparison of stiffness between the octet truss, cubic foam, and the cuttlebone structures, all with a volume fraction $V_f = 0.07$ (the volume of septa is accounted). The chambers noted as straight bottom and straight top are generated by extruding the bottom and the top profiles of the wavy walls, respectively. $t$ is the thickness, and $E$ is Young’s modulus, subscript $s$ and $w$ refer to septa and wall, respectively. The cuttlebone-based structure (#3) is 5 times stiffer than the octet truss and is comparable to the cubic foam. Structures #1-6 are constructed utilizing the same material ($E_s = E_w$). The thickness and stiffness ratios adopted here are based on experimental data of cuttlebone. The Hashin-Shtrikman upper bound (62) presents the upper limit of the stiffness of isotropic composite materials, and it is plotted as a reference for comparison. b, Stress-strain curves of three cuttlebone chambers (#4) under compression compared to chambers with straight walls (#5 and #6). (Figure credit: Zian Jia)
**Figure 3-20** Post fracture behavior at single chambers. Post fracture morphology comparison between single chambers with wavy walls (a) and straight walls (b). The wavy structures exhibit more progressive failure and fracture into larger pieces. More specifically, the fractured upper walls of the wavy structures generally have a greater height than the bottom part, $h_t > h_b$. $A_{top}$ and $A_{bot}$ refer to the total areas of the walls in the top and bottom portions, respectively. $L_{top}$ and $L_{bot}$ refer to the length of the wall profiles on the top and bottom septa, respectively. The post-fracture measurements reveal the asymmetric fracture of the wavy walls, with $h_t/h_0 = 0.1833$ and $h_b/h_0 = 0.1263$. c, Fracture of walls in a cuttlebone chamber, the color and number present the failure sequences of wall sections and wall clusters, respectively. (Figure credit: Zian Jia)

By comparing the septa deformation of the original wall overlaying pattern (from $\mu$-CT data) with that of offset wall overlaying pattern (**Figure 3-21a-c**), we further explore how wall overlayer patterns affect the mechanical performance in cuttlebone. As shown in **Figure 3-21d**, the offset model exhibits larger septum deformation than the original model, suggesting that an improper overlay pattern could lead to premature septum failure, unfavorable for post-fracture performance. By contrast, the naturally formed overlapping, presumed as the optimal design, balances the deformation between septa and walls through a proper alignment (**Figure 3-5h**). Here quantitative
descriptions of the optimal alignment have not yet been achieved. Nevertheless, our results show that adding an arbitrary offset to the naturally formed pattern is generally unfavorable.

Figure 3-21 | Effect of the wall overlaying pattern on the septa stress. a, 3D view of the original wall overlaying pattern on the top and bottom of a septum, with the top and bottom walls highlighted. b, The original wall overlaying pattern based on μ-CT scan. c, The wall overlaying pattern with 50 μm offset based on the original μ-CT model. The offset model exhibits larger septum deformation than the original μ-CT based pattern (displayed with x50 magnification), indicating that an improper interlayer packing is disadvantageous to the integrity of septa. d, Comparison of the septum deformation in the original wall overlapping pattern of cuttlebone (original) and the overlay pattern with 50 μm offset (offset). (Figure credit: Zian Jia)

Finally, the stress distribution of a three-chamber structure reconstructed from μ-CT is compared to its straight-wall counterpart in Figure 3-22a-c. The results reveal that the straight walls exhibit much more significant stress concentration at multiple locations near the septa (marked by white arrows) compared to the wavy-wall model (Figure 3-22b,c). Statistical analysis of the stress magnitudes at wall middle, wall end, and septa are further conducted to gain a quantitative understanding of the stress distribution (Figure 3-23). Compared to the straight wall
structure, the wavy design, on the one hand, reduces stress on the septa and the wall ends, while on the other hand, raises the stress at the middle portion of the walls. Remarkably, all these stress shifts are beneficial: 1) the reduced stress on the septa improves septa integrity, which leverages a higher plateau stress level and a more pronounced densification, 2) the higher stress level at the middle portion of the walls facilitates crack initiation therein, which controls the damage to a consistent location and thus improves structure robustness, and 3) the asymmetric stress distribution at the two wall ends facilitates asymmetric wall fracture, which is critical to the enhanced post-fracture contact performance. These stress shifts explain the asymmetric fracture, and significant densification observed experimentally, demonstrating nature’s ingenious design to achieve a stiff and damage tolerant material by arranging proper waviness gradients to the “wall-septa” structure.

Figure 3-22 | Stress distribution comparison between the three-chamber models with (a) wavy walls, (b) straight walls based on the bottom profile, and (c) straight walls based on the top profile under increasing compressive strains (see Figure 3-2 for methods of generating these models). As marked by the white
arrows (high-stress regions), the straight wall-based models exhibit much more significant stress concentration than the wavy wall-based model. Unfavorably, these stress concentration regions are near the septa, which makes the septa prone to premature penetration. (Figure credit: Zian Jia)

Figure 3-23 | Statistical analysis of the stress distributions on the septa, in the middle portion of the walls and at the ends of the walls. Compared to the straight wall-based structures, the wavy wall-based structure presents reduced stress on the septa and at the ends of the walls but increased stress in the middle portion of the walls. Note that 1. The reduced stress on septa improves septa integrity. 2. The improved stress at the middle portion of the walls localizes wall fracture to the middle region. 3. The asymmetric stress distribution at the two wall ends of the wavy wall facilitates asymmetric fracture. Also, note that 1IQR represents the range within 1 interquartile range (minimum + 1IQR ~ maximum data – 1IQR). (Figure credit: Zian Jia)
3.3.5 Structure designs of cuttlebone

*Balance for stiffness, damage tolerance, low density, and openness*

Combining the experimental and computational results, a representative stress-strain response of the cuttlebone under compression is summarized schematically in [Figure 3-24a](#), which exhibits a two-scale characteristic behavior. Macroscopically, it presents an elastic response followed by a large, serrated stress plateau and then densification. The plateau has a strain regime that consists of regularly shaped periods, \( n\varepsilon_{pp} \) (\( n \) and \( \varepsilon_{pp} \) shown in [Figure 3-24](#)), which is determined by the chambered microstructure. More specifically, each chamber contributes to a fluctuated period ([Figure 3-24b](#)) characterized by three stages discussed earlier. As illustrated in [Figure 3-24c](#), the enhanced contact (green arrows) and directional septa penetration (red arrows) are characteristic at the chamber level. Ultimately, the balance between stiffness and damage tolerance in cuttlebone is contributed by micro-walls, where the wavy corrugated shape leverages both effective stress transfer and extensive densification ([Figure 3-24d](#)). Based on these schematics, the macroscopic response of the cuttlebone can be correlated to its microstructure by

\[
\begin{align*}
\varepsilon_{pp} &= (h_0 - h_d)/h_0 \\
\varepsilon_{vp} &= (h_c - h_d)/h_0,
\end{align*}
\]

where \( n, \varepsilon_{pp}, \varepsilon_{vp}, h_t, h_b, h_d, \) and \( h_0 \) are parameters introduced earlier and noted in the figure. These equations verified that enhanced contact (larger \( h_c \)) and improved septa integrity (smaller \( h_d \)) are critical for the macroscopic response.

Finally, we highlight the outstanding performance of cuttlebone through a comparison with other cellular materials, aiming to provide insights for bio-inspired microstructural designs. Compared to the cancellous bones and echinoderms’ stereom based on open-cell branch-node designs\(^{105,199}\), the cuttlebone-like structure exhibits notably higher porosity. Higher specific stiffness could also be expected, as we have shown that the cuttlebone-like structure is three times
stiffer than the octet truss with the same porosity. On the other hand, the wall-based yet open structure of cuttlebone offers notable fluid permeability, which is distinct to other wall-based cellular materials such as woods, honeycombs, and metafoams. This wall-septa design where each chamber is completely separated from others through septa also ensures the skeleton’s buoyance regulation function even if some chambers are damaged. Such a balance of low density, stiffness, damage tolerance, openness, and functional robustness in cuttlebone makes it a remarkable design motif for potential applications such as sandwich cores, heat exchangers as well as spacecraft, and engine rotator blades.

Figure 3-24 | Correlation between the macroscopic response and the microstructure of cuttlebone. a, Cuttlebone exhibits the typical stress-strain response of cellular materials macroscopically. b, Each period in the plateau regime is characterized by local penetration (LP), expansion (EXP), and...
densification (DENS). The chamber level deformation exhibits local wall fracture, extensive wall contact (green arrows), and septum fracture induced by fractured wall segments (red arrows). Asymmetric fracture at the wall level. $h_0$, $h_t$, $h_b$, and $h_d$ are wall heights noted in the plot. $H_0 = nh_0$ is the sample height, and $h_c = h_t + h_b$ is the contact height. The geometric parameters are related to the peak-to-peak, and peak-to-valley strains by $\varepsilon_{pp} = (h_0 - h_d)/H_0$ and $\varepsilon_{vp} = (h_c - h_d)/H_0$. (Figure credit: Zian Jia)

3.4 Discussion and conclusion

Combining multiple experimental techniques, we reveal that cuttlebone with a honeycomb-like structure derives high energy absorption and damage tolerance from its asymmetric wall fracture, extensive densification, and chamber-by-chamber failure. Our parametric simulations further provide quantitative knowledge of how wall waviness, wall overlaying, and their statistic variations enhance the mechanical performance synergistically. Together, our analysis establishes the relationship between the macroscopic response of cuttlebone and its microstructure and reveals that the cuttlebone is optimized for lightweight, high stiffness, and high energy absorption simultaneously. Here we highlight several important strategies learned from this study on cuttlebone for the design of novel honeycomb-like engineering cellular ceramics and lattice metamaterials. First, the wavy corrugated walls possess stiffness close to straight walls (>95%), yet they control maximum stress to well-defined locations, opening an avenue to “manipulate” the fracture path. This approach can be utilized to improve the reliability of ceramics lattice materials whose fractures often initiate at random unknown defects. Second, utilizing asymmetric structural characters like waviness gradient in cuttlebone, asymmetric fracture and directional damage propagation can be introduced. Here, we have shown that asymmetric fracture contributes to
remarkably better post-fracture performance in cuttlebone. Similar designs could be useful in lightweight, protective systems where high energy absorption is desired, and the direction of protection is of more importance. Third, statistical variations of the microstructure play an important role in damage tolerance. In cuttlebone, both the wall shapes and wall alignment have statistical variations and are found to facilitate more progressive failure. Finally, we note that further research is required to elucidate the effects of the intrinsic mechanical properties of the biogenic aragonite in cuttlebone, particularly the contribution of the intracrystalline organics, which may also contribute to the observed mechanical performance of cuttlebone.

In this chapter, I performed synchrotron experiments and electron microscopy measurements. Ziling Wu and I developed the cellular network analysis algorithm together.

The mineralized skeletons of echinoderms are characterized by their complex, open-cell porous microstructure (also known as stereom), which exhibits vast variations in pore sizes, branch morphology, and three-dimensional (3D) organization patterns among different species. Quantitative description and analysis of these cellular structures in 3D are needed in order to understand their mechanical properties and underlying design strategies. In this paper series, we present a framework for analyzing such structures based on high-resolution 3D tomography data and utilize this framework to investigate the structural designs of stereom by using the spines from the sea urchin *Heterocentrotus mamillatus* as a model system. The first paper here reports the proposed cellular network analysis framework, which consists of five major steps: synchrotron-based tomography and hierarchical convolutional neural network-based reconstruction, machine
learning-based segmentation, cellular network registration, feature extraction, and data representation and analysis. This framework enables the characterization of the porous stereom structures at the individual node and branch level (~10 μm), the local cellular level (~100 μm), and the global network level (~1 mm). We define and quantify multiple structural descriptors at each level, such as node connectivity, branch length and orientation, branch profile, ring structure, etc., which allows us to investigate the cellular network construction of *H. mamillatus* spines quantitatively. The methodology reported here could be tailored to analyze other natural or engineering open-cell porous materials for a comprehensive multiscale network representation and mechanical analysis.
4.1 Introduction

The skeletons of echinoderms, such as sea stars, sea urchins, sand dollars, and brittle stars, are among the most remarkable biomineralized cellular structures in nature. As shown in the sea urchin spine structure in Figure 4-1, these skeletons are characterized by their complex bicontinuous porous structure, also known as stereom. The stereom structures also often display controlled gradients in porosity and structural variations. Remarkably, despite their complex internal microstructure, individual stereom-based skeletons are single crystals based on magnesium-bearing calcite \((\text{Ca}_{x}\text{Mg}_{1-x}\text{CO}_3)\) (mineral content > 99 wt\%). Although calcite is inherently weak and brittle, echinoderm skeletons exhibit high strength and excellent damage tolerance. Previous studies indicated that the strength-to-weight ratio of sea urchin spines is greater than that of brick and concrete, which is attributed to the crack confining effect from the highly porous structure. Such mechanical behavior contributes to the protection of echinoderms from the impact, wear, and fracture resulting from the hydrodynamic forces of waves (many echinoderms live in the intertidal zone) as well as predators. Additionally, some researchers recently demonstrated that such mechanical robustness can be translated to synthetic systems, further highlighting the importance of the structural designs of echinoderms’ cellular structures.

The echinoderms’ stereom structures were previously examined by 2D microscopic imaging techniques. Following Smith’s classification on stereom’s structural types, the stereom structures in \textit{H. mamillatus}’ spines have been qualitatively described as the laminar stereom with a multilayered construction in the center region (also known as medulla), the labyrinthic stereom with radial alignment (known as septa) between center and growth rings, and the micro-perforate stereom with a much lower porosity in the growth rings, which represents one
of the most delicately designed stereom structures among all sea urchin species\textsuperscript{93,101,145,149}. Analysis of morphological variations in sea urchin spines’ stereom has later been conducted by using parameters such as pore diameter and branch size\textsuperscript{211,212}. X-ray micro-computed tomography (μ-CT) has been used for structural visualization of the stereom morphology in 3D and porosity estimation\textsuperscript{150}. Local extracted 3D volumes are also utilized for computational mechanical modeling\textsuperscript{146,213}. More recently, Grun and Nebelsick conducted an in-depth investigation of the stereom structure from an echinoid’s plate by using a commercial tomography data analysis code \textsuperscript{214}. This study analyzed structural parameters such as node configuration, branch length, length ratio, tortuosity, radius, orientation, and inter-branch angle for local regions\textsuperscript{214}. Currently, we still lack a systematic methodology that allows for 3D structural quantification of stereom structures from individual branch and node level to the global network level. It further limits our understanding of stereom’s mechanical designs and capability of developing stereom-inspired cellular material systems.

In this chapter, a comprehensive analysis pipeline for acquiring, processing, and analyzing the 3D cellular structures of stereom in echinoderms is presented. By using synchrotron-based μ-CT data, this methodology allows for efficient feature recognitions of the complex cellular structures at three length scales, \textit{i.e.}, individual node and branch level (~10 μm), local cellular level (~100 μm), and global network level (~1 mm). The structural descriptors at each level allow for an accurate and comprehensive representation and analysis of the stereom’s 3D porous structure. This chapter is the first part of the work series, which presents the detailed methodology of this data analysis pipeline and demonstrates its capability by using a representative volume from sea urchin spines. The second part of this work analyzes large-volume structural variations in sea urchin
spines enabled by this methodology and investigates the underlying mechanical design strategies\textsuperscript{202}.

Figure 4-1| The cellular structure in the spine of *H. mamillatus*. a, Photograph of a live *H. mamillatus* sea urchin (Image credit, Eric Noora). b, Scanning electron microscopic (SEM) image of the biomineralized cellular structure.

4.2 Methods

4.2.1 Overview of the network analysis pipeline

For the 3D structural quantification of echinoderm’s cellular microstructure, we combined existing and customized 3D imaging analysis algorithms into a five-step cellular network analysis
pipeline based on the high-resolution tomography data (Figure 4-2). This pipeline enables the high-fidelity rendition of echinoderm’s complex 3D cellular microstructure with a dramatically reduced data volume (compression ratio, ca. 1000). This pipeline is also modular and can be further modified for network analysis of other biological or engineering open-cell porous structures. The five-step analysis pipeline is briefly summarized below:

(1) **Tomography reconstruction.** The porous microstructure from sea urchin spines was imaged with a high-resolution synchrotron-based µ-CT and subsequently reconstructed by using a Fourier grid reconstruction algorithm implemented in the open-source software TomoPy. We also developed a hierarchical convolutional neural network (CNN)-based reconstruction method, where the multiscale structural characteristics of sea urchin spines are imposed as priors during the reconstruction process to improve reconstruction quality.

(2) **Image segmentation.** The segmentation of the reconstructed µ-CT images is achieved through a customized machine learning method based on the training of CNN networks at multiscale length scales or using an open-source software Ilastik.

(3) **Network registration.** The binarized volumetric data after segmentation is used to construct a skeletonized network structure, which is further refined through adaptive trimming and node-merging treatments. This step allows the registration of branches, nodes, and connectivity for sea urchin spines’ cellular structure.

(4) **Multiscale feature extraction and analysis.** The quantitative information of the registered cellular network is established at three length scales, i.e., the individual branch and node level, the local cellular level, and the global network level, by defining and analyzing a series of structural descriptors.
(5) Data representation, visualization, and additional analysis. A number of custom-written codes are developed to present and visualize the results of structural descriptors and to convert them into data formats for additional analysis (such as finite element modeling) and visualization.

![Workflow of the cellular network analysis pipeline developed in this work.](image)

**4.2.2 Tomography data acquisition and reconstruction**

Dry spine specimens from sea urchin *H. mamillatus* (diameter, 10-15 mm, **Figure 4-3a**) were used as a model system. A cylindrical coordinate system ($L =$ longitudinal, $C =$ circumferential, $R =$ radial) was used throughout this work. A typical procedure for sample preparation and tomography measurement is summarized below. Transverse sections (thickness, ~1 mm) were cut from spines by using a low-speed diamond saw (**Figure 4-3b**). Subsequently, a rectangular block along the radial direction was cut from the circular section (boxed area in **Figure 4-3b**). Two strips
were then imaged via synchrotron-based μ-CT measurements at the beamline 2-BM at Advanced Photon Source, Argonne National Laboratory. A monochromatic beam with an energy of 27.4 keV was used. Each tomography scan consisted of 1500 projection images for a 180-degree rotation with a rotation speed of 0.5 degrees/second. The projection images were collected by using a PCO Edge high-speed CMOS detector (2560 × 2160 pixels). For the first strip, the system was equipped with a 10x long-working-distance objective lens, which resulted in an isotropic voxel size of 0.65 μm. This ensures enough resolution for analyzing the local branch morphology with at least 10 voxels sampled across branch cross-sections. For larger samples, we extended the field of view by collecting multiple scans with ~90 μm overlaps and then stitching them using Fiji/ImageJ\textsuperscript{168}. For the second strip, the system was equipped with a 5× long-working-distance objective lens, resulting in an isotropic voxel size of 1.3 μm in the reconstructed tomography data with the field of view of 3.33 mm × 1.69 mm. By collecting multiple tomography scans in the R direction with an overlap of approximately 200 μm and stitching the volumes together, the field of view was extended in the R direction, and a single 3D dataset for the entire rectangular block was thus obtained. Scanning electron micrographs were obtained from FEI Quanta 600 FEG with a typical working distance of 8 mm and an acceleration voltage of 5 keV.
Figure 4-3 | **Overview of the spine structure.**  

**a.** The isolated individual intact *H. mamillatus* spine (left) and a spine cross-section cut along the longitudinal direction (right). The longitudinal direction (L) is defined as the axial direction of the spine.  

**b.** An optical image of the transversely-cut cross-section of a spine, as illustrated in (a). A cylindrical coordinate is used in this work with two additional orientations defined: $C =$ circumferential and $R =$ radial directions. The dark circular lines are the growth rings.
Figure 4-4 | Overview of the cellular structure in sea urchin spines. The boxed region in Figure 4-3 is shown in high magnifications with different imaging techniques, including (c) optical image, (d) SEM image, (e) stitched projection image from synchrotron X-ray measurement, and (f) reconstruction image. The yellow arrows and yellow lines indicate the growth rings and septa, respectively. The white arrow indicates in (f) the location where the representative volumes used in this work were extracted.

The tomographic reconstruction of the collected projection images was achieved through two methods: a standard Fourier grid reconstruction algorithm implemented in TomoPy\textsuperscript{166,215} and a custom-developed hierarchical CNN method\textsuperscript{216}. For both methods, the CT imaging process is modeled as the Radon transform $R$, where the projection measurement (also known as sinogram) $g$ is obtained through the integral along each projection line $l$ of the object function $f(r)$ as

$$g(s, \theta) = R[f(r)] = \int_{l: r \cdot \theta = s} f(r) \, dr$$  \hspace{1cm} \text{Equation 4-1}
where $\theta$ is the projection angle, $s$ denotes the position on the detector, $f(r)$ represents the X-ray linear attenuation coefficients at different voxels of the object. A CT reconstruction problem is formulated as retrieving the unknown function $f(r)$ based on the observed sinogram $g(s, \theta)$. TomoPy provides an analytical reconstruction method based on Fourier grid reconstruction algorithm\textsuperscript{215}. The reconstruction artifacts and noises associated with this reconstruction is suppressed by removing abnormal pixels and stripes from the sinogram $g(s, \theta)$ before reconstruction\textsuperscript{217,218}. To minimize the diffraction-induced blurring at the sharp interfaces in the porous stereom, a phase retrieval step was conducted to sharpen the boundaries of the reconstructed objects\textsuperscript{219–223}. Desired sharpness of edges was obtained by manually tuning the relative strength between the absorption and the phase diffraction (Figure 4-5a,b).

Instead of manually tuning parameters analytically, the hierarchical CNN reconstruction algorithm relies on a data-training process to separate the artifacts and noise from the structure. Due to the adaption to the targeted dataset, this method can greatly increase reconstruction efficiency. We have noticed that the porous spine specimens display structural features on three different length scales, including

1. at the individual voxel level, each voxel is categorized into one of the two phases, i.e., solid from the branches and nodes, void from the porosity. The boundary between the two phases is clear and smooth;

2. at the local cellular level, the branches and nodes are formed with similar shapes. The structure is bicontinuous, which means each phase is interconnected without the presence of isolated elements, i.e., isolated solid particles or completely embedded holes. Moreover, the branches within a local region (~100 $\mu$m) are characterized by a similar thickness and length;
3. and at the long-range network level, the arrangement of branches and nodes are uniform and semi-periodic.

The hierarchical CNN architecture allows us to learn the structural characteristics of stereom at these three length scales, respectively. This is achieved by filtering the reconstruction images through three different spatial frequency filters and training individual CNNs on each scale, respectively. These learned structural characteristics $\delta_i$ are imposed as priors during the reconstruction process, which is implemented by formalizing a constraint optimization problem by

$$\hat{f} = \arg\min_x \frac{1}{2} \left| \left| Rf - g \right| \right|^2 + \sum \lambda_i \| \delta_i(f) \|^2$$

Equation 4-2

Here the first term represents the fidelity term (fitness), which contains the difference between the object $f(r)$ and the sinograms $g(s, \theta)$. The second term is the regularization term that describes the structural priors. These learned structures are then combined to generate the full-scale $\mu$-CT reconstruction that suppresses noises and artifacts. This method allows for high-fidelity reconstruction with reduced projection images and exposure time. The reconstruction based on TomoPy with 75 projections exhibits severe noise and artifacts in the background (Figure 4-5c). In contrast, our reconstruction method can achieve comparable reconstruction based on 75 projections with that from the TomoPy method with 1500 projections (Figure 4-5d). In order to demonstrate the capability of the cellular network analysis algorithm, all the results reported in this work are based on the reconstructions by using the standard TomoPy method.
**Figure 4-5** Comparison of tomography reconstruction results. Representative reconstruction images from TomoPy with 1500 projection images (a) without and (b) with phase retrieval treatment. Corresponding reconstruction images with 75 projection images obtained from (c) Tomopy and (d) the custom-developed hierarchical CNN reconstruction method.

### 4.2.3 Image segmentation

Two approaches were utilized to segment the reconstruction data for further cellular network analysis. The first approach was based on the open-source software, Ilastik\(^{167}\). A customized machine learning-based segmentation method was also developed to reduce segmentation artifacts using a data-driven learning process. In this method, we first generated the training dataset, consisting of the reconstruction slices with manually labeled segmentation. An image translation CNN is trained on the training dataset, which produces segmentation estimates for future input of reconstruction slices. Similar to the hierarchical reconstruction algorithm, we focused on the three featured length-scales of the sea urchin spines during the training process. This method yields comparable results as Ilastik but with much reduced manual work (**Figure 4-6**). This hierarchical CNN approach proved to be robust in reducing various noises and artifacts in our \(\mu\)-CT reconstructions\(^{224}\). In this paper, our cellular network analysis is based on the segmentation results from Ilastik, following the same argument regarding the reconstruction step.
Figure 4-6 | Comparison of segmentation results. a, Original reconstruction image via Tomopy and corresponding segmented results by (b) Ilastik and (c) customized machine learning method. 3D view of (d) original reconstruction result, segmented result from (e) Ilastik, and (f) customized method.

4.2.4 Network construction and registration

The segmented volumetric data was used as the input for the cellular network analysis, followed by the procedures below.

**Skeletonization:** A custom-written 3D thinning algorithm based on an iterative erosion method was developed to extract the 3D skeleton of the cellular structure from the binarized data, which represents individual branch as a line with one voxel thickness225,226 (Figure 4-7a-c). The
skeleton of the cellular structure consists of a network of branches interconnected with nodes. The
node of the skeleton is defined as the voxel with more than two neighbor voxels. The position of
the node is also used to register individual branches by specifying the starting and ending nodes.
The network after the direct skeletonization treatment suffers from several inherent artifacts,
including extrusions from dangling branches (Figure 4-7d) and node clusters characterized by
unrealistic short branches, which will be addressed in the following steps (Figure 4-7e, f). The
current skeletonization method works well with the sea urchin spine’s porous structures consisting
of rod-like branches; however, for porous structures with “plate-like” morphologies as in some
trabecular bone, further improvement of the algorithm is required.

Adaptive trimming: To refine the skeletonized network, we applied an adaptive trimming
process to remove extruded branches that are not connected to the network at one end (Figure
4-7e). These extruded or dangling branches may be due to the local surface variation of the cellular
structure. In addition, branches that are cut off by the volume boundaries also exhibit extruded
branches (red arrows, Figure 4-7e). Extruded branches are identified by inspecting the
connectivity and lengths of branches within the analyzed network. As the side effect of this
removal step, nodes connected by two branches, which are referred to as N-2 nodes, emerge. We
identified each pair of branches connected by these N-2 nodes and connected these branch pairs
by replacing these N-2 nodes with branch points. The connectivity of the network is updated after
each trimming step until all the extruded branches are removed, as shown in an example in Figure
4-7g.

Node merging: After removing the extruded branches with the adaptive trimming, further
refinement was conducted on nodes. The node cluster that is composed of nodes connected with
very short branches is considered as artifacts of skeletonization (Figure 4-7f). We first dealt with
one special case of node cluster, *i.e.*, adjacent nodes that are directly connected without branch points between the nodes. Connected nodes are results from the skeletonization of the non-smooth discrete volume, where extrusions are recognized as branches with unnecessary nodes. We replaced connected nodes with one single node, the coordinate of which is in the center coordinate of the node cluster. The connectivity of the network is updated after this merging procedure. For the general case of node clusters, we removed short branches and merged the node cluster into one node in the center of the cluster. In our analysis, we set the threshold of merging distance $d_m = 10$ voxels (= 6.5 $\mu$m) to be roughly the mean branch radius (6.2 $\mu$m). As shown by a typical example in Figure 4-7h, many node clusters are present in the original skeletonized network, and this merging process is critical to clean the unnecessary nodes and short branches.

The aforementioned steps establish the cellular network of the original tomography data of the sea urchin spine’s porous structure. The network information, including nodes, branches, and their associated surface profiles, is registered in terms of a weighted graph $G = \{N, B, P\}$, where $N$ and $B$ represent nodes and branches, respectively. The associated surface profiles, $P$, for individual branches are registered by using the binarized volumetric data. This allows for further structural quantification, such as branch thickness, surface curvature, and node diameter. The size of this combined network representation is 0.1% of the original tomography data, yet it carries the essential geometric information of the original structure.
Figure 4-7 Network construction. a, A representative volume based on the segmented binary data. b, 3D rendering of the cellular structure, and corresponding (c) cellular network consisted of branches (gray lines) and nodes (red dots) after initial skeletonization and (d) after adaptive trimming and node merging. e, Schematic illustration and (g) a corresponding representative example of the adaptive trimming process, which removes extruded dangling branches and associated nodes. f, Schematic illustration and (h) a corresponding representative example of the node merging process, through which node clusters with distances between adjacent nodes smaller than a critical distance, $d_m$, will be merged to a single node.

4.2.5 Feature extraction and analysis
In this step, we developed an automatic computer vision-based pipeline to extract, classify, and analyze the structural descriptors of the registered cellular network and surface morphology at three length scales, namely, the individual node and branch level, the local cellular level, and the global network level (Figure 4-8).

**Individual node and branch level:** The network connectivity registration identifies individual branch, $B_{ij}$, which is bounded with two end nodes, $N_l$ and $N_j$, as shown in Figure 4-8-i. The branch length is calculated with two metrics, the Euclidean distance, $l_{ij}$, defined as the straight distance between the starting and ending nodes and the physical branch length, $l_{o,ij}$, obtained by accumulating the distances in all neighboring voxels on the branch. The physical length $l_{o,ij}$ records the curviness of the branch and therefore is usually greater than the Euclidean distance $l_{ij}$.

We define the branch length ratio as $s_{ij} = l_{o,ij}/l_{ij}$. The branch orientation is characterized by two angles, the misorientation angle, $\theta$, and the in-plane rotation angle, $\omega$. $\theta$ is defined as the angle between the branch vector and a global reference direction (e.g., the $L$ direction). For calculation of the in-plane rotation angle $\omega$, the branch vector is first projected to the plane perpendicular to the defined global direction (e.g., the $R-C$ plane for the $L$ direction). $\omega$ is then defined as the angle between the projection direction and another selected in-plane reference direction (e.g., the $R$ direction). The in-plane misorientation angle $\omega$ ranges from $0^\circ$ to $360^\circ$.

The branch profile, $P_{ij}(l)$, is used to describe the cross-sectional size and morphology along the length of each branch (Figure 4-8-i). This is achieved by registering the intersecting line profiles between the cross-sectional planes (perpendicular to $B_{ij}$ at the sampling point) and branch surface at a given location of $l$. This allows for the determining the branch profile, $P_{ij}$, by using the radius of an equivalent circle with the same area of the intersecting profile. The thickness in
the middle of a branch is denoted as branch thickness, \( t_{o,ij} \). The local branch thickness profile \( P_{ij} \) is fitted with a second-order polynomial

\[
P_{ij}(l)/t_{o,ij} = 1 + a(l/l_{o,ij}) + b(l/l_{o,ij})^2,
\]

Equation 4-3

where \( a \) and \( b \) are fitting parameters.

Following the previous work on the inter-trabecular angle measurement method developed by Reznikov et al. for the analysis of trabecular bone \(^{227,228}\), we quantified the node connectivity and characteristics of the cellular structure of sea urchin spines (Figure 4-8-ii). First, the node type is categorized by the number of branches connected to a given node as N-3 (with three connecting branches), N-4 (with four connecting branches), ..., and N-n (with \( n \) connecting branches). The inter-branch angle for different node types is defined following the similar approach described in \(^{227,228}\). For example, for N-3 nodes, the three inter-branch angles are denoted as \( \gamma_{3,k} \), where \( k \) (1, 2, and 3) indicates the three angles in an ascending order. The average inter-branch angle for a N-3 node is denoted as \( \gamma_{3} \). Similarly, the inter-branch angles for N-4 nodes are represented by \( \gamma_{4,\text{min}}, \gamma_{4}, \gamma_{4,\text{max}} \) representing the minimum, average and maximum values.

To analyze the orientation of N-3 nodes, we defined a central axis as the node orientation that shared the same angle with each individual branch of an N-3 node. The misorientation angle \( \theta \) and the in-plane rotation angle \( \omega \) are defined in a similar way for the branch orientation. The direction of the central axis is selected so that the angle between the central axis and each branch is less than 90 degrees. Due to this convention, the misorientation angle \( \theta \) ranges from 0° to 180°, which is different from the \( \theta \) range of branch orientations. Following the previous work on trabecular bone \(^{227,228}\), we defined the cosine of the angle between the central axis and each branch as the planarity.
index, which varies between 0 and 1. The plane formed by N-3 nodes is flatter when the planarity index is closer to 0.

*Local cellular level:* In this scale, we investigated the local interconnection characteristics of adjacent branches. An algorithm was developed to quantify the number of branches required to form a complete ring structure (*Figure 4-8-iii*). Briefly, the analysis process is as following: starting from a given node, a “tree-shape” structure is constructed by identifying the neighboring nodes that are connected to the starting node. As this process continues to expand the “tree” structure, a ring is identified when the original node is included in the new layer of connections. This indicates that the node is connected back to itself in a non-repeated manner. The number of branches included in a complete ring is then used to define the ring type. For instance, a 5-B ring refers to a ring composed of 5 branches. We calculated the area enclosed by the ring and fitted it into a circle. The diameter of the fitted circle is defined as the ring size (\(d_{\text{ring}}\)). All branch points that form a ring are fitted into a plane, the normal direction of which is defined as the ring direction. The ring orientation is characterized by following the same definition of branch orientation and N-3 node orientation.

*Global network level:* The alignment of connecting branches over a long distance is investigated by defining a branch alignment factor as 
\[
CAF_{ij}^k = \max \prod_{k=\text{i},i+1\ldots}(c_k \cdot c_{k+1})\beta_{k,k+1},
\]
where \(c_k\) is the unit vector of the normal direction of the \(k_{th}\) branch in a “chain” structure starting from the branch of interest. By including the inter-branch angle \(\beta_{k,k+1}\) between two neighboring branches in the chain structure, \(CAF\) measures the degree of alignment for the most aligned \(k\)-node chain structure (*Figure 4-8-iv*). An iterative algorithm is implemented to detect aligned chain structure with minimized \(CAF\). The quantification of inter-branch angle \(\beta\), length and thickness
for branches in the detected chains, and offset angle $\phi$ (the angle away from a defined global direction) can be performed to evaluate the structural characteristics of these chains.

We further proposed to quantitatively describe the structural ordering at the global scale by conducting the long-range 3D fast Fourier transform (3D-FFT) analysis of the structural descriptors extracted from the full-volume registered network. In particular, we applied Fourier analysis on registered node positions over large volumes ($>0.1$ mm$^3$) to investigate its long-range periodicity. Note that directly implementing a Fourier analysis on the original 3D tomography data with such large volume is computationally expensive and not necessarily informative due to the mixing of structure information on all different scales. The 3D-FFT analysis based on the node distribution resolves these challenges and provides a clear representation of the global orderliness or randomness of the cellular network.
Figure 4-8 | A multiscale representation scheme for the cellular network of sea urchin porous structures. On the individual branch and node level (panel i and ii), each branch is denoted as $B_{ij}$ bounded by two nodes $N_i$ and $N_j$. The branch is characterized by its length ($l_{o,ij}$), Euclidean distance ($l_{ij}$), orientation ($\theta_{ij}$ and $\omega_{ij}$), thickness ($t_{o,ij}$) and morphology profile. $\theta_{ij}$ is the misorientation angle of the branch from the $L$ direction. $\omega_{ij}$ denotes the angle between the branch projection and the $R$ direction in the R-C plane. The branch morphology along a branch is captured and fitted into a quadratic function ($P_{ij}(l)$). On the local cellular level (panel iii), the node types (represented by colored dots) and ring structures formed by connected branches (highlighted in red) are registered. The ring diameter is denoted as $d_{ring}$. On the global network level (panel iv), the long-range alignment of branches, denoted as “branch chain”, can be identified. Two angles are defined: $\varphi$, misorientation angle of a branch in the chain from the $L$ direction, $\beta$; inter-branch angle between two adjacent branches in the chain.

4.2.6 Data presentation, visualization and additional analysis

A variety of data representation schemes were developed with custom-written MATLAB scripts to interrogate the results of cellular network analysis. These include (1) distribution of individual descriptors, (2) polar plots for orientation-dependent correlative analysis among multiple descriptors, and (3) direct representation of descriptors within the 3D skeletonized cellular
network. We also developed several 3D visualization strategies of the analyzed cellular network: (1) presentation of selected descriptors (such as branch thickness, length, or node connectivity, etc.) with a color scale in the 3D skeletonized cellular network in MATLAB directly. The binarized original tomography data can also be imported simultaneously to interrogate the cellular network results. (2) Similar results can be achieved by generating image stacks with selected descriptors (e.g., N-3 nodes or $l_{o,ij} >$ a critical value) and imported into and visualized with a commercial tomography analysis software Avizo (Avizo 9.5, Thermo Fisher Scientific, USA). (3) Beam models can be generated using a customized Python script to join intersecting beams based on the branch connectivity and assign thickness for each branch individually. The third visualization scheme also enabled us to conduct systematic mechanical modeling on the cellular network by using a commercial finite element analysis code Abaqus (Abaqus 2016, Simulia, USA), which will be discussed in the following paper of this work series.

4.2.7 Implementation and accessibility

The customized cellular network analysis algorithm has been implemented in MATLAB. It is available for download at https://github.com/Ziling-Wu/Quantitative-3D-structural-analysis-of-the-cellular-microstructures.

4.3 Results

In this work, the spines were chosen from sea urchin *H. mamillatus* as our model system. The *H. mamillatus* spines exhibit a porous gradient microstructure, where the porosity gradually reduces from 80 vol% in the center to 60 vol% in the edge region\(^{150}\). However, the detailed network organization of this structure on both local and global scales still remains elusive. In the current paper of this work series, a small region (200 μm (R) 250 μm (C) × 250 μm (L)) of the *H.*
*mamillatus* spines was used to demonstrate the capability of our cellular network analysis method. The representative volumes used in this work were extracted close to the center region of a spine (Figure 4-4).

4.3.1 Results of Network registration

The representative volume after segmentation is shown in Figure 4-9a. After the initial skeletonization, this volume contains 6342 nodes and 4574 branches. The trimming process reduces the numbers to 5126 nodes and 3584 branches, and the final merging treatment further refines to 1249 nodes and 2233 branches. This significant reduction in numbers also indicates the importance of our iterative trimming and node merging process. The density of nodes and branches are \( \approx 124900/\mu m^3 \) and \( 223300 /\mu m^3 \), respectively. Figure 4-9b shows the final registered network with the nodes and connected branches highlighted as red dots and grey lines, respectively.

4.3.2 Node characteristics

Due to the incomplete branches at volume boundaries, nodes (N = 289) and branches (N = 1030) within 20 \( \mu m \) from the boundaries are not considered in our further analysis. Within this volume, N-3 and N-4 nodes are dominating node types (50\% and 35\%, respectively) (Figure 4-9c,d). N-5 and N-6 nodes comprise 12\% and 3\%, respectively, where no N-7 and higher-branched nodes are detected. The relative frequencies of N-3: N-4: N-5: N-6 nodes are approximately 17: 12: 4: 1. Different types of nodes are uniformly distributed within this volume (Figure 4-9c).
Figure 4-9 | Distribution of nodes. a, 3D rendering and (b) corresponding skeletonized network of the analyzed volume close to the center region of an *H. mamillatus* spine. c, Skeletonized network with node types indicated with colored dots. d, Distribution of node types in this volume.

As the N-3 and N-4 nodes comprised the majority of all nodes, we further analyzed their inter-branch angles and node orientations. As shown in Figure 4-10a, the smallest, median, and largest inter-branch angle for N-3 nodes are $115.5^\circ \pm 20.3^\circ$, $119.4^\circ \pm 19.1^\circ$, and $119.4^\circ \pm 18.4^\circ$, respectively. The mean value of all inter-branch angles for N-3 nodes is $118.1^\circ \pm 3.5^\circ$ ($N = 447$) with the most frequent value of $120^\circ$. The measurement result is close to the ideal three-branched node with $120^\circ$ as the ideal inter-branch angle, which indicates that the three branches for an N-3 node in sea urchin spines are generally evenly orientated to span as much as possible in space.
The orientations of N-3 nodes are presented in a polar distribution plot by using the misorientation angle $\theta$ and in-plane rotation angle $\omega$. As shown in Figure 4-10b, most of the N-3 nodes are oriented perpendicular to the $L$ direction as they cluster around a circle with $\theta = 90^\circ$. In contrast, the distribution of $\omega$ is rather uniform between 0 and $360^\circ$, indicating a random distribution in the $R-C$ plane. Figure 4-10c shows the planarity index distribution of N-3 nodes. As introduced earlier, the planarity index describes the flatness of the plane formed by the N-3 nodes. 90% of the N-3 nodes have their planarity index less than 0.17, which is within $10^\circ$ offset from the ideal plane; about 95% of the N-3 nodes have their planarity index less than 0.27 (corresponding to $16^\circ$ offset). This indicates that the branches in N-3 nodes are roughly oriented in the same plane, consistent with the inter-branch angle measurement results.

For N-4 nodes, the smallest and largest inter-branch angles are $85.9^\circ \pm 13.6^\circ$ and $134.7^\circ \pm 16.7^\circ$, respectively, with a mean inter-branch angle of $110.0^\circ \pm 9.4^\circ$ ($N = 335$) (Figure 4-10d). This result approaches the ideal tetrahedral angle of $109.5^\circ$, which indicates that the branches connected to an N-4 node also tend to maximally span the space.
4.3.3 Branch characteristics

The branches in the sea urchin spines' stereom structure are characterized by curved surfaces. The registered skeleton network allows us to quantify the branch length and morphology reliably. The branch length can be color mapped on the skeletonized cellular network (Figure 4-11a). Long branches ( > 40.0 \( \mu m \)) are further extracted and displayed in Figure 4-11b, respectively. The correlation between branch orientation and length can be further visualized by using the polar scatter plot (Figure 4-11c). The long branches (cyan and yellow data points) typically have \( \theta = \)
90°, indicating that they are orientated in the $R-C$ plane. In addition, the uniform distribution of in-plane rotation angles $\omega$ shows no orientation preference in the $R-C$ plane.

The calculated branch thickness $t_o$ is shown in a colored 3D diagram mapped on the cellular network (Figure 4-11d). The thinner branches with $t_o < 3.3$ $\mu m$ are mostly oriented in the $R-C$ plane (Figure 4-11e), which is consistent with the polar plot in Figure 4-11f. The branch thickness as a function of their orientation distributions, the thicker branches (red and yellow data points) concentrate in the center of the plot (Figure 4-11f). This suggests that the branches aligned in the $L$ direction tend to be thicker, whereas the branches with $\theta$ values close to 90° typically have thickness smaller than 5 $\mu m$ (blue data points).
Figure 4-11 | Distribution of branch length and branch thickness. **a**, Network representation of branch length distribution, and corresponding volume (**b**) with branches $> 40.0 \, \mu m$. **c**, Correlation of branch orientation and length. (**a-c**) are based on the same color scale as in (**c**). **d**, Network representation of branch thicknesses ($t_o$). **e**, Distribution of branches with thickness $t_o < 3.3 \, \mu m$. **f**, Correlation between branch orientation and thickness. (**d-f**) are based on the same color scale for branch thickness.

More specifically, examples of these long branches are shown in **Figure 4-12a**, which confirms the preferred orientation in the $R-C$ plane. Short ($< 15.0 \, \mu m$) are further extracted and displayed in **Figure 4-12b**. These 3D representations reveal that short branches are distributed evenly in the volume. The physical branch length $l_{o,ij}$ and the Euclidean distance $l_{ij}$ are calculated, and their distributions are shown in **Figure 4-12c**. The mean physical length and Euclidean distance are
21.7 ± 7.9 μm and 18.0 ± 6.2 μm (N = 1203), respectively. The length ratio $s_{ij}$ is 1.2 ± 0.1, indicating that most of branches slightly deviate from straight connections (Figure 4-12d).

**Figure 4-12** Quantitative analysis of branch length and orientation. **a**, 3D rendering and SEM image of representative examples of long branches. **b**, Network representation of branch length distribution with branches < 15.0 μm. **c**, Distribution of branch length $l_o$ and Euclidean distance $l$. **d**, Distribution of the length ratio $s$.

The mean branch thickness is 6.2 ± 1.5 μm (N = 1203), and most of the branch thicknesses range from 4 to 8 μm (Figure 4-13a). In addition, the similarity between Figure 4-11c and Figure 4-11e leads us to further evaluate the correlation between the branch thickness and length. A negative correlation between the two parameters is found ($t_o = -0.14l_o + 8.14, R^2 = 0.39$,
**Figure 4-13b**. This result is consistent with the observations of some long (>40.0 μm) and thin (<3.3 μm) branches as demonstrated in **Figure 4-12a**.

In addition to the single branch thickness value at the branch middle point, the entire cross-sectional profile of each branch can be extracted as \( P_{ij} \) and then fitted with a 2\(^{nd} \) order polynomial according to Eq. 3 (**Figure 4-13c**). To study the general morphology characteristics of all branches, we normalized each individual branch profile with its own length \( l_o \) and thickness \( t_o \). The fitting result is \( P(l)/t_o = 1 - 0.026(l/l_o) + 1.23(l/l_o)^2 \) (\( R^2 = 0.899 \), **Figure 4-13d**). With previously measured \( l_o = 22 \) μm and \( t_o = 6.2 \) μm, this fitted profile represents the branch morphology well, as shown in a 3D rendering of a typical branch in the volume (**Figure 4-13e**). This finding indicates that the branch morphology in the sea urchin spines’ stereom is highly curved. In addition, the cross-sectional profiles of branches in stereom may be slightly non-circular (**Figure 4-13e**). These structural features are in stark contrast to the branch morphologies in synthetic cellular solids produced through the standard foaming process. The cross-section of the branches in these foams is characterized by the form of a three-cusp hypocycloid known as Plateau border\(^{229–232}\).
4.3.4 Local cellular level: ring characteristics

The ring detection algorithm is used to study the local branch organization characteristics. Figure 4-14a depicts four examples of detected ring structures with different ring types and the corresponding 3D renderings. As shown in Figure 4-14b, the complete ring detection in this representative volume can be achieved, which can be labeled and mapped to the cellular skeleton.
This volume contains 213 4-B rings, 577 5-B rings, 614 6-B rings, and 476 7-B rings (Figure 4-14c). All the ring types seem to distribute uniformly within this volume. The diameters of the 4-B, 5-B, 6-B and 7-B rings are 23.1 ± 5.8 μm, 28.2 ± 13.6 μm, 30.4 ± 5.0 μm, and 34.7 ± 7.0 μm, respectively (Figure 4-14d). This indicates that the ring size increases with the number of branches contained in a ring. The measured average diameter of all rings is 30.0 ± 9.6 μm (N = 1880). The ring size obtained here is broadly consistent to the pore diameter measurement based on 2D measurement of fully-grown H. mamillatus spines211. Lastly, our algorithm allows for determining the orientation of these rings. As shown in Figure 4-14e, most of the detected rings, regardless of their ring types, are aligned perpendicular to the R-C plane.
4.3.5 Global network level: chain alignment

We investigate the branch alignment by detecting “branch chains” and evaluating the misorientations among adjacent branches as well as with respect to a global reference orientation. As shown in Figure 4-9a and Figure 4-15a, the branches in this location appear to have preferred alignments in the $L$ direction. Our algorithm successfully detected these branch chains aligned in this direction (total number of detected chains = 62, Figure 4-15b). From the top view of the same volume, these branch chains appear to form a Voronoi-like pattern, which may be a result of the
biomineralization formation process along the $L$ direction\textsuperscript{233} (Figure 4-15c). The misorientation angle $\varphi$ with respect to the $L$ direction ranges from 0 to $50^\circ$, while the inter-branch angle $\beta$ between adjacent branches are $90^\circ - 180^\circ$. The length and thickness of the branches in these chains are $19.0 \pm 6.5 \, \mu m$ and $6.8 \pm 1.2 \, \mu m$ ($N = 957$), respectively (Figure 4-15d-f), which are shorter and thicker compared to the mean branch length and thickness of the whole volume, respectively. The preferred alignment of branches along the $L$ direction with shorter and thicker branches revealed here is expected to have a significant impact on the anisotropy of the mechanical properties, which will lead to enhanced resistance to withstand loads in the $L$ direction.
Figure 4-15 | Analysis of chain structures. a, 3D rendering of the cellular structure observed in the analyzed region close to the center of the sea urchin spines, from which the longitudinal alignment of branches can be qualitatively observed. b,c, Detected branch chains along the longitudinal direction viewed (b) from the side and (c) from the R-C plane. d, Distributions of the misorientation angles between a branch and the L direction (ϕ) and inter-branch angle between two adjacent branches (β) in the chain structures. Distribution of (e) branch length and (f) thickness for the branches in the detected chains in (b).

4.3.6 Global network level: 3D-FFT analysis

On the global network level, we utilize the 3D-FFT analysis to investigate the long-range organization characteristics of the cellular network. A larger region (530 μm (R) × 500 μm (C) × 500 μm (L)) in a similar location was used for this analysis. The same skeletonization and network
cleaning procedure were first applied to obtain the skeletonized network, which consists of 11540 nodes and 21049 branches (Figure 4-16a,b). The 3D-FFT analysis was performed on the node array of the skeletonized network. As shown in Figure 4-16c, localized high-intensity Fourier peaks in multiple directions with different periodicity are observed, which demonstrates the large-scale orderliness of the cellular network. Visualizing the FFT pattern in the L-C plane, the first-order Fourier peak in the L direction is at 0.034 μm⁻¹ away from the origin (d₁), corresponding to the diameter of the ring structures (~30 μm) aligned in this direction (Figure 4-16d). The second-order peak appears at 0.068 μm⁻¹ (d₂), corresponding to the average branch length detected in the chain structures in this direction (~15 μm). In the R-C plane, the circular distribution of the high-intensity region is observed at 0.022~0.052 μm⁻¹, which matches well with the branch length distribution (19~45 μm) in this plane (Figure 4-16e). The circular peaks also indicate random distributions of branches in the R-C plane, consistent with the result shown in Figure 4-11c.
Figure 4.16 | 3D-FFT analysis of nodes in the cellular network. a, Skeletonized network and (b) corresponding node distribution of the selected volume. c-e, Volumetric rendering of the 3D-FFT analysis of the volume shown in (b), shown in (c) side view, (d) view in the L-C plane, and (e) view in the R-C plane.

4.4 Discussion and conclusion

In this work, we introduced a computational tomography data analysis pipeline for investigating the 3D structure of natural porous materials, particularly the stereom structure for echinoderms. By using sea urchin spines as a model system, we systematically presented the development and capability of this multi-step methodology. For the preprocessing steps related to reconstruction and segmentation of tomography data, we developed a hierarchical CNN-based method by taking advantage of the inherent structural characteristics of the stereom structure exhibited at multiple length scales. We demonstrated that this approach not only can greatly minimize noises and artifacts but also allow for efficient compressive sensing compared to
conventional reconstruction and segmentation approaches. In terms of network construction and registration, although the iterative erosion method is standard and widely used for skeletonization, post-processing is required, especially for complex 3D porous structures like stereom. Our iterative, adaptive trimming and merging treatments are essential to achieve a clean and reliable cellular network. This step is crucial for robust analysis of structural descriptors later and for providing a reliable network model for mechanical modeling, as shown in the second paper of this work series 202. For the feature extraction and analysis step, we developed a series of descriptors to quantify the structural features at three different length scales, including 1) node type, inter-branch angle, node orientation and planarity, branch length, distance, length ratio, thickness, orientation, and profile at the individual node and branch level, 2) ring number, orientation, and size at local cellular level, and 3) branch chain and long-range orderliness based on 3D-FFT analysis at the global network level. This multiscale structural information for the 3D network organization can provide important insights for the understanding of the mechanical designs of biological cellular solids. In addition, as shown in Supplementary Information, the pipeline introduced here can be easily tailored to analyzing 3D printed lattice-like structures, providing an efficient tool for mechanical modeling and quality control of these structures.

Within the analysis volume, the porous stereom structure of sea urchin spines is primarily constructed with N-3 and N-4 nodes. A recent study revealed that the porous trabecular bone is also dominated by similar low-connectivity nodes227. The underlying principles for the formation of such low-connectivity nodes in natural systems are currently unclear. Regarding their mechanical implications, it is known that low-connectivity nodes tend to result in bending-dominated lattice structures, which is generally considered less effective compared to stretch-dominated structures8,25. Further study is required to better elucidate the mechanical roles of this
design strategy. In addition, similar to trabecular bone, the inter-branch angles for N-3 and N-4 nodes are very close to the values for idealized triangle and tetrahedron structures. This suggests that the branches connected to a common node tend to maximize the 3D space in stereom, similar to trabecular bone. As proposed by the previous study, such structural design may be beneficial to resist multidirectional loading.

The combined analysis results indicate the mechanical anisotropy of stereom structures can be controlled in a number of ways. First, branches can form preferred orientational alignment, as revealed by our branch chain analysis. Such strategy follows the concept of Wolff’s law, which suggested that the human femur may be strengthened in specific loading directions through the preferred alignment of individual trabeculae. Moreover, thanks to this branch chain analysis, we directly demonstrated that branches along these chains are thicker and shorter compared to the average values. This will further enhance the structural stiffness and strength in the alignment direction, i.e., the $L$ direction. Here we note that this preferred alignment along the longitudinal direction should be closely related to the fact that the analysis volumes are near the center region of the sea urchin spines. Such alignment may be directly affected by the microscopic biomineralization process, as a number of previous studies have shown that the stereom formation in the center region of spines initiates from the branches along the longitudinal direction. The Voronoi-like organization of branch chains in the transverse cross-section may also be closely related to this process. As shown in the second study of this work series based on the large-scale analysis, such structural alignment exhibits gradual local variations, leading to gradient mechanical properties within sea urchin spines.

Despite relatively small analysis volumes, our results reveal a number of important differences between sea urchin spines’ stereom structure and synthetic open-cell foams fabricated...
through conventional foaming processes\(^8,32\). First of all, most branches in the analysis volume are highly curved. The thickness profile can be fitted with a second-order polynomial. In contrast, the branches in synthetic foams produced through standard foaming processes often exhibit large portions with relatively constant thickness\(^{229-232}\). We also note that some long branches (length \(> 40 \mu m\)) in stereom exhibit relatively constant thickness over a long distance (Figure 4-11a). These branches appear to be reinforcing structures for the large holes in the Voronoi-like patterns in the R-C plane. Second, the cross-sections of stereom’s branches are generally circular, whereas, in synthetic foams, the branches exhibit a three-cusp hypocycloid known as Plateau border during the foaming process \(^{229-232}\). Third, our ring analysis indicates that 5-B and 6-B rings are dominating types in stereom, and the average number of branches per ring is estimated to be 5.7. This value is higher compared to that for synthetic foams with random polyhedral cell geometries (~5.1), which is governed by the well-known Euler’s law\(^{8,236-239}\). The difference revealed here is believed due to the difference in underlying formation mechanisms of the stereom’s porous structure and synthetic foams.

Increasing interests have been recently directed to mimicking the “biological forms” of cellular solids in engineering material systems to improve their mechanical performance\(^{240,241}\). In particular, 3D printing has been demonstrated as an effective approach for fabricating cellular solids with structural precision down to individual branch level\(^{19,46,242}\). 3D printing allows for the production of cellular structures not bounded by the physical constraints in the conventional synthetic foaming processes \(^8,32\). However, most of the current 3D-printed cellular materials are based on periodic tessellations of unit cells with idealized geometries, such as cylindrical beams with a constant cross-sectional area. These structures at both local and global scales are still far more simplified and less controlled compared to biological cellular structures. In this regard, the
methodology introduced here holds great potential in providing important insights for improved “mimicking” of the natural cellular structures via quantitative analysis of these materials in 3D.

In summary, a computational tomography analysis pipeline for multiscale structural representation and quantification of biological cellular materials is presented by using sea urchin spines as an example. At the individual branch and node level, this methodology allows for the quantification of a series of structural parameters, such as node type, inter-branch angle, orientation and planarity of nodes, branch orientation, length, thickness, and branch morphology profile. The analysis indicates that the porous structure of sea urchin spines is primarily composed of three- and four-branched nodes, which resembles ideal branched structures that span 3D space maximally. The thickness and length of branches are highly correlated with their orientation, where shorter and thicker branches are aligned with the longitudinal direction of the spines in the analyzed regions. The algorithm is able to extract the morphology profiles of individual branches, from which the highly curved branches can be fitted with a parabolic function, significantly different from synthetic foams. At the local cellular level, the algorithm allows for the investigation of the interconnections and alignments of adjacent branches by detecting branch rings and chains. 3D-FFT analysis of registered nodes over large 3D volumes is developed. The analysis results suggest that the cellular structure has a well-controlled alignment along the longitudinal direction while maintaining a relatively isotropic organization in the transverse direction. We expect that this algorithm can be utilized to conduct a structural analysis of a variety of synthetic or biological cellular solids, such as other echinoderms’ stereom structures, enabling quantitative comparative investigation. Further insights in understanding the structure-property of the biological cellular materials such as sea urchin spines enabled by this study could lead to the development of bio-inspired lightweight structures with structural controls at multiple length scales.
Chapter 5 Echinoderm stereom: A strong and damage-tolerant biological ceramic foam

The work based on the results is now being prepared for publication.

Despite significant strengthening and toughening enabled by incorporating nanoscale features, ceramic cellular solids, both traditionally synthesized and additively manufactured with upscaling features, still suffer from catastrophic failure. On the contrary, natural ceramic cellular solids are simultaneously strong, stiff, and tough despite their brittle constituent materials. In this work, we report a biomineralized cellular structure, echinoderm stereom (mineral content > 99%, relative density 0.2~0.4), that maintains the structural integrity and exhibits a graceful failure behavior upon loading. By the combination of micro-computed tomography, finite element analysis, and 3D failure process quantification, we correlate the remarkable mechanical performance of the stereom to its optimized morphology design in the microscale. For instance, the optimized surface curvature reduces the stress concentration and randomly distributed small throats that jam the fragments and localize the damage. As a result, echinoderm stereom achieves high strength superior to most synthetic and architected ceramic foams and high energy absorption outperforming many metallic foams. The design principles extracted from echinoderm stereom are significant for the development of strong and damage-tolerant engineering ceramic cellular solids.
5.1 Introduction

In Chapter 4, the multiscale structure characterization has been conducted on the stereom. However, the mechanical contribution of the elaborate meshwork and strut morphology of the stereom has not been fully appreciated. Previous studies have attributed the high strength and the graceful failure to the stereom-microstructure\(^8\) and hierarchical design\(^{145}\). Crack tip blunting was reported to be the primary toughening mechanism\(^{149,243}\). However, a quantitative correlation has not been established between stereom morphology and mechanical performance. Pursuing such a correlation is impeded by limited knowledge of the cracking and damage process of the stereom.

In this chapter, we use the stereom in the spine of sea urchin *Heterocentrotus mammillatus* (*H. mammillatus*) as a model system to correlate stereom morphology to its mechanical performance, including strength and damage tolerance. The 3D structure of stereom is quantified and directly compared with synthetic foams, and 3D printed octet trusses using micro-computed tomography (\(\mu\)-CT). The geometric measurements are further coupled with finite element analysis to predict stress concentrations within the porous structures. With synchrotron-based \(\mu\)-CT coupled with *in-situ* mechanical testing, the detailed failure process of the stereom is visualized and quantified. Our results reveal that the stereom achieves high strength and energy absorption simultaneously by evolving optimized surface curvature distribution that reduces the stress concentration and optimized strut thickness distribution that introduces the jamming of the fragments and localization of the damage after a fracture. Unlike the recently reported strong and tough ceramic mechanical metamaterials that require the nanoscale features, our finding can shed light on the design of strong and damage-tolerant ceramic cellular solids upscaling structure features.

5.2 Methods
Samples: Dried spine specimens from the sea urchin *H. mammillatus* were purchased from Etsy hobby shop. The Alumina synthetic foam was purchased from eBay. The architected octet truss foam was printed in photopolymer ceramic resin, Formlabs 2 ceramic (FLCEWH01), using a desktop 3D-printer FormLabs 2 (Formlabs Inc, Somerville, MA). The layer thickness of 3D printing is 100 μm. After printing, these printed architected octet trusses were rinsed in isopropyl alcohol for 10 mins and then fired according to the profiles described.

Compression Test: Cube-shaped samples of the stereom from the *H. mammillatus* spine (edge length: 5 – 9 mm, \(N = 76\)) and synthetic ceramic foam (edge length ~10 mm, \(N = 5\)) were cut by using a low-speed diamond wheel saw (MTI corporation). Cube samples of the octet truss foam were printed using Formlabs2 with ceramic resin (edge length ~12 mm, \(N = 3\)). Uniaxial compression experiments were performed on these samples in a quasi-static compression mode by applying load at a rate of 0.2 mm/min with a universal testing machine (Instron 5984; Instron, Norwood, USA). The videos of the compression test were recorded at a frame rate of 500 frames/min for additional analysis of the deformation process. The compression residues, including the samples with cracks initiated in the volume and the damage band formed in the volume, were saved for further SEM imaging. Moreover, five cube stereom specimens (sample size: 5 mm × 5 mm × 6 mm, \(N = 5\)) were tested with the same universal testing machine (Instron 5984; Instron, Norwood, USA). The cube specimen is constrained in a glass cube (cube ID: 5mm, thickness: 1.75 mm, Friedrich & Dimmock Inc). A cubic steel rod is inserted into the glass tube and placed on top of the specimen. The compression rate is ~0.2 mm/min.
Three-point bending: three-point bending tests were performed on the rectangular stereom specimens (5 mm × 5 mm × 40 mm, \(N = 22\)) using a universal testing machine (Instron 5984; Instron, Norwood, USA). The load rate is 0.2 mm/min.

Micro indentation test: Indentation experiments were performed on the cubic stereom specimens in a quasi-static mode with a steel tip. The load is applied at 0.2 mm/min with a universal testing machine (Instron 5984; Instron, Norwood, USA). The broken sample with residues was saved for electron microscopy and nanoindentation tests.

Electron microscopy: SEM imaging was performed on dried specimens, including fractured surfaces of the stereom, compression residue, and indentation residue. Before imaging, all specimens were coated with Pt/Pd (ca. 10 nm) to reduce charging effects. The fractured surfaces and compression residue were imaged using a Quanta 600 FEG Environmental SEM (FEI, OR). The indentation residues were imaged using an LEO 1550 Field Emission SEM (Zeiss, Munich). The SEM images were taken at acceleration voltages of 2-10 kV and working distances of 4-10 mm.

Synchrotron-based in-situ mechanical tests: Cube samples (2.2 mm × 2.2 mm × 1.7 mm) were cut from the stereom of \(H.\ mammillatus\) spine. The \textit{in-situ} mechanical tests combined with synchrotron-based \(\mu\)-CT were conducted at the beamline 2BM from Advanced Photon Source, Argonne National Laboratory. A monochromatic beam with an energy of 27.4 keV was used for the measurements. A customized \textit{in-situ} mechanical loading device was used for both synchrotron-based compression and indentation tests, through which the samples can be mechanically tested.
while allowing for X-ray imaging through an X-ray transparent window. For the \textit{in-situ} compression tests, the samples were compressed by a steel platen through a stepwise fashion (typical step size, ca. 0.1 mm). For the \textit{in-situ} indentation tests, a ceramic tip was used to induce localized deformation. Once the displacement is stopped, the sample stage together with the \textit{in-situ} device was rotated for a full tomography scan. The beamline was equipped with a single-crystal LuAg:Ce scintillator for converting X-ray into visible light, which was further magnified with a 2× or 5× long-working distance objective lens. For a typical scan, 1500 projection images were acquired during a 180° rotation with the exposure time of 0.1 s (corresponding to the total scan time for a single tomography scan of 2.5 mins). The projection images were collected using a PCO-Edge high-speed CMOS detector (2448 × 1024 pixels), which resulted in a typical voxel size of 1.725 or 0.69 µm depending on the objective lens used. The reconstruction of the obtained \textit{µ-CT} data was conducted with the open-source software \textit{Tomopy}\textsuperscript{166}. The reconstructed data was used for 3D volume rendering and quantitative analysis, such as structure thickness and surface curvature measurements, via a combination of methods, such as \textit{Avizo} (Thermofisher, scientific, USA), \textit{Fiji}\textsuperscript{168}, open-source software \textit{Blender} (www.blender.org), and customized \textit{MATLAB} codes.

\textit{Nanoindentation:} The dried \textit{H. mammillatus} spines were cut into cylindrical sections (ca. 10 mm) and embedded in cured Epofix\textsuperscript{TM} epoxy (Electron Microscopy Sciences, Hatfield, PA, USA) at room temperature. The embedded samples were then polished step by step (15 µm, 9 µm, 6 µm, 3 µm, and 1 µm) on diamond lapping films and finally with 40 nm colloidal silica suspension on a polishing cloth. The polishing machine used is MultiPrep\textsuperscript{TM} System from Allied High Tech Products, Inc. Load-controlled nanoindentations were performed on the polished sample surface and the damage band in the indentation residue using Berkovich (trigonal pyramid, semi-angle =

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65.3°) diamond probe tips on a Micro Material indentation system (Nano Test Vantage, Wrexham, UK). Maximum loads varied from 5 mN to 25 mN. Typical load functions include successive loading (15 s), holding (10 s), and unloading (15 s) sections. The thermal drifting was monitored when the load was unloaded to 10% of the maximum force for 30 s. The hardness $H_{O-P}$ and indentation modulus $E_{O-P}$ of the biogenic calcite and the damage band were quantified based on the standard Oliver-Pharr (O-P) methodology.245

Deep learning-based damage detection: The damaged struts in the complex cellular network are identified via deep learning (DL)-enabled process. Damaged struts are first labeled manually in small volumes. Common features of these damaged struts are extracted by learning with the convolutional neural network in a semi-3D manner.246,247 The semi-3D manner is applied by learning three consecutive slices in three orthogonal directions, enabling accurate damage region detections in new cases. The choice of the deep learning neural network architecture in terms of convolution filter size and network depth is adjusted depending on the integrality degree and distribution scope of damaged struts.

The slightly damaged struts, which usually behave like separated intact struts with high contrast micro-notches (cracks), are distributed locally and thus identified by a DL-based local feature recognition algorithm. Crack locations and rough crack shapes are firstly detected by filtering the three consecutive slices with a manual-established multi-scale feature library, which consists of multiple observed 2D crack morphologies. The detailed construction of a manual-established multi-scale feature library is discussed in our previous work.248 The proposed crack candidates are then further classified by the 3D DL-based algorithm, which enhances detection accuracy based on nonstandard features learned from manually labeled cracks beyond the feature
library and corrects the influence of complex cellular structures. Here we adopt a simplified VGG16 network as the fracture classification network for each orientation through learning the distinct features between the crack and non-crack. The input to each network is three-channel tomography slices for that specific orientation. Taking the input to the classification network in the x-y direction as an example, it is composed of 32×32×3 voxels with the middle channel as rough crack locations identified from the first step surrounded by its neighboring voxels in the x-y direction. The other two channels are two adjacent slices in the same direction. Finally, 3D image dilation and erosion are implemented to the summation of identified fractures with damaged struts as faked intact struts. The real crack shape is refined by subtracting faked intact struts from undamaged struts.

With the accumulation of multiple cracks, struts are separated into multiple pieces, which are named fragments. Fragments are distributing more dispersedly and globally compared to cracks. Here we adopt a conventional U-net architecture as the fragmentation segmentation algorithm to learn such large-scale fragmentation features in the previously mentioned semi-3D manner. With the combination of segmentation results from three different orientations, we obtain the high-fidelity fragmentation identification in 3D.

**Automatic multi-scale damage structure analysis:** Cracks are further registered to undamaged hosting struts by overlapping detected cracks with original undamaged volume, determined by the digital volume correlation (DVC). Each voxel is cropped by including its neighboring voxels in damaged volume and correlated with the undamaged volume voxel by voxel. We thus obtain the 3D correlation coefficients map, where the location with the maximum correlation coefficients represents the corresponding undamaged place of this voxel. For quantitative analysis of crack
locations and orientations, the undamaged microstructure is represented as a cellular network consisting of individual nodes and labeled connecting struts. Therefore, identified connected crack voxels are registered to each labeled strut as each individual crack by calculating the distance of each crack voxel to the struts and selecting the one with minimum distance. The center of each individual crack is identified by averaging all voxels in such crack, and its direction is recognized via finding its minimum principal component with principal component analysis. With identified individual crack center and crack direction, individual crack properties are analyzed in terms of individual crack orientation $\alpha$ and $\beta$, inter crack-strut angles $\theta$, and relative individual crack locations with respect to the whole struts $l/L$. Individual crack orientation is represented with the angle away from the force direction $\alpha$ and the angle of the projected individual crack in the plane perpendicular to the force direction and away from the x-direction $\beta$. The inter crack-strut angle $\theta$, is denoted as the intersection angle between strut direction and each individual crack direction. The relative individual crack location with respect to the hosting struts is defined as $l/L$, where $l$ is the shorter distance between the center of the crack plane and two connected nodes, and $L$ is the length of the hosting strut.

**FEA analysis:** Abaqus/Standard 2016 (Dassault Systems, Vélizy-Villacoublay, France) was implemented to simulate the mechanical response of five structures with the same non-dimensional length (5-characteristic length $S_v$ in each dimension) from the stereom ($\rho/\rho_s = 0.37, 0.27$), the synthetic foam ($\rho/\rho_s = 0.29$) and the architected foam ($\rho/\rho_s =0.26, 0.34$) under uniaxial compression. The geometry of the model was based on CT reconstruction, and the edges of the cubic model are controlled to have at least 5-characteristic length $S_v$ in each dimension. The constructed models were then discretized tetrahedral elements with a modulus of 109 GPa and an
aspect ratio of 0.291. The elastic and fracture performances were calculated utilizing the explicit dynamic module. The brittle cracking model and the element deletion were implemented to capture the fracture process. In the simulation, the strain rate of loading was controlled to guarantee a quasi-static condition. Cracks initiated when the maximum principal tensile stress exceeded 102 MPa. The fracture energy of forming a unit area of the crack surface in Mode I, \(G_{If}\), was used as the criteria of element deletion to avoid unreasonable mesh sensitivity. A linear shear retention model was also included, considering the reduction of post-cracked shear modulus as the crack opened. The elastic performance was calculated with general statics. In the explicit simulations, the applied pressure of loading was set as \(P = 6.02\) MPa. (The FEA analysis is conducted by Zian Jia).

5.3 Results

5.3.1 Structure characteristics

Sea urchin are characterized by their dome-shape body, known as test, decorated with long, cylindrical spines (Figure 5-1a). The model system used in this study, \(H.\ mammillatus\), commonly known as pencil sea urchin, have large spines with typical length and diameter of 4.42 - 8.58 cm and 0.79-1.23 cm, respectively. When viewed in cross sections, the \(H.\ mammillatus\) spines have several concentric, often dark pigmented rings (Figure 5-1b). These so-called “growth rings” are resulted from successive thickening during growth, which also exhibit low porosity locally in comparison to normal stereom microstructure. Scanning electron microscopy (SEM) of fractured spines reveals their characteristic bicontinuous, highly porous internal microstructure (Figure 5-1c).
Figure 5-1| Microstructure of the stereom in *H. mammillatus* spine. a, Photograph of a *H. mammillatus* sea urchin. b, An optical image of the transversely cut cross-section of a spine. c, SEM image of the stereom.

The structural characteristics of the stereom were analyzed using a representative volume from *H. mammillatus* spine ($\rho/\rho_s = 0.37, 205 \mu m \times 205 \mu m \times 205 \mu m$) (Figure 5-2a). The inverse structure, i.e., the void structure, is extracted to explore the morphology of voids in the stereom (Figure 5-2a, inset). Firstly, the stereom in echinoderm is a bicontinuous open-cell foam structure and can be skeletonized into a network composed of nodes and struts using the method proposed in Chapter 5. The node densities for the original structure and inverse structure are 60,000 nodes/mm$^3$ and 35,000 nodes/mm$^3$, respectively (Figure 5-2b-c). The node type is determined by the number of connected branches. The N-3 nodes are the dominant nodes in the original structure (271 N-3 nodes out of 412 nodes), while the dominant node types in the inverse structure are N-3 and N-4 (64 N-3 nodes, 65 N-4 nodes out of 245 nodes, Figure 5-2d).
Figure 5-2 | Network analysis of the stereom cube and the corresponding inverse structure. a, 3D \( \mu \)-CT reconstructed stereom structure and the inverse structure (inset). \( r_s \) and \( r_{is} \) are the thickness of the stereom and inverse structure, corresponding to the radius of the struts and inverse struts, respectively. The throat size \( r_t \) is calculated based on the radius of a circle with the equivalent throat area. b, c, 3D network of the stereom and the inverse structure with nodes highlighted by node type (classified by the number of struts connected to the node, interconnectivity). d, Node type distribution in the stereom and the inverse structure.

The distribution of structure thickness, \( r_s \) and the \( r_{is} \), characterizes the space distribution of the original volume and the inverse structure. \( r_s \) and \( r_{is} \) are defined as the radius of the struts and inverse struts, respectively (Figure 5-2a). The structure thickness in the original volume and inverse structure shows a rather uniform distribution with 7.7 ± 1.8 \( \mu m \) and 12.9 ± 1.8, respectively (Figure 5-3a,b, Table 5-1). The strut in the original and inverse structure has a minimum radius in the strut middle, \( t_{s0} \) and \( r_t \), respectively. The regions with the smallest strut radius in the inverse structure, defined as the throats (Figure 5-3c). The throat size shows a wider distribution compared to the structure thickness, with \( r_t \sim 11.3 \pm 3.9 \mu m \). Even if the mean throat size is larger than the
stereom thickness, many of the throats are smaller than the stereom thickness. As shown in Figure 5-3c, the overlap between the stereom thickness and throat thickness distribution is 57.7%. The small throats ($r_t < 12 \mu m$) are distributed in the entire stereom (Figure 5-3d).

Figure 5-3 | Comparison of thickness distribution in the stereom. a,b, The thickness distribution of the stereom (a) and inverse structure (b). c, A comparison of the structure and inverse structure thickness ($r_s, r_{is}$) to throat size ($r_t$) based on the volume. The overlap of the throat size distribution and structure thickness distribution is 57.7%. d, 3D rendering of the small throats ($r_t < 12 \mu m$) in (e) with each throat colored by its throat size.

The strut morphologies in the original volume and the inverse volume are quantified by the strut profile (Figure 5-4a). For all the struts in the original volume and the inverse volume, the strut radius $r_s$ and the $r_{is}$ are plotted against the positions $l$ on the strut. $L$ is the strut length. Each
individual strut shows a second polynomial strut profile, as shown in Figure 5-4b. By normalizing the position \( l \) with its strut length \( L \) and the strut radius with the smaller strut radius on the strut, the fitting strut profile for the struts in the original volume and inverse volume are obtained in Figure 5-4c and Figure 5-4d, respectively. The normalized strut profile in the inverse structure shows a smaller strut radius change (0.75 vs. 1.82) on the struts.

**Figure 5-4| Strut profile in the stereom.** a, Schematic of a strut in the stereom. \( r_s \) and \( r_{s0} \) are the strut thickness and the minimum strut thickness, respectively. \( l \) and \( L \) are the measured position and the strut length, respectively. b, Measurements of branch profiles and the fitting results for individual struts in
this volume. e,d, Normalized strut profiles of all struts and the fitting result (red line) for the stereom (e) and inverse structure (d).

The structure thickness distribution in the synthetic foam and architected foam shows a significantly different distribution (Figure 5-5a-l). For the synthetic foam, the original structure shows a thickness distribution with a larger range of $422.7 \pm 140.9 \mu m$ and the inverse structure are connected by spherical grains (Figure 5-5a-b). The connections between different grains are defined as throats in the synthetic foam. And the overlap between the stereom thickness distribution and throat size distribution is smaller than the stereom (34.9%, Figure 5-5c-d). The struts in the architected foam have identical cylindrical strut shape and throat shape (Figure 5-5e-l). The throat size is always smaller than the strut thickness for both the architected foams with $\rho/\rho_s = 0.26$ and $\rho/\rho_s = 0.34$. For the stereom, even if the relative density is reduced to $\rho/\rho_s = 0.27$, there are still many throats distributed in the volume and the overlap reaches 55.1% (Figure 5-5m-p). Therefore, the large overlap between the stereom thickness distribution and the throat size distribution is a structural feature of the stereom structure.
Figure 5-5 | Comparison of thickness distribution in the synthetic foam with $\rho/\rho_s = 0.29$ (a-d), the architected foam with $\rho/\rho_s = 0.26$ (e-h) and $\rho/\rho_s = 0.34$ (i-l), and the stereom with $\rho/\rho_s = 0.27$ (m-p). a,e,i,m, Structure thickness $r_s$. b,f,j,n, Inverse structure thickness $r_{is}$. c,g,k,o, The comparison of structure thickness $r_s$, the inverse structure thickness $r_{is}$, and throat size $r_t$ in the volume. d,h,l,p, Distribution of throats with small maximum throat sizes ($r_t < 12 \mu m$).
Table 5-1 | Thickness and throat size measurements for different foams.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Ste. (0.37)</th>
<th>Ste. (0.27)</th>
<th>Syn. (0.29)</th>
<th>Arc. (0.26)</th>
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<tbody>
<tr>
<td>( r_s (\mu m) )</td>
<td>7.7 ± 1.8</td>
<td>5.9 ± 1.2</td>
<td>422.7 ± 140.9</td>
<td>164.6 ± 27.4</td>
<td>148.9 ± 49.6</td>
</tr>
<tr>
<td>( r_t (\mu m) )</td>
<td>11.3 ± 3.9</td>
<td>9.2 ± 4.5</td>
<td>647.2 ± 229.6</td>
<td>243.9 ± 27.4</td>
<td>283.3 ± 12.4</td>
</tr>
<tr>
<td>( r_{is} (\mu m) )</td>
<td>12.9 ± 1.8</td>
<td>14.5 ± 4.1</td>
<td>1064.7 ± 219.2</td>
<td>333.0 ± 115.3</td>
<td>374.9 ± 77.2</td>
</tr>
<tr>
<td>( S_v (\mu m) )</td>
<td>38</td>
<td>31.2</td>
<td>2139.8</td>
<td>817</td>
<td>864</td>
</tr>
</tbody>
</table>

In addition, the surface curvature distribution of the synthetic foam, architected foam and the stereom are investigated through the interfacial shape distribution (ISD). Before comparison, the characteristic length \((S_v)\) of different foams are calculated to eliminate the effect of structure size (Figure 5-6, Table 5-1). \( S_v \) is determined as \( S_v = 2\times(r_t+r_s) \). The interfacial shape distribution is determined by the local normalized maximum \((S_vK_1)\) and normalized minimum curvatures \((S_vK_2)\) on the surface. The ISD plot indicates that the surface of the stereom is the hyperbolic surface with smaller range (Figure 5-7).

![Figure 5-6](image_url) | Characteristic length measurements of the stereom, the synthetic foam, and the architected foam. \( r_s \): structure thickness, \( r_t \): the radius of a circle that has the same area as the throat.
Figure 5-7] Interfacial shape distribution plots for the stereom with $\rho/\rho_s = 0.27, 0.37$, the synthetic foam with $\rho/\rho_s = 0.29$, the architected foam with $\rho/\rho_s = 0.34, 0.26$ and the schematic of the surface corresponding to different regions in the curvature distribution (b). Green: synthetic foam, blue: architected foam, red: stereom.

Therefore, compared with the surface and the throat shape in the synthetic foam and architected foam, the stereom surface is smoother in terms of more uniform maximum curvature distribution (Figure 5-8, Table 5-2). Another noticeable structural characteristic is that the stereom surface has dense strut with nanometer-scale surface roughness, distinct from the porous strut with micrometer-scale surface roughness of the discrete particles in synthetic and architected ceramic cellular solids due to sintering process$^{42,252}$ (Figure 5-9, Figure 5-10, Appendix A-2).
Figure 5-8| Normalized maximum curvature $K_1S_v$ distribution on five ceramic foams. a, The stereom with $\rho/\rho_s = 0.37$. b, The stereom with $\rho/\rho_s = 0.27$. c, The synthetic foam with $\rho/\rho_s = 0.29$. d, The architected foam with $\rho/\rho_s = 0.34$. e, The synthetic foam with $\rho/\rho_s = 0.26$.

Table 5-2| Quantitative results of maximum curvature distribution in five foams.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Ste. (0.37)</th>
<th>Ste. (0.27)</th>
<th>Syn. (0.29)</th>
<th>Arc. (0.26)</th>
<th>Arc. (0.34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_1S_v$</td>
<td>3.6 ± 1.4</td>
<td>5.1 ± 2.6</td>
<td>6.1 ± 20.6</td>
<td>5.8 ± 1.6</td>
<td>5.2 ± 2.1</td>
</tr>
</tbody>
</table>
Figure 5-9 | The cellular structure and cell edge of stereom, synthetic foam, and architected foam.

a-c, SEM images of the stereom (a), synthetic foam (b) and architected foam (c) and their corresponding solid struts (d-e).
5.3.2 Quantitative mechanical properties

Comparative uniaxial compression tests were performed on the stereom of *H. mammillatus* spine, synthetic ceramic cellular solids, and architected octet truss (Figure 5-11 and Figure 5-12). Unlike the engineering synthetic foam and the architected octet truss, where the stress drops significantly after the peak stress (Figure 5-12), the stereom exhibits graceful failure. More specifically, the stress-strain curve of the stereom comprises an elastic regime, a relatively high-stress plateau regime, and a densification regime (Figure 5-11a), which are classic behaviors observed in foams made of plastic materials such as metals and glassy polymers. During compression, we observe several damage bands transverse to the loading direction (yellow arrows, Figure 5-11b). The structure remains relatively intact until spallation occurs (white arrows, Figure 5-11b). For the synthetic foam, spallation occurs instantaneously after the peak stress (Figure 5-12a,c). For the architected foam, the structure fractures along weak planes, leading to the catastrophic failure and clean crack planes (Figure 5-12b,d).
The statistical results reveal that the stereom achieves high strength $\sigma_c = 40.4 \pm 12.4$ MPa and high energy absorption $W = 13.0 \pm 3.4$ MJ/m$^3$ ($N = 76$) simultaneously (Figure 5-11c,d). Additional samples with growth rings have also been tested, showing a similar graceful failure behavior with similar compressive strength MPa but smaller energy absorption capacity (Appendix Fig. A-3, Fig A-4). This is due to the early spallation of growth rings, which reduces the cross-section area under compression (Appendix Fig. A-3).

![Figure 5-11](image)

**Figure 5-11** Uniaxial compressive responses of the stereom. a, Compressive stress-strain responses of samples isolated from the stereom of *H. mammillatus* spine (red), the synthetic foam (green), and the
architected foam (blue). N is the number of tests. b, Deformation stages of cubic samples extracted from the stereom of *H. mammillatus* spine under uniaxial compression, corresponding to the spots highlighted in (a). The damage bands and the spallation are indicated by yellow and white arrows, respectively. c,d, Measurements of compressive strength $\sigma_c$ (MPa) (c) and energy absorption capacity $W$ (MJ/m$^3$) (d) of all tested cubic samples. The slope of the linear fitting line is 1.5 ($R^2 = 0.945$).

Figure 5-12 | Deformation behavior of cube samples from the synthetic cellular ceramics and the ceramic octet truss under compression. Stress-strain curves (a) and the corresponding deformation stages for samples from the synthetic foam (b) and architected foam (c).
The compressive strength of the stereom $\sigma_c$ is normalized by the fracture strength of the natural calcite $\sigma_s$ (100 MPa)$^{170}$ and plotted against the relative density $\rho/\rho_s$ in Figure 5-13a, indicating that the stereom is superior to most brittle synthetic foams$^{64,253–257}$ and architected foams$^{46,59,258,259}$, approaching the relative strength of the highest ceramic cellular composites ($\sim 1$)$^{62}$. In Figure 5-13b, the specific energy absorption (energy absorbed per unit mass) is calculated as $W/\rho = 17.7 \pm 4.0$ kJ/kg, which outperforms many stainless-steel foams, CNT/Al foams, steel foams, etc.$^{141,178–183,186,187}$. Moreover, the compressive strength of the stereom increases with the relative density and scales by $\sigma_c/\sigma_s \sim (\rho/\rho_s)^{1.5}$ ($R^2 = 0.95$), which is consistent with analytical scaling for the bending-dominant structures$^{25}$ (Figure 5-11c). However, results of 3-point bending show that the modulus of stereom increases with the relative density by $E/E_s \sim (\rho/\rho_s)^{1.77}$ ($R^2 = 0.99$), rather than $E/E_s \sim (\rho/\rho_s)^{2}$, revealing that the surface curvature can play a significant role in terms of the loading mechanism on the struts (Figure 5-14).

Figure 5-13| Ashby plots displaying relative compressive strength $\sigma_c/\sigma_s$ as a function of relative density $\rho/\rho_s$ ($\sigma_s$: fracture strength of solid struts), in comparison to the brittle synthetic foams$^{64,253–257}$ and architected foams$^{46,59,258,259}$ (a) and The energy absorption capacity $W (MJ/m^3)$ as a function of density
\( \rho \ (kg/m^3) \), compared with many stainless-steel foams, CNT/Al foams, steel foams, etc. \(^{178-184, 186, 187}\) (b).

The dashed lines represent different \( W/\rho \) values, with the shaded area highlighting the standard deviation of \( W/\rho \) for the stereom.

**Figure 5-14 | Mechanical performance of stereom under bending.** a, Schematic of the 3-point bending experiment. b, Stress-strain curves of samples isolated from *H. mammillatus* spine, \( N \) is the number of samples. c, Measurements of bending strength \( \sigma_b \) (MPa) and modulus \( E \) (GPa) of all tested *H. mammillatus* spine samples. d, An Ashby chart plotting relative modulus \( E/E_s \) (\( E_s \): modulus of
isotropic calcite) as a function of relative density $\rho/\rho_s^{46,258,268,269-267}$. Here the slope equals 1.77 ($R^2 = 0.99$).

5.3.3 Deformation mechanism

To gain a deeper understanding of the graceful failure process, additional $\mu-CT$ based in-situ compression tests are performed on the cubic samples (size: 2 mm $\times$ 2 mm $\times$ 1.7 mm, $N = 11$) to correlate the stress-strain response and the microstructural damage process (Figure 5-15a,b). The failure of the stereom does not occur instantaneously but progressively.

Figure 5-15] The stress-strain curves of stereom from in-situ synchrotron-based micro-computed tomography ($\mu$-CT) compression test (a) and (b) projection images corresponding to the stages indicated by red spots. The number of tests is 11.
In particular, during the damage process, the damage is constrained in a band volume (highlighted in yellow, Figure 5-16a,b). The rest of the stereom remains intact and can withstand increasing loads (Figure 5-16a,b). These damage bands are identified as local densified regions with a relative density of ~0.66 (Figure 5-16c,d, Table 5-3).

**Figure 5-16** Vertical slices showing the damage of stereom at applied stress $\sigma = 43.5 \text{ MPa}$ and $\sigma = 27.4 \text{ MPa}$, respectively. The damage bands are highlighted in yellow. e,f, 3D renderings of the relative density ($\rho/\rho_s$) in the damage volume at different applied stress $\sigma = 43.5 \text{ MPa}$ and $\sigma = 27.4 \text{ MPa}$, respectively. The color is set transparent when the relative density is smaller than 0.4.

**Table 5-3** The relative density ($\rho/\rho_s$) of the damage bands and rest of the undamaged structure at four different deformation stages upon compression.

<table>
<thead>
<tr>
<th>Stage</th>
<th>ii</th>
<th>iii</th>
<th>iv</th>
<th>v</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho/\rho_s$ (damage)</td>
<td>0.546 ± 0.126</td>
<td>0.600 ± 0.116</td>
<td>0.763 ± 0.121</td>
<td>0.796 ± 0.0126</td>
</tr>
<tr>
<td>$\rho/\rho_s$ (rest)</td>
<td>0.368 ± 0.151</td>
<td>0.361 ± 0.153</td>
<td>0.377 ± 0.155</td>
<td>0.364 ± 0.154</td>
</tr>
</tbody>
</table>
A small volume is extracted from consecutive deformation steps in Figure 5-15b to illustrate the microstructure's damage process (Figure 5-17a-d). Cracks firstly initiate in the volume, which is followed by the form of the damage bands by accumulating fractured debris and then expansion across their thickness direction. Giving the continuously increasing stress during this process (Figure 5-15a), the damage band formation does not lead to the structure's weakening. More specifically, the damage band exhibits a torturous 3D surface (Figure 5-17e). Such a 3D fracture path is similar to the cracks from the crack deflection mechanism in biological ceramic composites\textsuperscript{105}.

**Figure 5-17| Deformation behavior in a small cube.** A small volume extracted from the sample showing the failure process (a-d). The initial cracks are labeled in red, and the damage bands are labeled in yellow. e, 3D rendering of the damage band with the surface colored by the local mean curvature $(H)$. 
In order to investigate if cracks show preferred locations and orientations on the struts, the quantification of initiated cracks was conducted. The initiated cracks separate the cubs stereom into top and bottom parts. And the fracture surface of the bottom part is first identified based on the *in-situ* \( \mu \)-CT. The randomly distributed fractured branches or nodes on the fracture plane can be observed by overlapping the fracture surface with the original skeletonized network (Figure 5-18).

![Figure 5-18](image)

*Figure 5-18* | The fracture surface overlapped with the network of the original volume.

More specifically, based on the statistics, the cracks show no preferred location on the struts (Figure 5-19a), and the number of cross cracks is slightly higher than through cracks (Figure 5-19b), while both through and cross cracks show random orientations (Figure 5-19c). The crack location is defined as \( l/L \), where \( L \) is the length of the fractured strut and \( l \) is the shorter distance between the center of the crack plane and the end node) (Figure 5-19a). On the other hand, the
crack orientation is characterized by \( \theta / \theta_0 \), where \( \theta \) is the angle between the crack plane and the central axis of strut and \( \theta_0 \) is determined by the aspect ratio of the individual branch \( \theta_0 = \arctan(t_{s0}/L) \), \( t \) is the strut radius) (Figure 5-19b). Cracks are formed through the struts \( \theta / \theta_0 \leq 1 \) or cross the struts \( \theta / \theta_0 > 1 \), respectively.

**Figure 5-19** Crack position and orientation distribution in the stereom. 

- **a**, Distribution of the crack location \( l/L \). 
- **b**, Distribution of the crack orientation on the strut \( \theta / \theta_0 \). \( \theta_0 = \arctan(t_{s0}/L) \). \( \theta > \theta_0 \): cross crack; \( \theta < \theta_0 \): through crack. 
- **c**, The correlation between the crack location and crack orientation in space. \( \alpha \) is the angle between the normal vector of the crack plane and z-direction, \( \beta \) is the angle between the normal vector of the crack plane on the x-y plane and x-direction.

The measurements are consistent with the observations in the SEM images in Figure 5-20a,b. These random crack initiations in the volume suggest a uniform stress distribution on the surface, which will be further verified via finite element analysis.
Figure 5-20 | Experimental observation of the fracture surface on the stereom. **a,b,** An SEM image of one side of the compression residue after the crack initiation (**a**) and a zoom-in view showing the fracture surface with each crack colored in terms of crack location \( l/L \) (**b**).

Quantitative analysis of the damage band structure further reveals the damage band formation mechanism. In the damage band, the size of the fragments \( r \), characterized by the radius of the circle with the equivalent area, varies significantly from \( r < 1 \mu m \) (tiny particles) to \( r > 12 \mu m \) (large pieces) (**Figure 5-21a-d**). Notably, the size of the large pieces is close to or larger than the throat size of the stereom (**Figure 5-21c,d**).
Figure 5-21 | Fragment size distribution in the damage band. a,b, Compression residue of the sample after damage band formation (a), and the zoom-in view in (b). c, Fragment size distribution of the damage band. r: the radius of a circle with the equivalent area as the fragment. d, The fragments in the damage band with fragments colored in terms of their sizes.

In addition, in-situ indentation tests on the stereom reveal a similar deformation band, i.e., the damage is restricted to a thin layer (highlighted by yellow) around the conical indentation tip (highlighted by purple) in (Figure 5-22a,b).
Figure 5-22 | Deformation process of the sample under in-situ deformation. Vertical reconstruction slices (a) and horizontal reconstruction slices (b) of μ-CT data for samples at five stages under in-situ indentation.

The indentation residues exhibit a gradient deformation from the surrounding stereom to the location of direct contact, i.e., the locally fractured large fragments (red arrows), which is further jammed by the surrounding structure (yellow arrows), and a thin layer with a gradient size distribution (Figure 5-23a). The fragments in the densified region range from large, fragmented struts located close to the surrounding undamaged porous structure (red arrows, Figure 5-23b) to
nanosized particles (white arrows, Figure 5-23a,c) around the indentation tip. Moreover, the relative density of the damage band region is 0.6 - 0.8 (Figure 5-24, Table 5-4). The visualization and quantification of the damage band reveal the formation mechanism of the damage band: (1) Large fragments induced by random crack initiation are slightly rotated and shifted until quickly impeded by the throats of stereom, considering the large overlap between the structure thickness distribution and the throat size distribution (Figure 5-3c). (2) Continuous loading then breaks the large fragments into smaller pieces and even nanosized particles. The damage band formation process leads to high energy dissipation.
Figure 5-23| The damaged structure in the indentation residue. a-c, Indentation residue of the stereom showing the damaged structure around the tip (a) with zoom-in views showing the jamming of the fragments (highlighted in yellow) (b) and the small fragments (white arrows, a) (c), respectively. The locally fragmented structures and the jammed fragments are indicated by red arrows and white arrows, respectively.
Figure 5-24: The corresponding local density map at different deformation stages under *in-situ* indentation.

Table 5-4: The relative density ($\rho/\rho_s$) of the damage bands and rest of the undamaged structure at four different deformation stages upon indentation.

<table>
<thead>
<tr>
<th>Stage</th>
<th>ii</th>
<th>iii</th>
<th>iv</th>
<th>v</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho/\rho_s$ (damage)</td>
<td>0.546 ± 0.126</td>
<td>0.600 ± 0.116</td>
<td>0.763 ± 0.121</td>
<td>0.796 ± 0.0126</td>
</tr>
<tr>
<td>$\rho/\rho_s$ (rest)</td>
<td>0.368 ± 0.151</td>
<td>0.361 ± 0.153</td>
<td>0.377 ± 0.155</td>
<td>0.364 ± 0.154</td>
</tr>
</tbody>
</table>

Moreover, the structure of the densified region is less stiff with a modulus ~3.97 ± 0.59 GPa) and less hard with a hardness ~0.064 ± 0.025 GPa) (*Figure 5-25a-d, Table 5-5*), which can accommodate more structure deformation. As a reference, the hardness $H$ and modulus $E$ of the constituent biogenic calcite are 4.21 ± 0.28 GPa and 79.31 ± 3.61 GPa, respectively (*Figure 5-26, Table 5-6*). Despite the low density compared to the solid biogenic calcite (0.6-0.8), the strength of the stereom does not degrade significantly. This is attributed to the local densification and
structure stiffening in the damage region, which make the deformed microstructure intact and withstand high stress during the deformation process.

Figure 5-25| Summary of the indentation tests and the nanoindentation on densified bands. a, Experimental setup of the indentation tests. b, Load-displacement curves of the indentation tests. c, The densified region on the indentation residue. d, Load-displacement curves of the nanoindentation tests on the damage band (c).

| Table 5-5| Mechanical properties including hardness H and modulus E of the damage band measured from nanoindentation tests. |
| Load (mN) | | | | |
| H (GPa) | 5 mN | 15 mN | 25 mN | All |
| 0.041 ± 0.024 | 0.079 ± 0.011 | 0.076 ± 0.014 | 0.064 ± 0.025 |
| E(GPa) | 3.63 ± 0.76 | 4.19 ± 0.23 | 4.15 ± 0.46 | 3.97 ± 0.59 |
Table 5-6 | Mechanical properties including hardness H and modulus E of the biogenic calcite in the stereom measured from nanoindentation tests.

<table>
<thead>
<tr>
<th>Load (mN)</th>
<th>5 mN</th>
<th>15 mN</th>
<th>25 mN</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>H (GPa)</td>
<td>4.38 ± 0.27</td>
<td>4.04 ± 0.19</td>
<td>4.09 ± 0.21</td>
<td>4.21 ± 0.28</td>
</tr>
<tr>
<td>E(GPa)</td>
<td>81.92 ± 2.64</td>
<td>76.39 ± 3.59</td>
<td>77.76 ± 1.69</td>
<td>79.31 ± 3.61</td>
</tr>
</tbody>
</table>

5.3.4 Computational modeling

The fracture modeling was conducted by Zian Jia.

Both the in-situ and conventional mechanical experiments have demonstrated the importance of the damage band formation in maintaining structural integrity and achieving high damage tolerance. Additionally, finite element simulations, including the elastic modeling and fracture modeling, were conducted to provide a direct comparison of the mechanical behavior among the stereom ($\rho/\rho_s = 0.37, 0.27$), synthetic foam ($\rho/\rho_s = 0.29$) and architected foam ($\rho/\rho_s = 0.26, 0.34$) in terms of their structure.
The resultant maximum principal stress distribution from elastic simulation reveals that, compared with the synthetic foam and the architected foam, the stereom exhibits the lowest maximum principal stress concentration on both the volume and the surface (Figure 5-27, Table 5-7), corresponding to their lower maximum curvature distribution on the surface (Figure 5-28).

![Figure 5-27| Maximum principal stress $\sigma_1$ distribution of five structures under compression.](image)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Ste. (0.37)</th>
<th>Ste. (0.27)</th>
<th>Syn. (0.29)</th>
<th>Arc. (0.26)</th>
<th>Arc. (0.34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_p$</td>
<td>5.88 ± 0.66</td>
<td>5.46 ± 0.66</td>
<td>11.68 ± 1.03</td>
<td>13.15 ± 0.40</td>
<td>5.74 ± 0.31</td>
</tr>
</tbody>
</table>

Table 5-7| Stress concentration factor $C_p$ measured for five foams via static modeling, $C_p = \sigma_1/P$
Figure 5-28 Correlation between the maximum curvature distribution and maximum principal stress and five models under compression $P = 6.02 \text{ MPa}$. a, Maximum principal stress distribution. b, Maximum principal curvature distribution. c, Scatter plots of principal curvatures. Each point is colored with the stress concentration factor $C_p$.

According to the fracture modeling, normalized stress-strain curves are summarized in Figure 5-29a, where the normalized stress is defined as the relative stress $\sigma/\sigma_s$ divided by relative density $\rho/\rho_s$. Comparative analysis reveals that the stereom achieves higher energy dissipation, stiffness, and strength simultaneously (Figure 5-29a,b). The relative stiffness defined as the relative stiffness $E/E_s$ divided by relative density $\rho/\rho_s$, where $E_s$ (109 GPa) is the modulus for the isotropic calcite$^{249}$. 

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Figure 5-29] Simulation-based comparison of the stress-strain curves among the stereom ($\rho/\rho_s = 0.35$), synthetic foam ($\rho/\rho_s = 0.29$) and octet truss ($\rho/\rho_s = 0.34$). The stress is normalized by material strength and relative density ($\rho/\rho_s$). Stress-strain curves of 3 models with the stress normalized by density of the individual models. **b-f**, Comparison of the (b) stiffness, (c) strength, and (d-f) fracture behavior of those structures. (Figure credit: Zian Jia)

Moreover, the deformation behavior at the microstructure level is various (Figure 5-30). For the stereom, the damage starts with local crack initiation and then propagates to the neighboring struts progressively (Figure 5-30a). This is different from the synthetic foam, where multiple cracks initiate simultaneously at weak points, such as thin struts with locally high maximum curvature in the volume (Figure 5-30b). The synthetic foam collapses by spallation due to macrocrack, which are resulted from the coalescence of these cracks (Figure 5-30c). For the architected octet truss, the crack initiates on the strut close to the node region (Figure 5-30d). Further compression results in the breaking of struts along the \{110\} planes and, hence, the
structure's spallation (Figure 5-30e). Further examination of the crack distribution at stage iv of the stereom ($\rho/\rho_s = 0.37$) reveals that the structure is still intact to sustain a relatively high load (Figure 5-29a) since the initiated cracks are microcracks that do not fracture the structure (stage v, Figure 5-30f).

**Figure 5-30** Comparison of the fracture behavior among three foams. (a-f) show the distribution of maximum principal strains and displacement fields on the stereom (a,d), synthetic foam (b,c) and architected foam (d,e) at applied strains of (i) 0.0007, (ii) 0.0012, (iii) 0.0013, (iv) 0.0018, and (v) 0.0029. The circles and arrows mark the locations of microcracks. f,h,i, Displacement fields at stages corresponding to (a). (Figure credit: Zian Jia)

The measurements of the crack orientation and position show that these microcracks have no preferred orientation or position in the structure (Figure 5-31), consistent with previous fracture
observations in *in-situ* tests (*Figure 5-18* and *Figure 5-19*). Finally, all the fragments of the stereom are extracted and shown in *Figure 5-32a* and *Figure 5-33*. The sizes of the fragments are much larger than the throat size in *Figure 5-32b,c*, which further confirm the clogging mechanism observed in experiments (*Figure 5-34*).

*Figure 5-31* | **Analysis of crack orientation and locations in the fracture modeling.** The parameters in the plot are defined as the same as *Figure 5-18*. **a**, Distribution of microcracks (highlighted in red) in the stereom based on the simulation. **b,c**, Distribution of the crack location (*l/L*) (b) and orientation (*θ/θ₀*) (c). **d**, The correlation between the crack location and crack orientation in space. *α* is the angle between the normal vector of the crack plane and z-direction, *β* is the angle between the normal vector of the crack plane on the x-y plane and x-direction.
Figure 5-32 | Simulation-based fragments analysis. a, Simulation-based segmentation of the stereom using the location and orientation of the cracks. Different colors represent individual fragments. b, Representative fragments with each fragment described by three orthogonal dimensions ($2t_1 < 2t_2 < 2t_3$). Representative throats plotted in the same scale with the size defined by the diameter $r_t$. c, A comparison of the fragment sizes ($t_1, t_2, t_3$) to throat size ($r_t$) based on the volume (a). The relationship $r_t < t_2 < t_3$ indicates a clogging mechanism that facilitates densification.
Figure 5-33 | Comparison of the fragments (a) from the stereom in Figure 5-31a and the throats (b) in the stereom. Both (a) and (b) are plotted on the same scale.
5.3.5 Structure designs of stereom

Combining the experimental and computational results, a representative stress-strain response of the stereom under compression is summarized schematically in Figure 5-35a, which exhibits a global and local damage behavior. Globally, it displays an elastic response followed by a high-stress plateau regime and final densification. The high-stress plateau regime corresponds to the formation and propagation of damage bands in the stereom.

For the local deformation in microscale, the typical process of crack-induced damage band is illustrated by the extracted small volume in the stereom (Figure 5-35b, Figure 5-36). During the elastic deformation process (i), the stress concentration on the surface is not significant due to the
uniformly distributed strut thickness and the smooth surface. Beyond the elastic limit (ii), both the
experiment results (Figure 5-18 and Figure 5-19) and fracture modeling (Figure 5-31) show that
diffusive cracks are initiated randomly in the stereom due to low-stress concentration on the
surface. These microcracks propagate in the stereom (iii). The coalescence of these diffusive
microcracks further fractures the stereom (iv) and form large fragments (v). According to the in-
situ observation of the deformed volume (Figure 5-17), SEM examination of the residues (Figure
5-23), combined with the formed fragments in FEA, those large fragments can be easily rotated
and jammed by the small throats in the stereom (vi). These small throats with sizes smaller than
the structure thickness can be widely found in the echinoderm stereom\textsuperscript{93}. The jamming process
leads to the formation of a densely packed damage band. Load in the damage region is redistributed
to the entire fracture surface instead of concentrating on local nodes and struts. As a result, the
stress concentration in the damage band is low, allowing for further propagation of the damage
band without catastrophic crushing. Notably, the cracks surrounding the damage band show no
preferred position and orientations (vi). This facilitates local jamming and intact increasing local
bearing area (vii). Therefore, the strength of the deformed stereom is not reduced during the
formation and propagation of damage bands. This is further confirmed by the non-reduction in the
strength of the stereom when the stereom’s spallation is confined (Figure 5-37 and Figure 5-38).

In addition to the microstructural level toughening mechanisms discussed above, the biogenic
calcite also exhibits material level toughening mechanisms. For instance, the crack path of stereom
is observed to be conchoidal rather than brittle cleavage along the \{104\} plane as observed for
geological and synthetically produced crystals\textsuperscript{145,204,269}. The conchoidal behavior is also confirmed
by the random initiation of cracks (no preferred crack orientations and locations) under shear
loading (Figure 5-39). The conchoidal fracture of the biogenic calcite leads to the continuous
fracture of the calcitic lattices into micro-and nanoscopic pieces during the densification process within the deformation bands. It enhances the absorption capability in the damaged stereom\textsuperscript{270,271}.

**Figure 5-35** Correlation between the macroscopic response and the microstructure of stereom. a, Stereom exhibits the typical stress-strain response of cellular materials macroscopically. b, the deformed sample with a small volume cropped to show the failure process in **Figure 5-35** from stage i to stage viii. (Figure credit: Zian Jia)
**Figure 5-36** Small volume level deformation exhibits elastic deformation (i), microcracking (ii), microcrack propagation (iii), crack coalesce (iv), damage band formation (v), densification (jamming) (vi), damage band propagation (vii), further densification (jamming) (viii). inset, An SEM image of the damage band with the fragments colored by fragment size. (Figure credit: Zian Jia)
Figure 5-37 | Mechanical performance of the stereom upon compression. a, experiment setup of the compression test. b, stress-strain curves of the cube stereom under compression. The stereom is constrained in a glass tube here.
Figure 5-38 | Deformation behavior of the cube stereom constrained in the glass tube. a, stress-strain curve of the stereom, the spots in (a) correspond to the specimen at various deformation stages (b). $\varepsilon$ represents strain.
Figure 5-39 | Crack distribution in the shear-fractured stereom. a, volume rendering of the volume with cracks distributed in the volume. b,c, Distribution of the crack location ($l/L$) (b) and orientation ($\theta/\theta_0$) (c). d, The correlation between the crack location and crack orientation in space. $\alpha$ is the angle between the normal vector of the crack plane and z-direction, $\beta$ is the angle between the normal vector of the crack plane on the x-y plane and x-direction. e, An SEM image of the fracture surface.

5.4 Discussion and conclusion

In this chapter, in order to provide insights to address the challenge of catastrophic failure in the ceramic cellular solids, we investigate the structure-mechanical property relationship of
damage-tolerant and robust echinoderm stereom using *H. mammillatus* spine as an example. The stereom in *H. mammillatus* spine can achieve high strength 40.4 ± 12.4 MPa and high energy absorption 17.7 ± 4.0 kJ/kg. The design strategies in terms of their structures can be summarized as follows (1) The optimal strut surface morphology and randomly distributed skeleton enable a lower stress concentration on the stereom surface. This promotes high strength and facilitates the random crack initiations (no preferred locations or directions) in the volume and further leads to the formation of large fragments. (2) The randomly distributed small throats in the volume can jam the fragments and localize the damage in the damage bands. The damage band formation and propagation process does not weaken the structure and maintain the structural integrity. Further fragmentation, compaction, rotation, and friction of fragments contribute to high energy dissipation.

The strength and damage tolerance are mutually exclusive mechanical properties in both natural and synthetic materials\textsuperscript{195}. In natural biomineralized materials, they can be achieved by incorporating hierarchical structural design, such as the human cortical bone with key structure features from nanometer to millimeter scale. It can also be achieved by incorporating organic materials between mineral units, such as the mollusk shells\textsuperscript{272}. For echinoderm stereom, they achieve simultaneously high strength and high damage tolerance through a structured design on the key structural feature at the micrometer scale and with a mineral content > 99\%\textsuperscript{97–100}. Our findings shed light on the structure design of strong and damage tolerant ceramic cellular solids with upscaling key structure features.
Chapter 6 A natural dual-scale single-crystalline lattice material

The work based on the results is now being prepared for publication.

Cellular solids (e.g., foams and honeycombs) with high porosity are widely used in many applications such as sandwich structures, packaging, thermal and acoustic insulation. Architected cellular materials, enabled by the recent development of computational design and additive manufacturing, further extend the mechanical property space of conventional cellular solids and enable unusual physical properties. While remarkable micro-architectural control has been achieved in metals, glasses, ceramics, or polymers, current synthetic architected cellular structures are exclusively based on either polycrystalline or amorphous materials at the atomic scale. Here, we report a natural architected cellular structure found in the biomineralized skeletal units (ossicles) of the knobby starfish (Protoreaster nodosus), which exhibits a unique single-crystalline design at both atomic and lattice levels that have not been discovered in nature or realized synthetically before. Each millimeter-sized porous ossicle possesses a diamond triply periodic minimal surface (diamond-TPMS) structure with a typical lattice constant of ca. 30 µm. Remarkably, the [111] direction of the diamond-TPMS lattice is co-aligned with the $c$-axis of the underlying single-crystalline calcite at the atomic scale, exhibiting the dual-scale single-crystalline nature. This crystallographic co-alignment mitigates the compliance of calcite along the $c$-axis by utilizing the stiff [111] direction of the diamond-TPMS lattice. 3D in-situ mechanical characterizations further reveal that the presence of crystal defects such as 60° and screw
dislocations at the lattice level, which follow the dislocation types observed in atomic diamond crystals, suppresses slip-like fracture along the \{111\} planes of the diamond-TPMS lattice upon loading and hence enhances the energy absorption capability. This work provides new insights into the design of architected cellular materials that leverage controlled morphological and crystallographic coupling between atomic and lattice scales.
6.1 Introduction

Together with sea urchin, sand dollar, and brittle star, starfish belong to a group of marine organisms known as echinoderms. They possess a dermal endoskeleton consisting of numerous small porous calcareous skeletal elements (ossicles), which are connected by fibrous tissues. The interaction between the hard ossicles, muscles, and soft connective tissues allows for flexibility during locomotion and rigidity upon disturbance. While many stereom types have been observed, ranging from completely stochastic to anisotropically organized, fully periodic lattices have not been identified in stereom or other porous biomineralized structures in general.

Here, we report a natural architected cellular structure found in the biomineralized skeletal units (ossicles) of the knobby starfish (*Protoreaster nodosus*), which exhibits a unique single-crystalline design that has not been discovered in nature. Moreover, different from the synthetic and architected material with the structures exclusively based on either polycrystalline or amorphous materials, the ossicles in starfish exhibit a unique single-crystalline design at both atomic and lattice levels. Each millimeter-sized porous ossicle possesses a diamond triply periodic minimal surface (diamond-TPMS) structure with a typical lattice constant of 30 µm. Remarkably, the [111] direction of the diamond-TPMS lattice is co-aligned with the c-axis of the underlying single-crystalline calcite at the atomic scale, exhibiting the dual-scale single-crystalline nature. This crystallographic co-alignment mitigates the compliance of calcite along the c-axis by utilizing the stiff [111] direction of the diamond-TPMS lattice. 3D in-situ mechanical characterizations reveal that the presence of crystal defects such as 60° and screw dislocations at the lattice level, which follow the dislocation types as observed in atomic diamond crystals, suppresses slip-like fracture along the {111} planes of the calcitic diamond-TPMS lattice upon loading and hence enhances the energy absorption capability. This work provides new insights
into the design of architected cellular materials that leverage controlled morphological and crystallographic coupling between atomic and lattice scales.

6.2 Methods

Samples: Fresh and dried *P. nodosus* specimens were obtained from Gulf Specimen Marine Laboratories Inc. (FL, USA) and The Bone Room (CA, USA), respectively. Individual ossicle samples were prepared by removing the organic tissues through a two-step dissolution process: after the initial soaking of the frozen fresh starfish samples in bleach (6.05% sodium hypochlorite) for 20 mins to expose the dermal endoskeleton, the starfish skeleton was subsequently cleaned, labeled, and further soaked in bleach for another two hours to remove the connective tissues among ossicles. The as-obtained individual ossicles were rinsed with copious deionized water and finally dried at room temperature.

Microstructural analysis

*Electron microscopy:* SEM imaging was performed on dried specimens including intact starfish skeletons, isolated individual ossicles, fractured surfaces of ossicles, and ossicle samples after crystal overgrowth. Prior to imaging, all specimens were coated with Pt/Pd (ca. 10 nm) to reduce charging effects. The intact starfish skeletons and isolated individual ossicles were imaged using a Quanta 600 FEG Environmental SEM (FEI, OR). The fractured surfaces of ossicles and ossicle samples after crystal overgrowth were captured using a Helios Nanolab 600 Dual Beam electron microscope (FEI, OR). The SEM images were taken at acceleration voltages of 2-10 kV and at working distances of 4-10 mm.
Synchrotron µ-CT measurement: Dried individual ossicles were scanned at the beamline 2-BM (beam energy, 27.4 keV) at the Advanced Photon Source, Argonne National Laboratory. Each tomography scan consisted of 1500 projection images with an exposure time of 0.035s per projection and was acquired over a 180-degree sample rotation. Projection images were collected using a PCO Edge high-speed CMOS detector (2448×1024 pixels), which was equipped with 2× and 5× long-working-distance objective lens resulting in isotropic voxel sizes of 1.725 µm and 0.690 µm, respectively, in the reconstructed tomography data. The in-situ mechanical tests were conducted using a customized compression system designed for the µ-CT scanning experiments at the 2-BM beamline. The compression system was equipped with a hybrid linear actuator (resolution: 1.59 µm/step) and a load cell with a maximum load of 250 lbs (resolution: ±0.1%). Cubic samples with edge lengths of ~2 mm were cut from individual ossicles for the measurements. A displacement rate of 0.008 mm per step was used in this work. A time-lapse measurement was adopted, where the actuator was stopped at multiple nominal strain levels during the tests for tomography scans. The ossicle assembly was scanned using a high-resolution laboratory µ-CT Skyscan system (Model 1172, Aartselaar, Belgium). The X-ray tube was operated at 80 kV and 100 µA. Each tomography scan consisted of 273 projection images with an exposure time of 5.082 s per projection and was acquired over a 180-degree sample rotation. Projections were collected using a Hamamatsu camera (1280 x 1024 pixels) and the resulted isotropic voxel size is 17.42 µm in the reconstructed data. Multiple consecutive scans along the arm direction were acquired and subsequently stitched automatically with the analysis software provided by Skyscan.

µ-CT data analysis: Tomography reconstruction of the X-ray projection images was achieved using the open-source Python package Tomopy\textsuperscript{166}. The open-source machine learning-based
software, ilastik, was used for the segmentation of the reconstructed images\textsuperscript{167}. The segmented binary data was further imported to Avizo (Thermo Fisher Scientific. MA, USA) for 3D visualization, surface curvature analysis, and 3D-FFT analysis.

The principal curvatures, including the minimum curvature $\kappa_1$ and maximum curvature $\kappa_2$, were acquired through the curvature analysis module in Avizo. The curvature analysis was performed at individual nodes on the triangular mesh-based surface of the ossicle lattice. A quadratic surface is firstly fitted to the analysis node and three layers of surrounding neighboring nodes. The principal curvatures were then calculated from the eigenvalues and eigenvectors of the Hessian matrix defined at the node. Finally, the initial principal curvature values for the node were averaged four times with the curvature values of the surrounding neighboring nodes. The interfacial shape distribution of the as-calculated principal curvatures $\kappa_1$ and $\kappa_2$ was plotted in OriginPro 2016 (OriginLab. MA, USA).

3D-FFT analysis was performed on the 3D binary data of selected volumes of ossicle, individual intact ossicles, and a standard diamond-TPMS structure in Avizo. The 3D-FFT module in Avizo was used to compute the discrete Fourier Transform for scalar input data and center the results of 3D-FFT patterns. For visualization purpose, the logarithmic values of the original 3D-FFT data were calculated and used for volumetric rendering as shown in Figure 6-8 and Figure 6-9. The indexing process can be performed through the spherical integration of the original 3D-FFT pattern or the radial integration of the projected 3D-FFT pattern along a specific crystallographic orientation. For indexing with the spherical integration of the original 3D-FFT pattern, the specific wave vector positional ratios, $q/q_{\text{max}}$, for discrete peaks, which correspond to the high intensity spots in the 3D-FFT pattern, were determined through the spherical integration and subsequently obtained by dividing by the wave vector of the first peak ($q_{\text{max}}$). For the
representative volume in **Figure 6-6a**, with $q_{\text{max}} = 0.048 \mu m^{-1}$, diffraction peaks with $q/q_{\text{max}}$ of 1, 1.854, 2.689, and 3.530 were identified. As the first peak was indexed as the \{111\} planes from the diamond-TPMS structure, the entire set of high intensity spots can be indexed accordingly (**Figure 6-8**). Radial integrations of the projected 3D-FFT patterns along the [111] direction were also performed, and the indexing of the peaks followed the similar process for 3D spherical integration and indexing described above (**Figure 6-7, Figure 6-9**).

The lattice constant ($a$) and branch length ($l$) of the ossicles can be estimated from the 3D-FFT patterns. We first measured the reciprocal distance corresponding to \{HKL\} planes as $d_{\text{HKL}} (\mu m^{-1})$, which is the inverse spacing between the \{HKL\} planes, where $H, K, L$ are Miller indices for the plane. To convert this spacing in the reciprocal space into the lattice constant in the real space, the following equation was used

$$a^2 = (H^2 + K^2 + L^2)/d_{\text{HKL}}^2.$$  \hspace{1cm} \text{Equation 6-1}

With $l = \sqrt{3}a/4$ for the diamond lattice, the branch length $l$ can be obtained subsequently. Furthermore, the standard deviation of the lattice constant was calculated from the full width at half maximum (FWHM) of peak fitting of $d_{\text{HKL}}$ in OriginPro 2016 (OriginLab, Northampton, MA).

**Lattice network and dislocation analysis:** We analyzed the lattice network of ossicles by utilizing the quantitative network analysis algorithm that we recently developed for the analysis of the porous stereom structures in echinoderms\textsuperscript{284,285}. Following this algorithm, the porous lattice of ossicles can be represented by a network of branches and nodes after skeletonization. Here we defined a series of structural descriptors at branch and node level. Nodes are categorized according
to their coordination number, N. In the diamond-TPMS lattice analyzed here, due to the
 tetrahedron units, the dominant node type is N4, representing nodes connected with four branches,
 corresponding to a tetrahedron unit in the diamond-TPMS lattice. As shown in Figure 6-13 and
 Table 6-1, we further define branch lengths \( l \), thicknesses \( t \), and inter-branch angles
 for individual N-4 tetrahedral nodes. With a given global reference direction (typically the normal
direction of the starfish skeleton), the branch most aligned with the reference direction is denoted
as branch 1 and the other three inclined branches are denoted as branch 2, 3, and 4, respectively.
The orientation of branch 1 is registered as the [111] direction of the local tetrahedron unit of the
diamond lattice for consistency. The angle between branch 1 and the reference direction is defined
as the local crystal orientation, \( \theta \). The lengths and thicknesses of the four branches are denoted as
\( l_i \) and \( t_i \) \((i = 1, 2, 3, 4)\), respectively. We further calculated the average length and thickness of the
three inclined branches, i.e., \( l_m = (l_2 + l_3 + l_4)/3 \) and \( t_m = (t_2 + t_3 + t_4)/3 \), respectively.
Correspondingly, the length ratio and thickness ratio for a tetrahedron unit is given by \( \eta = l_1/l_m \)
and \( \lambda = t_1/t_m \), respectively. The inter-branch angles are calculated in two categories: \( \alpha_m \)
represents the average inter-branch angle between branch 1 and one of the inclined branches and
\( \beta_m \) represents the average inter-branch angle between two inclined branches. The mean inter-
branch angle for a tetrahedron unit is defined as \( \gamma_m = \alpha_m + \beta_m \). The histogram distribution of
\( \alpha_m \) shown in Figure 6-35a is converted according to the relationship \( A = 1/\tan (\pi - \alpha_m) \), where
\( A \) represents the corresponding location on the \( c \)-axis of calcite for each bin of \( \alpha_m \) values (Figure
6-35c). Matlab (MathWorks, Inc. USA) and the open-source software Ovito were used to generate
2D (Figure 6-14, Figure 6-16) and 3D (Figure 6-15, Figure 6-17) contour plots of the as-
calculated structural descriptors, respectively.
The nodal positions for individual intact ossicles obtained from the network analysis above were imported in Ovito for dislocation analysis using the Dislocation Extraction Algorithm (DXA), where the dislocation lines and the corresponding dislocation types with Burgers vectors can be identified. The DXA conducted on ossicles identifies the dislocation by comparing the distorted atomic configuration around the dislocation core to the strain-free local reference atomic configuration determined by an atomic structure identification algorithm. The total length of dislocation lines in the entire ossicle volume \( l_{\text{dislocation}} \) was then calculated. In addition to the conventional dislocation density \( \rho_{\text{dislocation}} = l_{\text{dislocation}}/V \) (\( V \) is the ossicle volume), we further define the normalized dislocation density \( \bar{\rho}_{\text{dislocation}} = (l_{\text{dislocation}}/a)/(V/a^3) \), where \( a \) represents the lattice constant. Note that \( \bar{\rho}_{\text{dislocation}} \) is dimensionless.

*Electron backscatter diffraction measurement:* Four superomarginal ossicles were mounted in Struers Epo-Fix epoxy. The mount was polished using 30 µm polishing paper to expose the interior of the ossicle. Once sufficiently exposed, the mount was progressively wet-polished for 10-minute intervals on 15 µm, 9 µm, 3 µm, 2 µm, and 1 µm polishing paper; between each step, samples were ultrasonicated for 3 minutes in deionized water to remove any particles. Prior to Electron backscatter diffraction (EBSD) analysis, the mount was weighted with a halved brass rod and chemically polished for 90 minutes in a non-crystallizing colloidal silica suspension on a Buehler Vibromet2 vibratory polisher. Samples were rinsed thoroughly with water and ethanol and dried overnight in a desiccator. After >12 hours of drying, samples were coated with ca. 10 nm carbon to minimize charging during EBSD analysis.

EBSD data were collected using an Oxford Instruments Nordlys Max3 detector attached to a Tescan Vega3 SEM. The ossicles were analyzed at a 70° tilt and the SEM was operated under high
vacuum at 20 kV using 15 nA beam current at working distances of 24–26 mm. The EBSD camera was positioned at 125 to 135 mm; EBSD data were acquired with AZtec 3.2 (SP1) and post-processed with Channel5.12 software. System settings for EBSD data acquisition include: Hough resolution of 65, 7 bands, 68 reflectors, 1x1 binning with a gain of 7. The entire superomarginal ossicle was mapped using a step size of 5 μm using a single frame (Figure 6-32b). The high-resolution map as shown in Figure 6-32c was collected using a 1 μm step size. Lattice constants used to create the reflector file were from Calcite (Calcite.cry) in the HKL phases database. The mean angular deviation (MAD) was 0.82° with a cutoff of 1.5°. Data were post-processed by iteratively interpolating unindexed pixels using the 8-, 7- and 6- nearest neighbors routine in Channel5. Next, aberrantly indexed, non-indexed points and wild spikes were removed. Wild spikes are non-systematic mis-indexed points and are classified as those with a significantly different measured orientation from neighboring grains and are usually the result of cracks or dislocation clusters within the mineral. Because post-processing can affect the integrity of the dataset, we compared processed data with raw data and found no obvious differences, indicating minimal impact from post-processing. (The EBSD measurement is conducted by Emily M. Peterman).

*Epitaxial overgrowth on ossicles:* The epitaxial overgrowth of calcite crystals on isolated starfish ossicles was achieved by following the previously reported procedures. Briefly, individual ossicles were placed in separated reaction wells of a 24-well culture plate. A 1.5 ml of the calcium chloride solution (7.5 mM, Sigma-Aldrich, 99%) was carefully added to each reaction well. The culture plate together with a petri dish filled with ammonium carbonate powder (Sigma-Aldrich, 30% NH₃ basis) were then placed in a sealed desiccator for 12 hours. The ossicles covered with
overgrown calcite crystals were then collected, rinsed with deionized water, and finally dried at room conditions for SEM imaging.

**Ex-situ mechanical test:** Cube-shaped samples with an edge length of ~2 mm were cut from the superomarginal and inferomarginal ossicles by using a low-speed diamond saw (MTI corporation, California, USA) and then manually polished with a rotating polisher (MultiPrepTM System, Allied High Tech Products, Inc., California, USA). The mass and size of each sample were measured before testing. The compression tests were conducted at a displacement rate of 0.5 mm/min by using a universal testing machine (Model 5944, Instron. MA, USA). Engineering stress ($\sigma$) and strain ($\varepsilon$) were obtained by using the original cross-sectional area and sample height of the specimens. The $\sigma$-$\varepsilon$ curves of the starfish ossicles exhibit three stages, including an elastic regime, a stress plateau regime ($\varepsilon$ up to ~60%), and a densification regime (Figure 6-37). The strength ($\sigma_p$, the peak stress before the first stress drop) is 46.48 ± 15.14 MPa, where the stress level in the plateau regime is approximately half of the strength. The energy absorption per unit volume ($W_p$) was calculated by integrating the area under the $\sigma$-$\varepsilon$ curve up to the strain where the densification begins ($\varepsilon_m$) (Figure 6-37). The mass and size of each sample were measured before testing. In addition, the indentation experiment shown in Figure 6-42b was performed on ossicle samples by using a steel tip on the same Instron system (displacement rate, 0.2 mm/min).
6.3 Results

6.3.1 Structure characteristics

6.3.1.1 Ossicles in *P. nodosus*

Starfish *P. nodosus* (knobby starfish) belongs to *Protoreaster* genus, *Oreasteridae* family, *Valvatida* order (*Figure 6-1a*). The body of *P. nodosus* is pentameral and composed of a central disc and five arms of equal length. Two global directions – radial and normal direction are used in this description. Radial direction starts from the peristome in the center to the arm tip, and the transverse direction is perpendicular to the disc. After removing the tissue in bleach, the skeleton of *P. nodosus* composed of ossicles is exposed for analysis (*Figure 6-1b-c*).

![Figure 6-1](image_url)

**Figure 6-1| Skeleton of *P. nodosus* with or without tissue.** *a*, Photo of live *P. nodosus*. *b,c*, Skeleton with (b) and without (c) soft tissue covered.

According to their morphologies and locations in the body, the ossicles can be classified into different types (*Figure 6-2*). Along the normal direction of *P. nodosus*, ossicles are on either the
oral or aboral side (Figure 6-2a,b). The central disc and the periphery of the arms consist of marginal ossicles, which are pairs of continuous ossicles in both interior and exterior, such as the superomarginal ossicle on the aboral side and the inferomarginal ossicles on the oral side (Figure 6-2c-f).

At the aboral side (Figure 6-2c,e), abactinal ossicles are extended from the arm tip to the central disc in regular series in Valvatida. Abactinal ossicles are thick and tabular, partially overlapping with primary tubercle ossicles and a few secondary ossicles. The madreporite is located beside the abactinal ossicles and the primary tubercle in the central disc for P. nodosus. In addition, secondary ossicles are superficial ossicles with varied morphology, including spinelets, granules, and tubercles. Spinelets are short and small ossicles, while granules are equidimensional and small. They are arranged densely, distributed everywhere, and hard to differentiate. Tubercles are massive, including the primary tubercle on the central disc and the arm tubercle on the arm.

At the oral surface (Figure 6-2d,f), five ambulacral ossicles meet at the peristome. Five jaws, each composed of a pair of mouth plates, are projecting over the peristome. The small interradial ossicles—odontophore—are adjacent to the mouth plate ossicles. The actinal ossicles on the oral side, arranged in an irregular mosaic pattern and surrounded by mouth plates, ambulacral and inferomarginal ossicles, are made up of small flat polygonal or sub-rounded ossicles.

Considering its pentameral symmetry, the ossicles from the central disc and one ray on both the oral and aboral sides are labeled (Figure 6-3).
Figure 6-2 | Classification of ossicles in the skeletal system of the starfish *P. nodosus*. a, Classification of ossicles in the skeletal system of the starfish *P. nodosus*. a, Optical images of the skeleton after soft tissue removal for both aboral and oral side, respectively. The normal direction is defined as the direction from oral to aboral side. b, Cross-sectional view of the skeleton based on μ-CT reconstruction data showing the ossicle assemblies. The dashed line in (a) indicates the location of the cross-sectional cut. Exterior (c,d) and interior (e,f) views of the aboral (c, e) and oral (d,f) sides of the skeleton, respectively, where ossicle types are labelled.281
Figure 6-3 | Labeling of individual ossicles in the aboral (a) and oral (b) side of the P. nodosus skeleton.

6.3.1.2 Diamond-TPMS structure

*Single-crystalline diamond lattice in a small cube*

A superomarginal ossicle is extracted from the skeleton for further visualization. Scanning electron microscopy (SEM) images reveal that these mm-sized ossicles exhibit a porous lattice-like structure (Figure 6-4), which is so ordered that atomic terrace-like morphology can be
observed at the surface of ossicles (yellow arrows, Figure 6-4a). Imaging of the fractured surfaces further indicates that the periodic lattice structure extends to the interior of the ossicles (Figure 6-4b,c).

Figure 6-4| SEM images showing the highly ordered lattice structure in ossicles. The arrows in (a) indicate the terrace-like morphology formed by the (111) planes on the ossicle surface. b, c, SEM image of the fracture surface of an ossicle.

The ossicle assembly extracted from one arm of *P. nodosus* reveals that the regular structures can be observed on the ossicle surfaces of different ossicles. Moreover, these regular lattice structures in ossicles are aligned in different orientations, as shown in Figure 6-5a. These planes are similar to the atomic single-crystalline configuration (Figure 6-5b-e, insets), showing the “atomic” distributions resembling transmission electron microscopy (TEM) images. The blue dots and the red dots in Figure 6-5b-e indicate the nodes on the lower and upper layer, respectively. The ossicle surface perpendicular to the normal direction was indexed after the lattice structure is clarified.
Figure 6-5 | Indexing crystallographic orientations on ossicle surfaces along normal direction. a, SEM image of the ossicle assemblies. Inset, the location of this region in the *P. nodosus* skeleton indicated by the red box. b-e, SEM images of selected ossicles with lattice orientations indexed. Their locations are indicated by the red boxes in (a). Insets, corresponding diamond crystal models when viewed in corresponding crystal orientations.

To determine the lattice structure and symmetry of the 3D structure, the quantitative 3D structural analysis was performed on a representative µ-CT volume (243×243×243 μm³) extracted from an ossicle (Figure 6-6a). The skeletonized lattice network demonstrates the periodic arrangement of the tetrahedron units with the branch length (*l*) of 15.5 ± 2.5 μm (number of measurements: 5,499) (Figure 6-6b-c). Moreover, the skeleton exhibits periodic patterns when visualized from different directions, as shown in Figure 6-6d-f.
Figure 6-6| Visualization of the diamond lattice structure in *P. nodosus*’ ossicles. 3D rendering (a) and corresponding skeleton (b) of a representative ossicle volume with three orthogonal edges of the [111], [110], and [112] direction. The inset in (c) highlights the tetrahedral units for a diamond lattice. d-f, Projections of the skeleton of ossicle volume on the [111] (d), [110] (e), [112] (f) directions, respectively.

The 3D-FFT analysis was performed on the 3D binary data of the representative volume (Figure 6-7a) and the diamond-TPMS structure (Figure 6-7b) with the same relative density and branch length. The logarithm of the magnitude of the 3D-FFT data was obtained. The radial integration of the 3D-FFT pattern was used to label diffraction peaks in terms of the wavevector positional ratios (Figure 6-7c). The reflection peaks of the 3D intensity plots were divided by the wave vector of the first peak with $q_{max}$, revealing discrete peaks with the scattering wave vector
positional ratios, $q/q_{\text{max}}$. The peaks for the intensity plot are on the same wavevector positional ratios as the diamond-TPMS structure. For the ossicle cube in Figure 6-7a, $q_{\text{max}} = 0.048 \, \mu m^{-1}$, while $q/q_{\text{max}}$ are 1, 1.854, 2.689, 3.530. The first peak was indexed as the \{111\} planes. Therefore, the discrete Bragg peaks can be indexed as \{220\}, \{222\}, \{331\}, \{440\}, confirming the diamond lattice$^{293}$.

Measurement of the reciprocal cell parameters from 3D-FFT indicates a lattice constant ($a$) of $34.0 \pm 5.9 \, \mu m$, which is in excellent correspondence with the real-space measurements ($a = \frac{4}{\sqrt{3}}l$ for a diamond lattice). The Bragg reflections for the volume extracted from ossicles have been indexed in 3D space (Figure 6-8) and 2D projections (Figure 6-9).

**Figure 6-7** Comparison of the diamond-TPMS lattice in ossicles with the standard diamond-TPMS structure. a, 3D volume renderings of a representative ossicle volume and a standard diamond-TPMS structure. b, Normalized radial integrations of the projected 3D-FFT pattern along the [111] direction for the two datasets. The wavevector positions are normalized with respect to the peak position of the \{111\} plane groups (see Methods for further details). The vertical lines denote the expected Bragg peaks for the cubic diamond structure (space group of $Fd\bar{3}m$). The dashed line indicates the position of the \{222\} forbidden reflections.
The pattern and indexing of the ossicle cube are directly compared with the FFT pattern and indexing from the analytical results and from the diamond-TPMS structure. In addition to the standard “Fd$\bar{3}m$” Bragg reflections, “symmetry-forbidden” intensity spots corresponding to the \\{hkl\} planes with $h + k + l = 4n + 2$ ($n$ is an integer) have also been observed, for example, the \{002\} family (Figure 6-8 and Figure 6-9)\textsuperscript{294}. In contrast to the atom-based diamond crystals, the presence of these symmetry-forbidden reflections results from the branches connecting neighboring nodes in the diamond lattice structure\textsuperscript{294,295}.

![Figure 6-8](attachment:image.png)

**Figure 6-8| Comparison of 3D-FFT patterns from a theoretical diamond crystal (a), a standard diamond-TPMS lattice, and an ossicle.** The lattice constants ($a$) of the theoretical diamond crystal and the standard diamond-TPMS lattice are set to be 34 µm, comparable with that of the starfish ossicles. Note that some diffraction spots are overlapped with multiple diffractions resulted from different plane groups. The indices labeled in blue, yellow, and red represent the diffraction spots located at the back, middle and front plane with respect to the viewer, respectively. The indices highlighted in green circles represent forbidden reflections in single scattering approximation for a diamond crystal lattice.
**Figure 6-9| Indexing 3D-FFT patterns for the standard and ossicles’ diamond-TPMS lattice structure.** a-c, Theoretical 3D-FFT patterns of a diamond lattice viewed in different directions. The diffraction spots are indexed on three consecutive planes along the [111] direction (a), six consecutive planes along with the [11\(\bar{2}\)] direction (b), and two consecutive planes along with the [1\(\bar{1}\)0] direction (c), respectively. The diffraction spots highlighted in the same color indicate those located in the same plane. Note that the locations of the \{002\} forbidden reflections are highlighted in green circles. d-i, 2D projections of the 3D-FFT patterns for the standard (d-f) and the ossicles’ diamond-TPMS structure (g-i) along [111], [11\(\bar{2}\)], [1\(\bar{1}\)0] directions, respectively. In (d-i), the color of circles represents diffraction spots located in corresponding planes shown in (a-c), respectively. Note that the diffraction spots on the central planes are highlighted in yellow consistently. The forbidden reflections of \{222\} plane group on the central planes are highlighted in orange instead. ZA, zone axis.

**TPMS surface characterization**

In addition to the superb lattice periodicity, the surface morphology of the diamond lattice in ossicles exhibits remarkable resemblance to the diamond-TPMS structure when viewed in low-index planes such as the (111), (1\(\bar{1}\)0), (11\(\bar{2}\)) planes (**Figure 6-10**).
Figure 6-10| A comparison of the cross-sectional patterns between the ossicle (left) and a standard diamond-TPMS structure (right) on (111), (112), and (110) planes.

Moreover, with the synchrotron µ-CT data, we quantified the minimum and maximum principal curvatures (\( \kappa_1 \) and \( \kappa_2 \), respectively) of the lattice surface and represented their distributions with an interfacial shape distribution (ISD) plot (Figure 6-11a). \( \kappa_1 \) and \( \kappa_2 \) are estimated as \(-0.135 \pm 0.050 \mu\text{m}^{-1}\) and \(0.137 \pm 0.043 \mu\text{m}^{-1}\), respectively. The corresponding mean curvature \( H = (\kappa_1 + \kappa_2)/2 \) is \(0.001 \pm 0.032 \mu\text{m}^{-1}\), which explains a single narrow peak centered along the line \( \kappa_1 + \kappa_2 = 0 \) in the ISD plot (Figure 6-11a). The contour plot of \( H \) directly mapped onto the original structure shown in Figure 6-11b further reveals the uniform distribution of nearly zero mean curvature values. In the 1960s, Donnay and Pawson postulated that the bicontinuous morphology of echinoderms’ stereom may be a minimal surface structure\(^{83}\). This has been subsequently cited in later publications\(^{82,207}\), although quantitative validation has not been demonstrated. Our analysis here indicates that the periodic lattice in the \( P. nodosus \)’ ossicles is, to
the best of our knowledge, the first known biomineralized diamond-TPMS structure found in nature.

Figure 6-11 | Surface curvature distribution on ossicle. a, Interface shape distribution map (i.e., \( \kappa_2 \) v.s. \( \kappa_1 \)) of an ossicle volume. Inset schematics indicate the interface shapes in different regions. b, Contour map of the mean curvature \( H \) on the ossicle’s diamond-TPMS structure.

Structure characteristics in the ossicle

The structure characteristics are demonstrated using ossicle No. 7 (superomarginal ossicle) located at the edge of one arm as an example (Figure 6-12a,b). The pattern (111), (11\( \bar{2} \)), (1\( \bar{1} \)0) planes resemble diamond-TPMS structure (Figure 6-12c). The \( \mu \)-CT projection images and corresponding 3D-FFT patterns clearly demonstrate that the diamond lattice structure is conserved in a single ossicle (Figure 6-12d,e). The diamond-TPMS lattice fully comprises the entire volume of ossicles. This makes it possible to possible to “index” specific lattice planes of their outer surfaces (Figure 6-5). As shown in Figure 6-5, the normal directions of ossicles are primarily oriented along the [111] direction of the diamond lattice.
Figure 6-12 | Visualization of the diamond-TPMS lattice structure at the ossicle level. a, 3D volume rendering of a representative ossicle (No. 7) with three orthogonal planes of (111), (112), and (110). b, The location of ossicle No. 7 in the *P. nodosus* skeleton. c-e, Reconstruction slices (c), volumetric projection images (d), and corresponding 3D-FFT patterns (e) along the [111], [112], and [110] directions, respectively.

We further utilized our recently developed network analysis algorithm to quantify a range of structural descriptors within individual ossicles. The local structural variations were characterized by the structural descriptors shown in Figure 6-13, Table 6-1. The structural descriptors include nodal configuration (N) and crystal orientation (θ: angle between the local [111] orientation and global reference orientation) that examine the single crystallinity of the local tetrahedron, and
branch length, thickness, the inter-branch angle that characterize the local tetrahedron morphology, and the relative density \( \rho \) that reflects local volume fraction.

![Schematic diagram of a tetrahedron unit showing the structural descriptors used in this study.](image)

**Figure 6-13** Schematic diagram of a tetrahedron unit showing the structural descriptors used in this study. See Method (Lattice network and dislocation analysis) and Table 6-1 for further details.

<table>
<thead>
<tr>
<th>Table 6-1</th>
<th>Definition of all structural descriptors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td><strong>Definition</strong></td>
</tr>
<tr>
<td>N</td>
<td>Nodal configuration, or node type</td>
</tr>
<tr>
<td>( \theta ) (°)</td>
<td>Crystal orientation (angle between the local [111] orientation and global reference orientation)</td>
</tr>
<tr>
<td>( \bar{\rho} )</td>
<td>Volume fraction, or relative density</td>
</tr>
<tr>
<td>( l_1 ) (µm)</td>
<td>Length of branch 1</td>
</tr>
<tr>
<td>( l_m ) (µm)</td>
<td>Mean branch length of branch 2,3 and 4</td>
</tr>
<tr>
<td>( \eta )</td>
<td>Length ratio, ( \eta = l_1/l_m )</td>
</tr>
<tr>
<td>( t_1 ) (µm)</td>
<td>Thickness, or radius, of branch 1</td>
</tr>
<tr>
<td>( t_m ) (µm)</td>
<td>Mean thickness, or mean radius, of branch 2,3, and 4</td>
</tr>
<tr>
<td>( \xi )</td>
<td>Thickness ratio, ( \xi = t_1/t_m )</td>
</tr>
<tr>
<td>( \alpha_m ) (°)</td>
<td>Mean value of three inter-branch angles between branch 1 and three inclined branches</td>
</tr>
<tr>
<td>( \beta_m ) (°)</td>
<td>The mean value of three inter-branch angles among branch 2,3 and 4</td>
</tr>
<tr>
<td>( \gamma_m ) (°)</td>
<td>Mean value of the inter-branch angle in a unit tetrahedral structure, ( \gamma_m = (\alpha_m + \beta_m)/2 )</td>
</tr>
</tbody>
</table>
The local structural variations of ossicle No. 7 can be visualized in the 2D contour plot of different planes (Figure 6-14) and the 3D contour plot of the volume (Figure 6-15). The corresponding structure descriptor measurements of ossicle No. 7 are summarized in Table 6-2. The relative density $\bar{\rho}$ is uniformly distributed on the entire ossicle with the range of 0.5-0.6. This is due to local variations of branch length and branch thickness. For instance, the inclined branches, their length ($l_m$) and thickness ($t_m$) vary in the range of 13.0~17.6 µm and 3.5~5.0 µm (90% confidence intervals), respectively. Non-N4 nodes (indicated by the red arrow) are concentrated at the ossicle periphery due to the skeletonization artifacts on the boundary. Branch length and branch thickness, except for the distribution of $l_1$ that increases from the periphery towards the interior, decrease from the arc periphery towards the interior of the ossicle. The branch length ratio increases from the ossicle periphery to the ossicle interior. On the contrary, the branch thickness ratio increases from the ossicle periphery to the interior. The inter-branch angles $\alpha_m$ and $\beta_m$ remain uniformly distributed within an individual ossicle. The mean of the addition of $\alpha_m$ and $\beta_m$, defined as $\gamma_m$, however, distributes uniformly proving the complementary nature of $\alpha_m$ and $\beta_m$. 
Figure 6-14 | 2D contour plots of lattice structural descriptors for ossicle No. 7 when viewed along three orthogonal orientations, i.e., [111], [111], and [110], respectively. The yellow arrows in the node type (N) maps indicate the periphery regions of the ossicle with a high density of non-N4 nodes.
Figure 6-15] 3D contour plots of lattice structural descriptors for ossicle No. 7. In these plots, the values of each structural descriptor are calculated for each node and represented as a point.

The same structural characterization performed on ossicle No. 52 (abactinal ossicle) shows similar local variations from the ossicle periphery to the interior (Figure 6-16, Figure 6-17, Table 6-2).
Figure 6-16 | 2D contour plots of lattice structural descriptors for ossicle No. 52 when viewed along three orthogonal orientations, i.e., [111], [112], and [110], respectively. The yellow arrows in the node type (N) maps indicate the periphery regions of the ossicle with a high density of non-N4 nodes.
Figure 6-17| 3D contour plots of lattice structural descriptors for ossicle No. 52. In these plots, the values of each structural descriptor are calculated for each node and represented as a point.

Table 6-2| Mean and standard deviation of the structure parameters of ossicle No. 7 and ossicle No. 52.

<table>
<thead>
<tr>
<th>Ossicle</th>
<th>N3</th>
<th>N4</th>
<th>Other nodes</th>
<th>( \bar{\rho} )</th>
<th>( \theta ) (°)</th>
<th>( l_1 ) (µm)</th>
<th>( l_m ) (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>79,023</td>
<td>384,681</td>
<td>4,763</td>
<td>54.90%</td>
<td>9.29±6.78</td>
<td>14.02±2.26</td>
<td>15.46±1.43</td>
</tr>
<tr>
<td>52</td>
<td>51,230</td>
<td>148,541</td>
<td>3,471</td>
<td>51.70%</td>
<td>10.23±7.85</td>
<td>14.02±2.59</td>
<td>16.05±1.58</td>
</tr>
<tr>
<td>Ossicle</td>
<td>( \eta )</td>
<td>( t_1 ) (µm)</td>
<td>( t_m ) (µm)</td>
<td>( \xi )</td>
<td>( \alpha_m ) (°)</td>
<td>( \beta_m ) (°)</td>
<td>( \gamma_m ) (°)</td>
</tr>
<tr>
<td>7</td>
<td>0.92±0.18</td>
<td>4.26±0.55</td>
<td>4.34±0.45</td>
<td>0.98±0.11</td>
<td>110.81±3.87</td>
<td>107.35±4.11</td>
<td>109.08±0.49</td>
</tr>
<tr>
<td>52</td>
<td>0.89±0.21</td>
<td>4.43±0.78</td>
<td>4.16±0.52</td>
<td>1.07±0.15</td>
<td>113.11±4.96</td>
<td>104.64±5.62</td>
<td>108.87±0.65</td>
</tr>
</tbody>
</table>
**Structural characteristics among individual ossicles**

The structures of eight individual ossicles form both the oral side and aboral side, including superomarginal ossicles (Ossicle No. 7, 98), inferomarginal ossicle (Ossicle No. 157), abactinal ossicles (Ossicle No. 37, 52, 129), actinal ossicles (Ossicle No. 184, 191), were further examined (Figure 6-18). Both the $\mu$-CT projection images and corresponding 3D-FFT patterns clearly demonstrate that the diamond lattice structure is conserved in ossicles from both aboral and oral sides.

**Figure 6-18** Visualization of the diamond-TPMS lattice structure at the ossicle level. a, Eight randomly chosen ossicles from the aboral and oral sides of a *P. nodosus* skeleton. b, Volumetric projections of the reconstructed volumes and corresponding 3D-FFT patterns along the [111], [112] and [110] directions, respectively.

Moreover, the radial integration of the FFT pattern among individual ossicles confirms the $Fd\bar{3}m$ symmetries in the ossicles (Figure 6-19a). The distance of the first peak in reciprocal space
and the corresponding full width at half maximum (FWHM) allows us to quantify the lattice constants variations among individual ossicles. The lattice constant is rather stable among different ossicles, i.e., 29.2–34.2 µm (corresponding to the branch length of 12.6 - 14.8 µm) in regardless of their relative density variation (0.5 - 0.6) and large volume variations (1 – 5 mm³) (Figure 6-19, Table 6-3).

![Figure 6-19](image)

**Figure 6-19| Analysis of lattice constants of the ossicles’ diamond-TPMS structure based on 3D-FFT data. a, Normalized radial integration of the 3D-FFT patterns projected along [111] direction for eight ossicles. The vertical lines denote the expected Bragg peaks for the diamond cubic structure with the space group of \( Fd\bar{3}m \). b,c, Distribution of lattice constants for eight ossicles as a function of relative density (b) and volume (c) of ossicles, respectively. The error bars are the minimum and maximum values, respectively (see Methods, \( \mu \)-CT data analysis).**

**Table 6-3| Lattice parameters among different ossicles**

<table>
<thead>
<tr>
<th>Ossicle</th>
<th>37</th>
<th>52</th>
<th>191</th>
<th>184</th>
<th>129</th>
<th>7</th>
<th>157</th>
<th>98</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume ((V, \text{mm}^3))</td>
<td>0.980</td>
<td>1.060</td>
<td>1.140</td>
<td>1.880</td>
<td>2.220</td>
<td>2.310</td>
<td>4.640</td>
<td>5.070</td>
</tr>
<tr>
<td>Lattice parameter ((a, \mu m))</td>
<td>34.0</td>
<td>34.2</td>
<td>30.7</td>
<td>32.2</td>
<td>33.0</td>
<td>32.5</td>
<td>29.2</td>
<td>31.5</td>
</tr>
<tr>
<td>(a_{\text{min}} (\mu m))</td>
<td>27.6</td>
<td>27.9</td>
<td>24.6</td>
<td>25.8</td>
<td>26.6</td>
<td>27.0</td>
<td>23.0</td>
<td>25.5</td>
</tr>
<tr>
<td>(a_{\text{max}} (\mu m))</td>
<td>44.2</td>
<td>44.1</td>
<td>40.9</td>
<td>42.7</td>
<td>43.3</td>
<td>40.7</td>
<td>39.8</td>
<td>41.3</td>
</tr>
<tr>
<td>Branch length ((l, \mu m))</td>
<td>14.7</td>
<td>14.8</td>
<td>13.3</td>
<td>13.9</td>
<td>14.3</td>
<td>14.1</td>
<td>12.6</td>
<td>13.6</td>
</tr>
<tr>
<td>(l_{\text{min}} (\mu m))</td>
<td>12.0</td>
<td>12.1</td>
<td>10.7</td>
<td>11.2</td>
<td>11.5</td>
<td>11.7</td>
<td>10.0</td>
<td>11.0</td>
</tr>
<tr>
<td>(l_{\text{max}} (\mu m))</td>
<td>19.1</td>
<td>19.1</td>
<td>17.7</td>
<td>18.5</td>
<td>18.7</td>
<td>17.6</td>
<td>17.2</td>
<td>17.9</td>
</tr>
</tbody>
</table>
6.3.1.3 Dislocations in ossicles

The analysis above demonstrates that the individual ossicle in the starfish *P. nodosus*’ skeletal system is a single-crystalline diamond-TPMS lattice. As it is well known that defects such as dislocations are ubiquitous in atomic crystals, affecting material properties and functionality, it is of high interest to investigate the structural defects at the lattice level within ossicles and compare them with those in atomic crystals with the cubic diamond symmetry (e.g., diamond, Si, and III-V semiconductors)\(^{296-298}\).

For the atomic crystals with diamond cubic structure, similar to many face-centered-cubic (FCC) structures in semiconductors, the slip system in the atomic diamond crystal is of the \{111\}<110> type (slip plane/glide direction)\(^{297-299}\), where the dislocation lines can originate at the substrate interface\(^{300}\), form from surface defects\(^{297,298,301}\) or a sharp corner\(^{299}\) or in the vicinity of crack fronts\(^{302}\). The two prominent types of dislocations are the screw dislocation with a dislocation line parallel to the Burgers vector, and the \(60^\circ\) dislocation with dislocation line forms a \(60^\circ\) angle with Burgers vector\(^{298,303-307}\).

For the \(60^\circ\) dislocation, the dislocation can terminate on two equivalent families of \{111\} planes, closely spaced \{111\} planes or the widely spaced \{111\} planes, resulting in the glide-set and shuffle-set dislocations, respectively\(^{308}\). The \(60^\circ\) shuffle dislocation is characterized by an 8-atom ring including a 3-coordinated atom with a dangling bond oriented along \<111\> direction\(^{303}\). The \(60^\circ\) shuffle dislocation has been identified in previous investigations of the nucleation of dislocations in silicon\(^{309-312}\). The \(60^\circ\) glide dislocation is composed of a five-atom ring and a seven-atom ring\(^{313}\).

For screw dislocations in the atomic diamond crystals, the \<110\> screw dislocation is usually classified into the \(A\), \(B\), and \(C\) (including \(C_1\) and \(C_2\)) types according to the position of core
Hornstra firstly proposed two possible core structures, $A$ type and $C_1$ type, in terms of the locations on shuffle $\{111\}$ planes and glide $\{111\}$ planes, respectively. For shuffle dislocations ($A$ type), the original coordination of atoms is conserved at the expense of strong bond distortions. For instance, bonds close to the core and linking atoms on both sides of shuffle planes are disoriented by about $20^\circ$ compared to the bulk. The simple-period glide configuration ($C_1$ type) corresponds a core composed of 3-coordinated atoms with bond-bond angles of approximately $120^\circ$ (i.e., $sp^2$ hybridized states). The mixed shuffle/glide core ($B$) involves the breaking of bonds along the dislocation line, resulting in two rows of dangling bonds. Finally, by atomic rearrangements of $C_1$ configuration, the double period glide configuration $C_2$ is formed with only 4-coordinated atoms. The relative stabilities of these four possible core configurations were investigated through different computational and analytical methods. The study based on first-principles calculations confirmed that $A$ type was energetically favored. However, an analytical study on the effects of pressure on the dislocation core properties confirmed that the $C_2$ configuration is most stable. Furthermore, $B$ configuration is most stable using the Stilling-Weber potential for interatomic interaction method. However, electronic structure calculations all indicate that the B core is most unstable due to the existence of dangling bonds.

The dislocation lines are deviated from the straight line in most crystals and under most circumstances due to the existence of kinks and jogs. Kinks are steps in the dislocation line that are fully contained in the slip plane of dislocation. In contrast, jogs are steps on the dislocation which move it from one atomic slip plane to another. In silicon, the kink structure has been widely investigated using atomistic numerical simulations for partial dislocations. However,
there is less understanding about the kink structure of undissociated dislocations. The kink structure in the screw dislocations was characterized by an N5 node\textsuperscript{327}.

The dislocation densities in various single-crystalline atomic diamond crystals have been previously measured (Table 6-4). The dislocation densities are $10^4$ - $10^6$ cm\(^{-2}\) and $10^8$ - $10^9$ cm\(^{-2}\) in the synthetic Type-Ib diamond\textsuperscript{328} and the natural Type-IIa diamond\textsuperscript{301}. The dislocation densities in the commercially available diamond can be as low as $(10^3$ - $10^5$ cm\(^{-2}\))\textsuperscript{329}. In addition, the silicon sample at room temperature has high dislocation density (more than $10^3$ cm\(^{-2}\))\textsuperscript{330,331}. Considering the lattice constants in the diamond crystal and silicon crystal are $a = 3.57$ Å and $a = 5.4307$ Å at room temperature, respectively, the normalized dislocation density in the single atomic diamond crystals can be as high as $10^{-6}$ in the natural Type-IIa diamond\textsuperscript{301} and can be very low as $(10^{12})^3$ in the commercially available diamond\textsuperscript{329}. The dislocation density in the chemical vapor deposition (CVD) grown polycrystalline diamond can be very high $(10^{-3})^3\textsuperscript{304}.$

Table 6-4| Dislocation densities in the atomic diamond crystal

<table>
<thead>
<tr>
<th>materials</th>
<th>Line density (cm(^{-2}))</th>
<th>Lattice constant (Å)</th>
<th>Normalized density</th>
</tr>
</thead>
<tbody>
<tr>
<td>natural type IIa diamond\textsuperscript{301,328}</td>
<td>$10^4$-$10^9$</td>
<td>3.57</td>
<td>$10^{-7}$-$10^{-6}$</td>
</tr>
<tr>
<td>single-crystal plasma-deposited diamond\textsuperscript{332}</td>
<td>$10^6$</td>
<td>3.57</td>
<td>$10^{-9}$</td>
</tr>
<tr>
<td>synthetic type Ib diamonds\textsuperscript{328}</td>
<td>$10^4$ - $10^6$</td>
<td>3.57</td>
<td>$10^{-11}$-$10^{-9}$</td>
</tr>
<tr>
<td>commercially available diamond\textsuperscript{329}</td>
<td>$10^3$-$10^5$</td>
<td>3.57</td>
<td>$10^{-12}$-$10^{-10}$</td>
</tr>
<tr>
<td>Chemical vapor deposition (CVD) grown polycrystalline diamond\textsuperscript{304}</td>
<td>up to $10^{12}$</td>
<td>3.57</td>
<td>$10^{-3}$</td>
</tr>
<tr>
<td>mc-Si solar cell material\textsuperscript{333}</td>
<td>$10^4$-$10^6$</td>
<td>5.43</td>
<td>$10^{-11}$-$10^{-9}$</td>
</tr>
<tr>
<td>Si-samples with high dislocation density\textsuperscript{330,331}</td>
<td>more than $10^9$</td>
<td>5.43</td>
<td>$10^{-6}$</td>
</tr>
</tbody>
</table>

For ossicles, careful examination of the $\mu$-CT slices and SEM images of the fracture surface reveals the presence of dislocation-like lattice defects (Figure 6-20a,b). Moreover, taking
advantage of the 3D information of the lattice at the microscale, we further determine the type, core structure, and density of dislocations in ossicles. Such information in atomic crystals is extremely challenging to acquire since it requires 3D structural analysis at Angstrom-scale.

The dislocation analysis was performed using ossicle No. 52 as an example. Both the 60° dislocations and screw dislocations in ossicles were identified as the \{111\}/<110> (slip plane/glide direction) type. Remarkably, they are also the primary dislocation types in atomic diamond crystals\textsuperscript{298,303,304,307}. The nodal positions for ossicle No. 52 obtained from the network analysis were imported in the open-source software \textit{Ovito} using a Dislocation Extraction Algorithm (DXA), where the dislocation lines and the corresponding dislocation types with Burgers vectors can be identified. The DXA conducted on ossicles identifies the dislocation by comparing the distorted atomic configuration around the dislocation core to the strain-free local reference atomic configuration determined by an atomic structure identification algorithm. The total length of dislocation lines in the entire ossicle volume is calculated \(l_{\text{dislocation}}\). In addition to the

\[ \text{Figure 6-20} \mid \text{Dislocation-like defects on the } \mu\text{-CT reconstruction slice (a) and the SEM image in the fracture surface of an ossicle (b).} \]
conventional dislocation density $\rho_{\text{dislocation}} = l_{\text{dislocation}}/V$ ($V$ is the ossicle volume), we further define the normalized dislocation density $\tilde{\rho}_{\text{dislocation}} = (l_{\text{dislocation}}/a)/(V/a^3)$, where $a$ represents the lattice constant. Note that $\tilde{\rho}_{\text{dislocation}}$ is dimensionless.

Guided by the location of the dislocation lines detected in Ovito, the dislocation core structures in ossicles are further traced in Avizo. The tracking results are shown in Figure 6-21a,b. Globally, the dislocation line for the 60° dislocation is in zig-zag along [111] direction (Figure 6-21a). For the screw dislocations in ossicles, the dislocation line is along [112] direction (Figure 6-21b,c). Further examinations on the local structure reveal the Burgers vector for the 60° dislocation is $b_{60°} = 1/2[01\bar{1}]$ (Figure 6-21d, Figure 6-22a-c). The screw dislocation forms the spiral step pattern of a screw dislocation when viewed on a (11\bar{2}) plane with Burgers vector $b_s = 1/2[011]$ (Figure 6-21e, Figure 6-22d-f).
Figure 6-21 | Dislocation lines in the ossicle No. 52. a-c, 60° dislocation line (a) and screw dislocation line (b-c) in the ossicle No. 52. d,e, Magnified views of 60° (d) and screw (e) dislocations extracted from (a-c), where their locations are indicated in (a-c).
Figure 6-22 | Burgers vectors in the ossicle No.52. a,c, projection of thin slice in ossicle No. 52 along [112] direction with the 60° dislocation line and the screw dislocation line highlighted in red, respectively. b,e, Two layers of the skeleton with branches colored with the corresponding branch distance along [111] direction and [112] direction, respectively. c,f, zoom-in views in (b) and (e), the Burgers vector is indicated by green arrows.

In addition, similar to atomic diamond crystals, due to the presence of two inequivalent families of {111} planes, namely the glide set and shuffle set, two types of 60° dislocations are observed, i.e., G-type and S-type respectively, depending on where the inserted extra plane terminates (Figure 6-23 a,b)\textsuperscript{303,304}. When viewed in the $[\overline{1}0\overline{1}]$ direction, the G-type 60° dislocation is characterized with a 5-node ring and a 7-node ring (highlighted in green and cyan, respectively, Figure 6-23 a(1,2))\textsuperscript{307}. In contrast, the S-type 60° dislocation exhibits an 8-node ring (highlighted in cyan, Figure 6-23 b(1,2))\textsuperscript{304,307}. In addition, the two nodes beneath the inserted extra half-plane are always connected with a branch (dotted line, Figure 6-23a(1)), probably for mechanical
strengthening purposes. This is different from the core structure of the S-type 60° dislocation in atomic diamond crystals, where the connection depends on the bond length. The 3D configuration of the shuffle and glide dislocation is shown in Figure 6-24. The dislocation line for the local 60° dislocation is different (e.g., $\xi_{60°} = \overline{[101]}$), forms a 60° angle with the Burgers vector $b_{60°} = 1/2[01\overline{1}]$. Manual tracing of the core structure of a complete 60° dislocation within an ossicle (total dislocation length, ca. 1.1 mm) reveals that the ratio between the G- and S-type 60° dislocation is approximately 1:3 (Figure 6-25, Figure 6-26). Direct quantification of branch-level structural descriptors further reveals local distortions of the lattice surrounding the dislocation core. Similar to atomic crystals, the branches on the “compressive” and “tensile” sides of the dislocation core of 60° dislocations have reduced and increased lengths, respectively, whereas the branch thickness remains relatively uniform (Figure 6-23a(3,4),b(3,4))²⁹⁸.

**Figure 6-23 | 2D configurations of 60° glide (a) and shuffle (b) dislocations.** (a), (1), standard 2D models, (2), µ-CT reconstructions of the dislocation core structure in ossicles, and corresponding (3) branch length, l, and (4) thickness, t, maps. The 5-node ring in (a(2)) is shaded in green, while 7-node ring in (a(2)) and 8-node ring in (b(2)) are shaded in cyan.
Figure 6-24| 3D analysis of the core structures of 60° dislocations. a-f, 3D rendering (a,d), the supposition of the transparent 3D volume rendering and skeletonized network (b,e), and the corresponding theoretical core structures (c,f) of 60° glide dislocation (a-c) and 60° shuffle dislocation (d-f). The inserted plane in the dislocations are highlighted in red in (c,f). The Burgers vector ($b_{60°}$) and dislocation line ($\xi_{60°}$) are $1/2[01\overline{1}]$ (orange arrows) and $[\overline{1}0\overline{1}]$ (red dashed line), respectively.
Figure 6-25 | Analysis of the global core structures of 60° dislocations along the dislocation line in ossicle No. 52. **a**, Projection image of the entire ossicle along the [112] direction. The yellow line indicates the dislocation line of the 60° dislocation within the ossicle. The black lines indicate the locations of the analysis planes, i.e., (111), used in **(b)** and **(c)**. **b,c**, Overview (**b**) and zoomed-in (**c**) images of the dislocation core structures when viewed in the (111) plane. $\mathbf{b}_60^\circ = 1/2[01\overline{1}]$ is the Burgers vector. The corresponding dislocation type (shuffle and glide) are also indicated. The ⊥ symbols indicate the positions of dislocation cores. All branches in (**b-c**) are colored by their branch lengths.
Figure 6-26 | Analysis of the local core structures of $60^\circ$ dislocations along the dislocation line in ossicle No. 52. a, Projection image of the entire ossicle along the [11\(\bar{2}\)] direction. The yellow line indicates the dislocation line of the $60^\circ$ dislocation within the ossicle. The boxed region indicates the location of the consecutive dislocation core structures shown in (b) and the red arrow indicates the visualization direction [101]. b, Five consecutive 2D sections showing the core structures of the $60^\circ$ dislocation along the dislocation line. The $\perp$ symbols indicate the positions of dislocation cores. $b_{60^\circ} = 1/2[01\bar{1}]$ is the Burgers vector. The corresponding dislocation type (shuffle and glide) are also indicated. All branches in (b) are colored by their branch lengths.

While the core structure of screw dislocations exhibits multiple different configurations in atomic diamond crystals\(^{319}\), our analysis revealed that the dislocation core in the diamond lattice of ossicles is an undissociated screw dislocation in the shuffle set with kinks (Figure 6-27a, Figure 6-28)\(^{321,327}\). In addition, the N4 node in the standard kink structure is transformed into two N3 nodes to reduce the length of the stretched branch (Figure 6-27(1-3) and Figure 6-29). The dislocation line for the local screw dislocation is $\xi_s = [011]$, along with the Burgers vector $b_s = 1/2[011]$. The kink structure characterized by the N5 nodes in the dislocation core, similar to the
kink structure in the atomic diamond crystals\textsuperscript{327}. It helps deflect the overall dislocation orientation towards [112] direction, making the dislocation line a zig-zag pattern (Figure 6-27 (4) and Figure 6-29, Figure 6-30).

![Diagram showing 2D configurations of screw dislocations.](image)

**Figure 6-27| 2D configurations of the screw dislocations.** a, Mixed shuffle-glide screw dislocation: (1), standard 2D model, (2), \(\mu\)-CT reconstruction of the dislocation core structure in ossicles, (3), 3D schematic, and (4), corresponding 3D reconstruction. b, 3D core structure with the burger circuit highlighted in red.
Figure 6-28 | Structural reconstruction for the undissociated shuffle screw dislocation. a-f, 3D model (a,d), zoomed-in view (b,e), and corresponding 2D view in the (111) plane (c,f) of the original (a-c) and the reconstructed (d-f) screw dislocation. Here the Burgers vector ($\mathbf{b}_s$) and dislocation line ($\xi_s$) are $1/2[011]$ and [011], respectively. N3, N4, and N5 nodes are colored in green, red, and blue, respectively. Note that in the reconstructed configuration (d-f), the N4 nodes (highlighted in the red boxes in (b)) become two N3 nodes (highlighted in the red boxes in (e)). Also note that the kink structures, characterized by the presence of the N5 nodes, reorient the local dislocation line to the [101] direction, as shown in (c,f).
Figure 6-29 | Structural analysis of the screw dislocation in starfish ossicles. a-c, 3D rendering (a), superposition of transparent volumetric rendering and skeletonized network (b), and corresponding skeleton (e) of a representative screw dislocation extracted from an ossicle. d, Zoomed-in view of the 3D core structure, as extracted from the red box in (c). e, Visualization of the dislocation core structure in the (1̅1̅1) plane. Here the Burgers vector (\( \mathbf{b}_s \)) and dislocation line (\( \xi_s \)) are 1/2[011] and [011], respectively. N3, N4, and N5 nodes are colored in green, red, and blue, respectively. Note that the core structure of the screw dislocation in starfish ossicles resembles the undissociated shuffle screw dislocations with reconstructions (See Figure 6-28).
Figure 6-30 | **Long-range analysis of the screw dislocation in starfish ossicles.** a. Projection image of the entire ossicle along the [112] direction. The yellow line indicates the dislocation line of the screw dislocation within the ossicle. The boxed regions indicate the locations of the long-range dislocation core structures shown in (b). b, 2D view of the screw dislocation cores along the dislocation line. The branches are colored by their branch lengths (l).

On the ossicle level, the dislocation density is estimated in the range of 100-1200 cm⁻², corresponding to a normalized density of 0.001-0.011 (measurement based on eight ossicles randomly chosen from both aboral and oral sides).

**Table 6-5 and Figure 6-31, Methods.** This value is considerably higher than the typical dislocation density in the single atomic diamond crystals can be as high as 10⁻⁶ in the natural Type-IIa diamond³⁰¹ and can be as low as 10⁻¹² in the commercially available diamond³²⁹. The high dislocation density in the diamond lattice of *P. nodosus*’ ossicles is believed to result from its high structural tolerance surrounding the dislocation cores as described above.
Figure 6-31| Visualization of dislocations within individual ossicles. Eight ossicles are randomly selected and their positions in the *P. nodosus* skeleton can be found in Figure 6-3. The dislocations are colored according to the angle between their Burgers vector \(b\) and corresponding dislocation line \(\xi\).

Table 6-5| Dislocation density among different ossicles

<table>
<thead>
<tr>
<th>Ossicle</th>
<th>37</th>
<th>52</th>
<th>191</th>
<th>184</th>
<th>129</th>
<th>7</th>
<th>157</th>
<th>98</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>0.98</td>
<td>1.06</td>
<td>1.14</td>
<td>1.88</td>
<td>2.22</td>
<td>2.31</td>
<td>4.64</td>
<td>5.07</td>
</tr>
<tr>
<td>(\text{mm}^3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Line density</td>
<td>99.65</td>
<td>421.14</td>
<td>1215.61</td>
<td>683.51</td>
<td>877.49</td>
<td>237.85</td>
<td>523.64</td>
<td>202.54</td>
</tr>
<tr>
<td>(/cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normalized line density</td>
<td>0.0011</td>
<td>0.0049</td>
<td>0.011</td>
<td>0.0071</td>
<td>0.0095</td>
<td>0.0025</td>
<td>0.0045</td>
<td>0.0020</td>
</tr>
</tbody>
</table>

6.3.2 Co-alignment between calcite and diamond-TPMS lattice

Previous research indicates that individual calcitic skeletal element in echinoderms, such as sea urchins’ spines and brittle stars’ dorsal arm plates, typically diffracts as a single crystal. Here we further investigated the crystallographic characteristics of the underlying calcite in correlation
to the diamond-TPMS structure. First of all, ossicle-level crystallographic mapping based on electron backscatter diffraction (EBSD) confirms its single-crystalline nature at the atomic scale (Figure 6-32a-c). Moreover, the normal direction of the polished ossicle surface is estimated as the [2\(\bar{1}2\)] direction of the diamond-TPMS lattice, whereas the EBSD results show that at the atomic scale, the sample normal is tilted away from the c-axis of calcite by 56.31° (Figure 6-32b-d). As the angle between [2\(\bar{1}2\)] and [111] in a diamond lattice is 54.74°. This result suggests that the c-axis of calcite is aligned with the [111] direction of the diamond-TPMS lattice in ossicles.

![Figure 6-32](image)

**Figure 6-32** | Single-crystalline calcite in ossicles based on EBSD results. An SEM image (a), the corresponding orientation map (b,c) and \{0001\} pole figure (d) of the polished surface of the ossicle, indicating the single-crystal nature of the entire ossicle. (Figure credit: Emily M. Peterman)
To further verify this crystallographic co-alignment between calcite and diamond-TPMS lattice at the atomic and lattice scale, we utilized the epitaxial overgrowth strategy to induce the formation of synthetic calcite crystals on ossicle surfaces (Methods). As shown in Figure 6-33a-c, the overgrown calcite crystals (shaded in light blue) still retain the normal rhombohedral symmetry of calcite, while inheriting the crystallographic orientation of the underlying ossicles. Therefore, the crystallographic orientation of the ossicles can be determined by investigating the symmetry and orientation of the overgrown calcite crystals. When viewed along the [111] direction of the diamond-TPMS lattice, the overgrown calcite crystals exhibit a perfect three-fold symmetry, confirming that the $c$-axis of calcite is oriented along the [111] direction of the diamond-TPMS lattice (Figure 6-33c). Moreover, the edges formed by two adjacent $\{10\bar{1}4\}$ planes are aligned with the branches connected with the “higher nodes” in the $(11\bar{1})$ plane of the diamond-TPMS lattice (Figure 6-34a,b). This indicates that the in-plane three-fold symmetry of calcite (space group, $R\bar{3}c$) is aligned with that of the diamond lattice in the [111] direction.
Figure 6-33 | Crystallographic co-alignment at atomic and lattice scale. a,b, SEM image (a) and a zoom-in view (b) of the ossicle lattice with epitaxially overgrown calcite crystals (shaded in light blue) when viewed at the (111) plane of the diamond-TPMS lattice. The purple and cyan dots represent the lower and higher nodes on the (111) plane, characterized by their concave and convex surfaces, respectively. c, Co-alignment of a single overgrown calcite crystal and the branch orientations of the diamond lattice.

Figure 6-34 | 2D (a) and 3D (b) illustrations of the crystallographic co-alignment at atomic and lattice scale.

Lastly, the common edges formed by adjacent \{10\bar{1}4\} planes of calcite intersect with the c-axis by an angle of 116.6°, which is close to the measured inter-branch angle ossicles (α_m = 110.9° ± 10.2°) and the ideal tetrahedral angle in a diamond lattice (109.5°) (Figure 6-35a-c).
These results suggest that the formation of the diamond-TPMS lattice may be guided by the crystal symmetry and orientation of the underlying calcite at the atomic scale, similar to the crystallographically guided growth of three-branched sea urchin larval spicules and calcitic sponge spicules.\textsuperscript{337–339}

Figure 6-35| Measured and theoretical angles between crystallographic planes and c-axis. a, The distribution of measured angle $\alpha_m$ in ossicles. Eq. (5) in Methods indicates the conversion between $\alpha_m$ and distance $t$ of the c-axis as in (c). b,c, {1012}, {1014}, and {1018} planes in the hexagonal calcite crystal (b) and their angles with the c-axis of calcite (c) which coincides with the [111] orientation of the ossicle. The tetrahedral bond angle of 109.5° is for comparison.

6.3.3 Computational modeling

The theoretical modeling is conducted by Zian Jia, and the finite element analysis is performed by Hongshun Chen.
The diamond lattice exhibits long-range variations of structural parameters on the ossicle level, such as branch length and thickness (Figure 6-14, Figure 6-15, Figure 6-16, Figure 6-17 and Table 6-2). Figure 6-36a represents an extreme example where the branch thickness increases from 4.7 µm to 7.3 µm towards the ossicle surface within a distance of ~6 unit-cells, despite a relatively uniform branch length. Figure 6-36b shows the thickness and branch length variations across the entire ossicle.

These long-range variations on the ossicle result in the long-range variations on the mechanical properties, such as modulus and strength, of the local tetrahedron. The mechanical properties were quantified using the Timoshenko beam theory (Appendix B). Under the assumption of isotropic calcite as the constituent material, it’s concluded that the normalized modulus and strength along [111] direction of the tetrahedron unit are significantly affected by $\eta$ ($l_1/l_m$) and $\lambda$ ($t_1/t_m$). More specifically, as $\eta$ is higher and $\lambda$ is smaller, the inclined branches are shorter and thicker. Shorter and thicker inclined branches contribute to higher bending stiffness and hence higher stiffness of the lattice. At the individual ossicle level, *P. nodosus* adjusts local structural parameters to control the mechanical heterogeneity.
The mechanical anisotropy of solid calcite in the constituent materials and its co-alignment with the diamond lattice is further investigated through finite element modeling (Appendix C). The stiffness of the solid calcite along the c-axis is much smaller than the stiffness along the a-axes. However, the diamond-TPMS achieves the highest stiffness along [111] direction considering isotropic constituent materials. The co-alignment of [111] direction of the diamond lattice and the c-axis of the constituent calcite indicate the starfish exploits the stiff [111] direction of the diamond-TPMS lattice to mitigate the material-level compliance in the c-axis of calcite.

6.3.4 Quantitative mechanical properties

Due to the inherent brittleness of ceramics, synthetic ceramic foams as well as the recently-developed architected ceramic lattices, often suffer from catastrophic failure when the applied load exceeds a critical value. As calcite is well known as a brittle mineral, we further investigated the ossicle’s fracture behavior and energy dissipation mechanisms through a series of experimental approaches. Uniaxial compression tests on cube-shaped specimens cut from individual ossicles (edge length, ca. 2 mm) reveal the graceful failure behavior of ossicles as demonstrated by the large stress plateau after reaching the failure strength ($\sigma = 46.48 \pm 15.14$ MPa, the number of
tests: 14) (Figure 6-37a). This leads to energy absorption of $W_v = 14.25 \pm 2.50 \, MJ/m^3$ and hence a remarkable specific energy absorption ($W_v/\rho = 9.76 \pm 1.59 \, kJ/kg$), outperforming many synthetic ceramic or even metallic foams (Figure 6-37b, Figure 6-38).

Figure 6-37 | Energy absorption capability of starfish ossicles. a, Engineering stress-strain curves of ossicles obtained from ex-situ compression tests. One representative curve is highlighted in red. The behavior includes a linear elastic regime, a stress plateau regime, and a densification regime. The area under the curve is used to calculate the energy absorption ($W, MJ/m^3$). The strain level when densification is initiated is denoted as $\varepsilon_m$. b, Energy absorption capacity $W$ of the tested samples.
Figure 6.38 | Comparison of the energy absorption capability of starfish ossicles with foams reported in the literature, including AlSi_{10}Mg open-cell foams\textsuperscript{178,179}, titanium foams\textsuperscript{180,181}, sintered fiber stainless steel foams\textsuperscript{182}, carbon nanotube reinforced nanocomposites\textsuperscript{183}, stainless steel foam\textsuperscript{184,185}, carbon nanotube reinforced aluminum foams\textsuperscript{186}, nickel foams\textsuperscript{187}, Zn/Al/Cu alloy foam\textsuperscript{188} and epoxy foam\textsuperscript{189}.

6.3.5 Deformation mechanism

Intriguingly, the calcitic diamond-TPMS structure in ossicles exhibits a large stress plateau upon compression since this is typically observed in polymeric and metallic foams\textsuperscript{8}. We utilized synchrotron \textit{in-situ} mechanical characterization further to elucidate the underlying deformation mechanisms (Figure 6.39a-b). Multiple \(\mu\)-CT data was acquired during the compression test that allows us to track, visualize and analyze the deformation process in 3D. Surprisingly, the ossicle samples developed slip-like deformation bands upon initial yielding (yellow arrows, Figure 6.39c).
The sequential cross-sectional slices of the reconstructed data clearly demonstrate that the co-
parallel deformation bands undergo local densification and increase in band width (yellow arrows,
Figure 6-39d and Figure 6-40). In this representative data, the applied load is in the \([\bar{1}01]\) the
direction of the diamond lattice and the deformation bands are in the \((1\bar{1}1)\) plane (Figure 6-39d
and Figure 6-40). The preferred fracture in the \{111\} plane is expected because the spacing
between two neighboring \{111\} planes is largest, similar to the slip systems in a face-centered-
cubic (FCC) atomic crystal\(^{298}\). This is also one of the main reasons why synthetic ceramic lattices
often fracture in this slip-like form\(^{56}\).

![Figure 6-39](image)

**Figure 6-39l Synchrotron in-situ compression measurements.** a, Stress-strain curves on the *in-situ*
compression test. b, the 3D volume rendering of the specimen. c, A projection image of an ossicle sample
under *in-situ* compression with fracture bands indicated by yellow arrows \((\varepsilon = 0.11)\). d, Sequential cross-
sectional reconstruction slices showing the deformation and fracture evolution within the sample. The
yellow and red arrows indicate the slip-like fracture bands and the densified regions, respectively.
Figure 6-40 Structural visualization of the deformation bands formed in ossicles during an *in-situ* compression test. This is based on the same dataset shown in Figure 6-39. **a**, Vertical cross-sectional reconstruction slices of the compressed sample. The yellow boxes indicate the locations for **b-d**. The yellow arrows indicate the visualization directions. The red, green, and cyan lines in the yellow boxes indicate the locations of i-iii images in (b-d), respectively. **b-d**, Reconstruction slices across the damage bands along the [11\bar{1}] direction. Note this damage band is on the (11\bar{1}) plane. (Figure credit: Liuni Chen)

However, unlike the architected ceramic lattices in which the deformation bands fully propagate to the sample surface, the deformation bands in ossicles are often deviated and constrained within the specimen (Figure 6-39c,d). A correlative crystal lattice analysis of both undeformed and deformed volumes suggests that the deformation bands do not propagate to the regions with a high density of dislocations (Figure 6-41a). When encountering a lattice dislocation,
the \{111\} fracture plane has to “jump” to another \{111\} plane for further crack propagation (Figure 6-41b). This mechanism is similar to the “pinning” behavior of slips by the pre-existing dislocations in metals, which is responsible for their well-known strain hardening behavior\(^{298,340}\). It is remarkable to see that nature utilizes a similar concept to strengthen and toughen the ceramic lattice against catastrophic slip-like fracture at the \(\mu\)m scale.

![Figure 6-41](image)

**Figure 6-41 | Fracture deflection in the region with high dislocation density.** a. 3D rendering of the sample with the fracture bands colored in red and dislocations with Burgers vectors (yellow arrows). b, SEM image of a fracture surface deviated by a dislocation (indicated by the red arrow). The regions shaded in green and yellow represent two adjacent (111) fracture planes.

Finally, the underlying biogenic calcite in ossicles, despite its single-crystalline nature, does not undergo cleavage fracture along the \{10\14\} planes as in its geological counterpart\(^{336}\). Instead, it behaves as a glassy material with the non-cleavage “conchoidal” fracture (Figure 6-42a)\(^{203}\). This leads to continued fragmentation of the calcitic lattices into micro- and nanoscopic pieces during the densification process within the deformation bands (Figure 6-42b). Continuous loading leads to particle compaction, rotation, and friction, further contributing to its enhanced energy absorption capability\(^{270,271}\).
6.4 Discussion and Conclusions

In this chapter, ossicles with periodic open-cell foam structure in the starfish *P. nodusus* were investigated in terms of their structure, constituent materials, and mechanical performance. Combining the 3D FFT analysis on the μ-CT data and the surface curvature analysis, it can be confirmed that most of the ossicles in *P. nodusus* have the diamond-TPMS structure with a typical lattice constant of ca. 30 µm, making it the first known biomineralized diamond-TPMS in nature. In contrast, most natural single-crystalline structures are not mineralized and their lattice constants are in the sub-micron length range\textsuperscript{341}. For instance, the highly curved 3D periodic structures, cubic membranes (lattice constant: 100 nm\textsuperscript{342}), can be found in various lifeforms, including *Mitochondrion*\textsuperscript{343}, *Endoplasmic Reticula*\textsuperscript{344}, and *Chloroplast*\textsuperscript{345}. These cubic membranes are formed by folding up lipid bilayer under different conditions, such as cellular stress, viral infection, or disease\textsuperscript{346,347}. Other examples include the mesoscale biological photonic crystalline periodic structures (lattice constant:100-350 nm) found in the cuticular scales and setae of arthropods\textsuperscript{348--352}. They are composed of air, the cuticular protein, and polysaccharide chitin.
Moreover, the [111] direction of the diamond-TPMS lattice is further co-aligned with the \( c \)-axis of the underlying single-crystalline calcite at the atomic scale, exhibiting the dual-scale single-crystalline nature. This crystallographic co-alignment mitigates the compliance of calcite along the \( c \)-axis by utilizing the stiff [111] direction of the diamond-TPMS lattice. This is in contrast to the current synthetic architected cellular structures based exclusively on either polycrystalline or amorphous materials at the atomic scale\(^{46,275,276} \). These synthetic architected structures mainly achieve excellent mechanical properties through micro-architecture control.

3D *in-situ* mechanical characterizations further reveal that the presence of high density of crystal defects such as 60° and screw dislocations at the lattice level, which follow the dislocation types observed in atomic diamond crystals, suppresses slip-like fracture along the \{111\} planes of the diamond-TPMS lattice upon loading and hence enhances the energy absorption capability. This is similar to the strain hardening mechanisms used to strengthen metals, which results from the interactions of the strain fields of dislocations at the atomic scale\(^{338} \). By incorporating this mechanism into periodic metamaterials, it is possible to develop bioinspired mechanical metamaterials with enhanced damage tolerance.
Chapter 7 Summary and future directions

7.1 Summary

In this thesis, I studied three biomineralized cellular solid models with honeycomb-like, random open-cell foam, and periodic open-cell foam morphologies. The relationship between structural characteristics and their mechanical performance was elucidated through a combination of experimental and numerical approaches. The key findings of each model system are summarized here.

Chapter 3 investigates the internal biomineralized cuttlebone of *S. officinalis* (relative density: 7 vol%, constituent material: 90 wt% aragonite\textsuperscript{141}). The cuttlebone is a multichambered structure consisting of horizontal septa and thin vertical walls with corrugated cross-sectional profiles. Combining quantitative three dimensional (3D) structural characterization, four-dimensional (4D) mechanical analysis, digital image correlation, and parametric simulations, we reveal that the characteristic chambered “wall–septa” microstructure of cuttlebone, drastically distinct from other natural or engineering cellular solids, allows for simultaneous high specific stiffness (8.4 MN·m/kg) and energy absorption (4.4 kJ/kg) upon loading. We demonstrate that the vertical walls in the chambered cuttlebone microstructure have evolved an optimal waviness gradient, which leads to compression-dominant deformation and asymmetric wall fracture, accomplishing both high stiffness and high energy absorption. Moreover, the distribution of walls is found to reduce stress concentrations within the horizontal septa, facilitating a larger chamber crushing stress and a more significant densification.

Chapter 4 and Chapter 5 studies the stochastic open-cell foam-like structure, also known as stereom (relative density: 20-40 vol%, constituent material: 99 wt% biogenic calcite\textsuperscript{97–100}) in *H.*
In Chapter 4, we propose a cellular network analysis framework, which consists of five major steps: synchrotron-based tomography and hierarchical convolutional neural network-based reconstruction, machine learning-based segmentation, cellular network registration, feature extraction, and data representation and analysis. This framework enables the characterization of the porous stereom structures at the individual node and branch level (~10 μm), the local cellular level (~100 μm), and the global network level (~1 mm). We define and quantify multiple structural descriptors at each level, such as node connectivity, branch length and orientation, branch profile, ring structure, etc., which allows us to investigate the cellular network construction of *H. mammillatus* spines quantitatively. The methodology reported in chapter 4 could be tailored to analyze other natural or engineering open-cell porous materials for a comprehensive multiscale network representation and mechanical analysis.

In Chapter 5, I further show that the smooth, highly curved branch morphology with near-constant surface curvature in stereom results in low-stress concentration, which further leads to dispersed crack formation upon loading and promotes high strength (40.4 MPa). Furthermore, combined synchrotron *in-situ* analysis, electron microscopic analysis, and computational modeling further reveal that the fractured branches are efficiently jammed by the small throat openings within the cellular structure. This further leads to the formation of damage bands with densely packed fracture pieces. The continuous widening of the damage bands through progressive microfracture of branches at the boundaries contributes to the observed high plateau stress during compression, thereby contributing to its high energy absorption (17.7 kJ/kg).

Chapter 6 discovers a unique dual-scale single-crystalline porous lattice structure (relative density: 50 vol%, constituent material: 99 wt% biogenic calcite97–100) in the ossicles of *P. nodosus*. At the atomic scale, the ossicle is composed of single-crystal calcite. At the lattice scale, the lattice
is a diamond-triply periodic minimal surface (TPMS) structure. Moreover, the stiff [111] direction of the diamond-TPMS structure is co-aligned with the c-axis of the intrinsic single-crystalline calcite, compensating for the compliance of the c-axis at the atomic scale. Moreover, 3D in-situ mechanical characterizations reveal that the presence of crystal defects such as 60° and screw dislocations at the lattice level suppresses slip-like fracture along the {111} planes of the calcitic diamond-TPMS lattice upon loading, achieving an enhanced energy absorption capability. Even though the skeleton of the echinoderm is made up of single-crystal calcite, the structure fractures in a conchoidal manner rather than along the clipping plane. The continuous fracture of the fragments into small pieces enhance energy dissipation.

7.2 Future directions

Potential dual-scale single-crystalline lattice materials in ossicles from other starfish species

*Protoreaster nodosus* is probably not the only starfish species that have single-crystal structures at the lattice scale. Many species that belong to *Valvatida*, such as “*Goniaster” muelleri*, also potentially have fully periodic structures in their ossicles. Their structure periodicity at the lattice scale can be verified via 3D fast Fourier transform. Noting that the echinoderm stereom are composed of single-crystal calcite, more dual-scale single-crystalline lattice materials may be discovered.

Investigation of ossicles without fully periodic structures in starfish Protoreaster nodosus

Even though most ossicles from the oral side and aboral side are single-crystal diamond-TPMS structure at the lattice scale, some ossicles, including the ambulacral ossicles, pillar ossicles, madreporite, terminal plate, *etc.*, do not possess fully periodic structure. Among these ossicles,
ambulacral ossicles and pillar ossicles are located between the oral side and the aboral side, serving as structural supports. What are the features of these ossicles, and what are the corresponding specific functions? Their mysteries remain to be revealed. The mechanical design of these two types of ossicles can be further investigated. Moreover, despite the mm-sized ossicles, many secondary ossicles (with a much smaller size of ~0.1 mm) distributed on the entire endoskeleton are without fully periodic structures. The structure characteristics, as well as their functions, are also worth being studied.

(1) Gradient structural design in starfish ossicles

The ambulacral ossicles are composed of diamond lattice structures in the center and random open-cell structures with much higher relative density in the edge. How and why are these gradient structures formed? The mechanical properties of the ambulacral ossicles can be quantified through ex-situ mechanical tests. The correlation between the gradient structural design and its mechanical properties can be further investigated through the synchrotron-based in-situ mechanical tests.

(2) Natural polygrain-like lattice materials

As mentioned above, pillar ossicles are located between the oral side and the aboral side. Each pillar is made up of three ossicles, of which the top ossicle is directly inserted to the aboral sides, and the bottom ossicle sits on top of the oral side. Interestingly, the ossicles in pillars possess a polygrain-like structure at the lattice scale.

As is well known, for atomic polycrystals, changes in the orientation of the crystal lattice across the grain boundaries can prevent dislocations from moving from one grain to another\cite{354}, resulting in plastic deformation depending on the grain size\cite{355}. Inspired by mimicking the structures of atomic crystalline materials (e.g., grain boundaries, precipitates, and phases),
damage-tolerant architected materials printed with brittle polymers have been proposed recently\textsuperscript{56}. Pillar ossicles, as the natural polygrain-like lattice material, can potentially achieve high damage tolerance. Their mechanical design strategies could shed light on the development of bioinspired polygrain-like materials with high energy absorption.

Firstly, the mechanical properties, including the strength and the energy absorption, can be quantified for this polygrain-like structure to verify its potential high damage tolerance. Moreover, the fracture behavior of this polygrain-like structure upon loading can be visualized and quantified through the synchrotron-based \textit{in-situ} mechanical test. Secondly, to investigate the mechanism behind the unique mechanical properties, the orientation change from one grain to its neighboring grains, the crystal planes of two grains at the interface, the grain size, and the locations of different grains should be quantified in pillar ossicles. Especially, the interface structure that connects two grains should be extracted. The quantitative relationship between the polygrain-like structure and the deformation behavior can be further established.

\textit{Bioinspired mechanical metamaterials with dislocations}

In Chapter 6, the dislocation lines and the corresponding core structures for the diamond-TPMS structures have been studied. Through the synchrotron-based \textit{in-situ} mechanical test, we further observed that the deformation bands are deviated and constrained by the regions with a high density of dislocations when the ossicles are upon loading, which results in an enhanced energy absorption. By incorporating the dislocation structure in the diamond-TPMS structure, the bioinspired mechanical metamaterials with high energy absorption could potentially be developed.

Firstly, the model structure directly extracted from ossicles can be simulated. Based on the simulation results, the effect of dislocations on the stress distributions and the fracture behaviors
can be obtained and analyzed. Furthermore, by varying structure parameters such as dislocation line orientation and density, dislocation type, etc., the mechanical performances of the structures incorporating different dislocation features can be obtained. Finally, the bioinspired parametric model can be further developed and printed using a polymerization resin that is brittle after curing and heat treatment\textsuperscript{56}. The fracture behavior can be further validated through the \textit{ex-situ} tests of these models.

\textit{Quantification of the effect of the wall waviness and chamber pattern on the permeability and mechanical properties of cuttlebone structure}

Cuttlebone is a multifunctional chambered structure and serves as the structural supports and the buoyancy tanks for cuttlefish. The walls become wavier from the bottom to the top of the chamber. Compared with the bottom chamber, the top chamber has smaller spacings and could potentially exhibit better mechanical performance but reduced permeability. From the cuttlebone inlet to the inside, the patterns formed by vertical walls change from an aligned pattern to a labyrinthine pattern. Compared with the labyrinthine pattern at the inlet, the aligned pattern in the internal structure tends to have better permeability but degraded mechanical properties. The balance between permeability and mechanical properties of the cuttlebone structure is clever and interesting. By quantifying the permeability and mechanical property of cuttlebone at different regions, the multifunctional structural design principles of cuttlebone structure can be extracted.
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Appendix A. Additional supporting figures

Figure A-1| The distribution maps of wall spacings at the bottom (left) and the top (right) of a chamber.
Figure A-2 | μ-CT based analysis of the cell edge of the 3D printed ceramic foam using ceramic resin through Formlabs 2. a,b, Reconstructed volume of an unfired strut with the diameter of c.a. 2.8 mm (a) and the shrink sintered strut (b). c,d, Reconstruction slices of an unfired strut (c) and the shrink sintered strut (d). e, The pores in the sintered strut (b) with the largest pore colored in white and the rest pores colored in green. f, The size distribution of the pores shown in (e). \( d \) is the equivalent diameter of a sphere that has the same volume as the pore. The diameter of the sintered strut in (b) is 87% of the unfired strut in (a). The inner porosity of the sintered strut is 15.1%, which results from the sintered process. The deposited layer thickness is 100 \( \mu m \). The solid struts shrink more in the layer depositing direction, which leads to the formation of the largest pore (white pore in (e)). The density of the sintered beam is 1.9 \( g/cm^3 \).
Figure A-3| Quantitative mechanical characterization of the sections of *H. mammillatus* spine under compression. **a**, Stress-strain curves of samples isolated from *H. mammillatus* spine. **b**, Deformation stages of *H. mammillatus* spine sample under bulk compression, which is corresponding to the colored spots highlighted in (**a**). The damage bands and the spallation are indicated by yellow and white arrows, respectively. **c,d**, Measurements of compressive strength $\sigma_c$ (MPa) (**c**) and energy absorption capacity $W$ ($MJ/m^3$) (**d**) of all tested *H. mammillatus* spine samples.
Figure A-4 | Ashby plots displaying relative compressive strength $\sigma_c/\sigma_s$ of the specimens with growth ring as a function of relative density $\rho/\rho_s$ ($\sigma_s$: fracture strength of solid struts), in comparison to the brittle synthetic foams$^{64,253-257}$ and architected foams$^{46,59,258,259}$ (a) and The energy absorption capacity $W$ ($MJ/m^3$) as a function of density $\rho$ ($kg/m^3$), compared with many stainless-steel foams, CNT/Al foams, steel foams, etc.$^{178-184,186,187}$ (b). The dashed lines represent different $W/\rho$ values, with the shaded area highlights the standard deviation of $W/\rho$ for the stereom.
Appendix B. Theoretical micromechanical analysis of diamond lattice

The theoretical modeling is completed by Zian Jia.

Method: Analytical solutions of the effective modulus and strength of the diamond lattice structure along the [111] direction are obtained as follows. The diamond lattice is simplified as a beam network for theoretical modeling (Figure B-1a,b). The deformation of a unit cell loaded in the [111] direction is shown in Figure B-1c, where the displacement was calculated based on the deformation of the truss and the beam (Figure B-1d). As a general situation, the lattice branch along the [111] direction has a length $c_1$ and thickness $t_1$, while the other three inclined branches have the same length $c_2 = c_3 = c_4$ and thickness $t_2 = t_3 = t_4$ ($= t_m$). The deformation of branch 1 in the [111] direction under a compressive force of $3F$ is

$$\sigma_{x,eff} = -\frac{3Fl_1}{E_{iso}^{calcite}A_1}.$$  \hspace{1cm} Equation B-1

Here, $E_{iso}^{calcite}$ and $A_1$ denote the equivalent isotropic modulus of the solid calcite and the cross-sectional area of branch 1, respectively. The subscript “$x$” denotes the [111] direction. The corresponding deformation of branch 2 in the [111] direction can be evaluated by considering the deformation of an inclined branch with one end fixed while the other end is subjected to force $F$ and moment $M$. As shown in Figure B-2, the moment is related to force by equilibrium as,

$$M = (Fl_m cos \varphi)/2.$$ \hspace{1cm} Equation B-2
Note that $\varphi$ represents the angle between the branches 2, 3, or 4 with the horizontal direction. We assumed that the force $F$ is applied along the $x$-direction, which can be decomposed as the component along the beam, $F_1 = F \sin \varphi$, and the component perpendicular to the beam, $F_2 = F \cos \varphi$. The total displacement at the end is then obtained by adding the axial compression component $\delta_A$ contributed by $F_1$ and the bending component $\delta_B$ contributed by $F_2 F_1 F_1$. The axial displacement was calculated as

$$\delta_A = \frac{F l_m \sin \varphi}{E_{\text{calcite}} A}. \quad \text{Equation B-3}$$

$A (= A_2 = A_3 = A_4)$ is the cross-sectional area of the inclined branches. The bending effect is calculated by treating the branch as a Timoshenko beam, which is governed by the following differential equation\(^{47}\),

$$E_{\text{calcite}}^{\text{iso}} I \frac{d^4 w}{dx^4} = q(x) - \frac{E_{\text{calcite}}^{\text{iso}} I}{\kappa A G_{\text{calcite}}^{\text{iso}}} \frac{d^2 q}{dx^2}. \quad \text{Equation B-4}$$

Here, $I$ is the second moment of area of the cross-section, $G_{\text{calcite}}^{\text{iso}}$ is the shear modulus of the bulk calcite, $q(x)$ is the distributed load, $\kappa$ is the shear coefficient factor, and $w$ is the bending deflection of the branch. For circular cross-sections, $\kappa = 6(1 + \nu)/(7 + 6\nu)$, where $\nu$ is Poisson’s ratio.

Utilizing Equation B-5, we derived the bending displacement of branch 2 as,

$$\delta_B = -\frac{F l_m^3 \cos \varphi}{3E_{\text{calcite}}^{\text{iso}} I} - \frac{M l_m^2}{2E_{\text{calcite}}^{\text{iso}} I} - \frac{F l_m \cos \varphi}{\kappa A G_{\text{calcite}}^{\text{iso}}}. \quad \text{Equation B-5}$$

The total displacement of branch 2 is $\delta_2 = \delta_A + \delta_B$, whose component in the $x$-direction is,

$$\delta_{2,x} = \delta_A \sin \varphi + \delta_B \cos \varphi. \quad \text{Equation B-6}$$

Combining Equation B-6, we derived the total displacement of branch 2 along the $x$-direction,
\[
\delta_{2,x} = -\frac{Fl_3\cos^2\varphi}{12E_{iso}^c A} - \frac{Fl_m\sin^2\varphi}{E_{iso}^c A} - \frac{Fl_m\cos^2\varphi}{\kappa A G_{iso}^c A}.
\]

Equation B-7

Note that the geometric parameters satisfy the following relations,

\[
a = \sqrt{3}l_m \cos \varphi, \\
b = l_1 + l_m \sin \varphi, \\
\varphi = \alpha_m - 90^\circ, \\
\sin\left(\frac{\beta_m}{2}\right) = \frac{\sqrt{3}}{2} \sin \alpha_m.
\]

Equation B-8

\(a\) and \(b\) are the dimensions of the tetrahedron unit defined in Figure B-1b,c. Moreover, the effective strain and stress can be evaluated as,

\[
\varepsilon_{x,eff} = \frac{\delta_{1,x} + \delta_{2,x}}{b}. \\
\sigma_{x,eff} = -\frac{3F}{A_{eff}} = -\frac{2\sqrt{3}F}{a^2}.
\]

Equation B-9

Equation B-10

The displacement in the \(x\)-direction of branch 1, \(\delta_{1,x}\), is calculated as \(\delta_{1,x} = \frac{-3Fl_1}{E_{iso}^c A_{1}}\).

Combining the Equation B-7,9,10, the effective Young’s modulus (i.e., \(E_{Ossicle}^{11,iso} = \frac{\sigma_{x,eff}}{\varepsilon_{x,eff}}\)) was derived as,

\[
E_{Ossicle}^{11,iso} = \frac{24\sqrt{3}E_{iso}^c A_1 Ab}{a^2\left[A_1 A_1 l_3^3 \cos^2 \varphi + 12l_1 A_1 \left(\sin^2 \varphi + \cos^2 \varphi E_{iso}^c / \kappa G_{iso}^c A_{iso}^c \right) + 36l_1 A_1\right]}. \\
\]

Equation B-11

Substituting the geometric parameters in Eq. (9), we further derived the normalized effective stiffness,
When the diamond lattice is compressed in the [111] direction, the maximum tensile stress, which causes the failure of the lattice, results from the bending of branches 2, 3, and 4 (Figure B-1). The normalized tensile stress was derived to be the following equation,

\[
\sigma_{\text{Ossicle}}^{111,\text{iso}} = \frac{2 \sqrt{3} \pi t_m^3}{2 l_m^2 \cos^2 \phi \left[ 9 t_m^4 + 4 t_1^2 t_m \cos^2 \phi + 3 t_m^2 t_1^2 l_m \left( \sin^2 \phi + \cos^2 \phi E_{\text{Calcite}}^{111,\text{iso}} \right) \right]}
\]

Equation B-12

The notation “\(\hat{\cdot}\)” refers to the geometric parameters that are normalized by \(l_1\).

Based on the geometric dimensions measured from the experiment, the distribution of normalized stiffness and strength in the ossicle can be estimated theoretically according to Eq. (13) and (14). The evaluated distribution is summarized in Figure B-3. It is observed that the distribution of stiffness and strength is close to that of the ratio \(l_1/l_m\) (Figure B-4). Based on the developed theoretical model, the effect of four dimensionless parameters including \(l_1/l_m\), \(t_1/l_1\), \(t_m/l_1\), and \(\alpha_m\) on the normalized modulus are summarized in Figure B-5. The plots show that the effective stiffness is most sensitive to the ratio \(l_1/l_m\), and subsequently \(t_m/l_1\), \(\alpha_m\), and \(t_1/l_1\), suggesting that the bending effect dominates the stiffness of the diamond lattice.
Figure B-0-1 | Modeling of the diamond lattice loaded in the [111] direction. a, Diamond-TPMS structure with a load applied along the [111] direction. b, The simplified branch network of the diamond lattice. c, A tetrahedron unit with structural descriptors defined. Note that there are two types of branches, the branches parallel to the loading direction [111] (branch 1) and the branches inclined to the [111] loading direction with an interbranch angle $\alpha_m$ with branch 1 (branches 2, 3, and 4). d, Load and boundary conditions for the two categories of branches. (Figure credit: Zian Jia)

Figure B-0-2 | Deformation of the branch under applied force $F$ and moment $M$. The total displacement $\delta_{tot}$ is contributed by a bending deformation ($\delta_B$) and an axial compression deformation ($\delta_A$). (Figure credit: Zian Jia)
Table B-1 | Definitions and notations of the mechanical property parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{Calcite}^{Iso}$</td>
<td>Equivalent isotropic modulus of calcite</td>
<td>$E_{Calcite}^{Iso}$</td>
</tr>
<tr>
<td>$E_{Calcite}^{HKL,Ani}$</td>
<td>Modulus of calcite along a specific diamond lattice direction $[HKL]$</td>
<td>$E_{Calcite}^{Ani}$, $E_{Calcite}^{111,Ani}$, $E_{Calcite}^{130,Ani}$, $E_{Calcite}^{112,Ani}$</td>
</tr>
<tr>
<td>$E_{Ossicle}^{HKL,iso}$</td>
<td>Modulus of the ossicle along a specific diamond lattice direction $[HKL]$ calculated using $E_{Calcite}^{Iso}$</td>
<td>$E_{Ossicle}^{Iso}$, $E_{Ossicle}^{111,iso}$, $E_{Ossicle}^{110,iso}$, $E_{Ossicle}^{112,iso}$</td>
</tr>
<tr>
<td>$E_{Ossicle}^{HKL,Ani}$</td>
<td>Modulus of the ossicle along a specific diamond lattice direction $[HKL]$ calculated using $E_{Calcite}^{HKL,Ani}$</td>
<td>$E_{Ossicle}^{Ani}$, $E_{Ossicle}^{111,Ani}$, $E_{Ossicle}^{130,Ani}$, $E_{Ossicle}^{112,Ani}$</td>
</tr>
<tr>
<td>$E_{TPMS}^{HKL,iso}$</td>
<td>Modulus of the standard diamond-TPMS lattice along a specific diamond lattice direction $[HKL]$ calculated using $E_{Calcite}^{Iso}$</td>
<td>$E_{TPMS}^{Iso}$, $E_{TPMS}^{111,iso}$, $E_{TPMS}^{110,iso}$, $E_{TPMS}^{112,iso}$</td>
</tr>
<tr>
<td>$E_{TPMS}^{HKL,Ani}$</td>
<td>Modulus of the standard diamond-TPMS lattice along a specific diamond lattice direction $[HKL]$ calculated using $E_{Calcite}^{HKL,Ani}$</td>
<td>$E_{TPMS}^{Ani}$, $E_{TPMS}^{111,Ani}$, $E_{TPMS}^{130,Ani}$, $E_{TPMS}^{112,Ani}$</td>
</tr>
<tr>
<td>$\sigma_{Calcite}^{T,iso}$</td>
<td>Equivalent isotropic tensile strength of calcite</td>
<td>$\sigma_{Calcite}^{T}$</td>
</tr>
<tr>
<td>$\sigma_{Ossicle}^{111,iso}$</td>
<td>Strength of the ossicle along [111] direction of the diamond lattice direction calculated using $\sigma_{Calcite}^{T,iso}$</td>
<td>$\sigma_{Ossicle}^{111,iso}$</td>
</tr>
</tbody>
</table>
Figure B-0-3| Distribution of theoretically calculated mechanical properties of ossicle No. 7. 

(a, b) Distribution of locally normalized modulus (a) and normalized strength (b) on the cross-sections of ossicle along [111], [112], and [110] directions. The load is applied in the [111] direction. (Figure credit: Zian Jia)
Figure B-0-4| Distribution of the branch length ratio ($\eta$) and branch thickness ratio ($\lambda$) of ossicle No. 7. a,b. The distribution of length ratio $\eta = \ell_1/\ell_m$ (a) and thickness ratio $\lambda = t_1/t_m$ (b) on the cross-sections of ossicle along [111], [112], and [1\bar{1}0] directions.
Figure B-5: A parametric comparison of the sensitivity of normalized elastic modulus to different geometric parameters under loading in the [111] direction. The plots show the sensitivity of normalized modulus to $l_1/l_m$ and $\alpha_m$ (a), $\beta_m$ (b), $t_1/l_1$ (c), and $t_m/l_1$ (d). $t_m/l_1$ is inversely equivalent to $t_1/t_m$ with a constant $l_1$ and $t_1$. The branches 2, 3, and 4 are under bending while branch “1” or branch along [111] direction is in compression. (Figure credit: Zian Jia)
Appendix C. Finite element analysis of diamond-TPMS lattice in ossicles

The finite element analysis of diamond-TPMS lattice in ossicles is completed by Hongshun Chen.

Method: Static finite element simulations were carried out using Abaqus/Standard 2016 (Dassault Systems, Vélizy-Villacoublay, France) on representative ossicle volumes (ca. 5×5×5 unit cells) and standard diamond-TPMS lattice structures. For the generation of the standard diamond-TPMS structure, its iso-surface \( U \) is given by

\[
U = S_x S_y S_z + S_x C_y C_z + C_x S_y C_z + C_x C_y S_z - r,
\]

where

\[
S_i = \sin(k_i \cdot i), \quad C_i = \cos(k_i \cdot i), \quad \text{and} \quad i = x, y, \text{or} \ z \quad \text{representing three orthogonal directions of the Cartesian coordinates.}
\]

The periodicity \( k_i \) is defined as \( k_i = \frac{2\pi n_i}{L_i} \), where \( n_i \) and \( L_i \) here represent the number of cells and the size of the diamond-TPMS structure along the \( x \), \( y \) and \( z \) direction, \( r \) is a geometric parameter which controls the volume fraction of the generated diamond-TPMS structure\textsuperscript{356}. One can define either \( U > 0 \) or \( U < 0 \) as the primary or secondary solid region\textsuperscript{357,358}. We chose \( U < 0 \) and \( r = 0 \) to generate a diamond-TPMS lattice with a relative density of 50%.

Each simulation model typically consisted of ca. 4 million quadratic tetrahedral elements (C3D10). Compression simulations were conducted along the [111], [1\( \overline{1} \)0] and [11\( \overline{2} \)] direction of the diamond-TPMS lattice, respectively, with the maximum compression strain of 0.1%. To evaluate the effects of material anisotropy, we conducted comparisonal simulations by using both equivalent isotropic and anisotropic material properties of calcite (Table C-1). The full stiffness matrix for the single-crystalline calcite with the trigonal symmetry is given as,
\[
\begin{pmatrix}
c_{11} & c_{12} & c_{13} & c_{14} & 0 & 0 \\
c_{12} & c_{11} & c_{13} & -c_{14} & 0 & 0 \\
c_{13} & c_{13} & c_{33} & 0 & 0 & 0 \\
c_{14} & -c_{14} & 0 & c_{44} & c_{14} & 0 \\
0 & 0 & 0 & c_{44} & c_{14} & c_{66} \\
0 & 0 & 0 & 0 & 0 & 0
\end{pmatrix}.
\]

The values of the individual stiffness constants \(c_{MN} (M,N = 1 - 6 \text{ in Voigt notations})\) are given in Table C-1. Here the direction 1, 2, and 3 represents the [2\(\bar{1}\)0] (one of the a-axes of calcite), [01\(\bar{1}\)0], and [0001] (c-axis) directions of calcite, respectively (Figure C-1). By utilizing the crystallographic co-alignment between calcite and the diamond-TPMS lattice in ossicles, the direction 1, 2, and 3 coincide with the [1\(\bar{1}\)0], [11\(\bar{2}\)] and [111] directions of the diamond lattice, respectively. Therefore, the corresponding moduli of the solid calcite along the direction 1, 2, and 3 can be calculated and denoted as \(E_{\text{Calcite}}^{1\bar{1}0, \text{Ani}}\) (114.8 GPa), \(E_{\text{Calcite}}^{11\bar{2}, \text{Ani}}\) (119.1 GPa), and \(E_{\text{Calcite}}^{111, \text{Ani}}\) (63.5 GPa), respectively. Moreover, the equivalent isotropic modulus and Poisson’s ratio of the solid calcite has been obtained by using a mean-field homogenization method, which are denoted as \(E_{\text{Calcite}}^{\text{Iso}}\) (109 GPa) and \(\nu\) (0.29)\(^{249}\). We first conducted simulations on the diamond-TPMS lattice by using the equivalent isotropic properties of calcite, and the corresponding moduli along the [111], [1\(\bar{1}\)0] and [11\(\bar{2}\)] directions were denoted as \(E_{\text{Ossicle}}^{111, \text{Iso}}, E_{\text{Ossicle}}^{1\bar{1}0, \text{Iso}}, \) and \(E_{\text{Ossicle}}^{11\bar{2}, \text{Iso}}\), where the normalized moduli can be obtained by using \(E_{\text{Calcite}}^{\text{Iso}}\) as the reference (Table B-1). For the simulation of the diamond-TPMS lattice with anisotropic properties of calcite, the full stiffness matrix of calcite and the crystallographic co-alignment between calcite and the diamond-TPMS lattice were incorporated in the modeling. The corresponding moduli along the [111], [1\(\bar{1}\)0] and [11\(\bar{2}\)] directions of the “anisotropic” diamond-TPMS lattice were denoted as \(E_{\text{Ossicle}}^{111, \text{Ani}}, E_{\text{Ossicle}}^{1\bar{1}0, \text{Ani}}, \) and \(E_{\text{Ossicle}}^{11\bar{2}, \text{Ani}}\), respectively.
and $E^{111, Ani}_{Ossicle}$, respectively. The normalized moduli for the “anisotropic” diamond lattice are then calculated by normalizing with the moduli of the solid calcite in a specific direction. For example, for [111] direction, the normalized modulus is given by $E^{111, Ani}_{Ossicle} / E^{111, Ani}_{Calcite}$.

While the theoretical analysis above allows us to investigate the long-range mechanical heterogeneity within ossicles, it ignores the important effects of the mechanical anisotropy of calcite and the crystallographic co-alignment between the atomic and lattice scale. Calcite is a highly anisotropic material with the stiffness along the $c$-axis being smaller than that along the a-axes (Table C-1)249. We developed a finite element (FE) modeling approach that incorporates the crystallographic co-alignment deduced earlier and the full stiffness matrix of calcite (Methods). This allows us to estimate the effective Young’s moduli of the solid calcite along the lattice directions of [111], [110], and [112] (denoted as $E^{111, Ani}_{Calcite}$, $E^{110, Ani}_{Calcite}$, and $E^{112, Ani}_{Calcite}$, respectively) as $63.5, 114.8$, and $119.1$ GPa, respectively (Figure C-2, Table B-1, Table C-1). This again confirms the softness of calcite along the $c$-axis or the [111] lattice direction. However, the [111] direction is the stiffest for a diamond-TPMS lattice based on isotropic materials among other surveyed orientations, as evident from our modeling results and previous studies (Figure C-2)356. This indicates that the starfish exploits the stiff [111] direction of the diamond-TPMS lattice to compensate for the material-level compliance in the $c$-axis of calcite. Indeed, when the anisotropic properties of calcite are used in simulations, the increase in the normalized modulus of the diamond-TPMS structure is the highest along the [111] direction (Figure C-2). Furthermore, this dual-scale alignment generates a more uniform stress distribution compared to the case with isotropic material properties (Figure C-3).
Figure C-1| Crystallographic orientations in the atomic scale for simulation. a, The hexagonal calcite crystal. b, Crystallographic orientations of the (0001) plane of calcite crystal. (Figure credit: Hongshun Chen)
Table C-1 | Anisotropic and equivalent isotropic material properties of calcite used in finite element simulations (See Methods for additional details). (Table credit: Hongshun Chen)

<table>
<thead>
<tr>
<th>Anisotropic elastic constants (GPa)</th>
</tr>
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<tbody>
<tr>
<td>( c_{11} )</td>
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<table>
<thead>
<tr>
<th>Anisotropic elastic modulus along specific directions of the diamond lattice (GPa)</th>
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<tr>
<td>( E_{\text{Calcite}}^{[111, \text{Aniso}]} )</td>
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<td>63.5</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Equivalent isotropic material properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E_{\text{Calcite}}^{\text{Iso}} ) (GPa)</td>
</tr>
<tr>
<td>109</td>
</tr>
</tbody>
</table>
Figure C-2! Simulation results of elastic modulus of the anisotropic calcite and standard diamond-TPMS structure with isotropic and anisotropic calcite properties. 

**a**, Elastic modulus of the anisotropic calcite in [111] \(E^{111,\text{Ani}}_{\text{Calcite}}\), [110] \(E^{110,\text{Ani}}_{\text{Calcite}}\), and [112] \(E^{112,\text{Ani}}_{\text{Calcite}}\) directions, respectively. The dashed line indicates the equivalent isotropic modulus of calcite \(E^{\text{Iso}}_{\text{Calcite}}\).

**b**, Elastic modulus of the standard diamond-TPMS structure with isotropic and anisotropic calcite properties, respectively \(E^{HKL,\text{Iso}}_{\text{TPMS}}\) and \(E^{HKL,\text{Ani}}_{\text{TPMS}}\).

**c**, Relative modulus of the standard diamond-TPMS structure with isotropic or anisotropic calcite properties, respectively \(E^{HKL,\text{Iso}}_{\text{TPMS}}/E^{\text{Iso}}_{\text{Calcite}}\) and \(E^{HKL,\text{Ani}}_{\text{TPMS}}/E^{\text{Ani}}_{\text{Calcite}}\). The definitions and notations of the relevant parameters are also listed in Table B-1. (Figure credit: Hongshun Chen)
<table>
<thead>
<tr>
<th>Orientation</th>
<th>Model</th>
<th>Loading schematic</th>
<th>Diamond TPMS (Isotropic)</th>
<th>Diamond TPMS (Anisotropic)</th>
<th>Ossicle (Isotropic)</th>
<th>Ossicle (Anisotropic)</th>
</tr>
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<td>[112]</td>
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<td><img src="image17.png" alt="Diagram" /></td>
<td><img src="image18.png" alt="Diagram" /></td>
</tr>
</tbody>
</table>

**Figure C-3** Von Mises stress distribution of the standard diamond-TPMS and a representative volume in the ossicle with equivalent isotropic or anisotropic calcite properties compressed along the [111], [110], and [112] direction, respectively. (Figure credit: Hongshun Chen)