

Anisotropic design of a multilayered biological exoskeleton

Lifeng Wang

Department of Mechanical Engineering, Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Juha Song and Christine Ortiz^{a)}

Department of Materials Science and Engineering, Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Mary C. Boyce^{b)}

Department of Mechanical Engineering, Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

(Received 2 May 2009; accepted 29 September 2009)

Biological materials have developed hierarchical and heterogeneous material microstructures and nanostructures to provide protection against environmental threats that, in turn, provide bioinspired clues to improve human body armor. In this study, we present a multiscale experimental and computational approach to investigate the anisotropic design principles of a ganoid scale of an ancient fish, *Polypterus senegalus*, which possesses a unique quad-layered structure at the micrometer scale with nanostructured material constituting each layer. The anisotropy of the outermost prismatic ganoin layer was investigated using instrumented nanoindentations and finite element analysis (FEA) simulations. Nanomechanical modeling was carried out to reveal the elastic-plastic mechanical anisotropy of the ganoin composite due to its unique nanostructure. Simulation results for nanoindentation representing ganoin alternatively with isotropic, anisotropic, and discrete material properties are compared to understand the apparent direction-independence of the anisotropic ganoin during indentation. By incorporating the estimated anisotropic mechanical properties of ganoin, microindentation on a quad-layered FEA model that is analogous to penetration biting events (potential threat) was performed and compared with the quad-layered FEA model with isotropic ganoin. The elastic-plastic anisotropy of the outmost ganoin layer enhances the load-dependent penetration resistance of the multilayered armor compared with the isotropic ganoin layer by (i) retaining the effective indentation modulus and hardness properties, (ii) enhancing the transmission of stress and dissipation to the underlying dentin layer, (iii) lowering the ganoin/dentin interfacial stresses and hence reducing any propensity toward delamination, (iv) retaining the suppression of catastrophic radial surface cracking, and favoring localized circumferential cracking, and (v) providing discrete structural pathways (interprism) for circumferential cracks to propagate normal to the surface for easy arrest by the underlying dentin layer and hence containing damage locally. These results indicate the potential to use anisotropy of the individual layers as a means for design optimization of hierarchically structured material systems for dissipative armor.

I. INTRODUCTION

Hierarchical structural anisotropy is a salient feature of many biological materials, for example, bone,¹ nacre,² wood,³ tendon,⁴ skin,⁵ cartilage,⁶ tooth,⁷ etc. In such materials, two types of anisotropy may exist. Firstly, at the smallest length scale, there may be “inherent” anisotropy

of the fundamental building blocks, e.g., mineral crystallinity⁸ and for organic constituents, anisotropic molecular structures.^{9–11} Secondly, at a larger length scale, the controlled shape, orientation, and spatial placement of various structural elements in a composite or hybrid material,¹² e.g., as dictated by biomineralization processes,¹³ may also induce a “geometric” anisotropy. Specifics of these combined anisotropic contributions can result in macroscopic direction-dependent elastic and plastic mechanical properties (e.g., nacreous mollusk shell layers, mineralized tendon, etc.), while other biological materials are known to possess direction-independent mechanical

Address all correspondence to these authors.

^{a)}e-mail: cortiz@mit.edu

^{b)}e-mail: mcboyce@mit.edu

DOI: 10.1557/JMR.2009.0443

properties (e.g., echinoderm calcite, crossed-lamellar mollusk shell layers, lamellar bone, etc.) as needed for their particular biomechanical function.^{14,15} Even in the systems that exhibit direction-independent material properties, underlying inherent/geometric anisotropies exist that affect local stress and strain distributions, the corresponding energy dissipation contributions, and the ultimate fracture mechanisms. Lastly, many physiological loading cases are multi-axial and may produce properties that appear to be direction-independent, but are in fact anisotropic in uniaxial situations.

In most biological exoskeletons, or “natural armor,” a multilayered structure exists where each layer is composed of a different nanocomposite material with varying types and degrees of structural and mechanical anisotropy. Among juxtaposed layers of many exoskeletal structures, the outermost layers are frequently known to exhibit controlled orientation of anisotropically shaped mineral crystals of specific crystallographic orientations. For example, in the outer layers/shells of American lobster,¹⁶ mollusk shells,¹⁷ chicken eggshell,¹⁸ and giant barnacles,¹⁹ the crystallographic *c*-axis is found to be oriented approximately perpendicular to the surface and parallel to the long axis of the rodlike crystals. Similarly, in the outer enamel layers of teeth, the crystallographic *c*-axis of hydroxyapatite (HAP) is also parallel to the long axis of the crystals and approximately perpendicular to the surface.²⁰ Here, we examine the effect of this anisotropy

by focusing on ganoine, the outermost layer of the mineralized scales of the ancient fish *Polypterus senegalus* (Fig. 1). The scales of *P. senegalus* possess four distinct inorganic–organic nanocomposite layers as follows (from outer to inner surface): ganoine (~10 μm in thickness), dentin (~50 μm in thickness), isopedine (~40 μm in thickness), and bone (~300 μm in thickness).²¹ Ganoine is known to be enamel-like²² and possesses an anisotropic nanostructure composed of rodlike HAP nanocrystals²³ with their long axis aligned approximately perpendicular to the scale surface (Fig. 1) along with ~5% organic matrix.²⁴ As with the other natural armor systems described previously, this anisotropic ganoine layer serves as one of the first lines of defense against penetrating attacks. In this study, we investigate the mechanics of the ganoine anisotropy through experiments and modeling, in particular the role of structural anisotropy of the ganoine layer in the penetration/indentation resistance of the entire multilayered scale. First, the mechanical anisotropy of ganoine was experimentally assessed via instrumented nanoindentation in the surface and cross-sectional orientations (i.e., indentation modulus, hardness). An anisotropic elastic-plastic nanomechanical model, capturing a representative volume element (RVE) that idealizes the random heterogeneous structure as a periodic arrangement of hexagonally packed HAP rods surrounded by an organic matrix, was constructed through finite element analysis (FEA) and subjected to multiple loading conditions; these

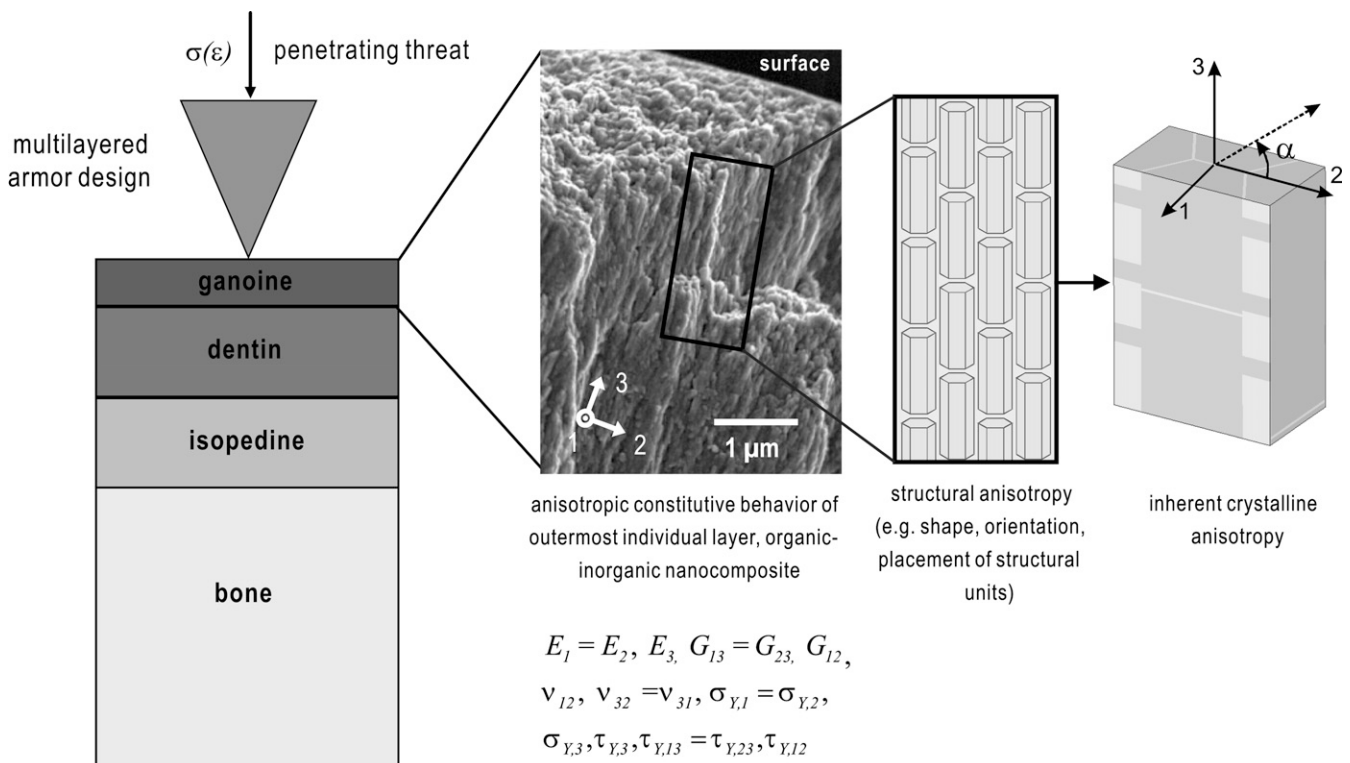


FIG. 1. The structure of the quad-layered armored scales of *P. senegalus* is one example of a protective mineralized biological exoskeleton where the anisotropy of the outermost individual layer (ganoine) is illustrated and studied.

nanomechanical models were then used to obtain the anisotropic elasticity and plasticity of the ganoine layer and also to reveal the contribution of the crystalline anisotropy of the HAP crystal (as opposed to geometric anisotropy) to the overall anisotropy of the ganoine. The anisotropic nanomechanical modeling results were used in simulation of the nanoindentation of ganoine in orthogonal directions and then compared with experimental nanoindentation data. Furthermore, the anisotropic elastic-plastic behavior was captured in a continuum-level constitutive model to reveal the role of ganoine anisotropy in the entire multilayered ganoine–dentin–isopedine–bone multilayered exoskeleton structure, especially in terms of the penetration resistance and deformation of the scale.

II. EXPERIMENTAL STUDY

A. Methods

1. Scale removal

A living *Polypterus senegalus* (length ~20 cm) was anesthetized, and a row of four scales surgically dissected off from the 49th row on the left flank (posterior region). Tricaine methanesulfonate (MS-222, Sigma-Aldrich, St. Louis, MO) was used for general anesthesia, prepared at the concentration of 1.6 g/500 mL H₂O with 3 pellets of KOH for neutralization. The fish was subsequently removed and immersed in a mixture of 50/50 MS-222 and water from the tank to maintain anesthetization. Afterward, the fish was treated with tetracycline (250 mg; Thomas Laboratories, Tolleson, AZ).

2. Sample preparation

Individual scales were initially cut in half with a razor blade. One half of each scale was designated for testing parallel to the surface normal (surface orientation), and the other half of the scale was designated for testing perpendicular to the surface normal (cross-sectional orientation), as shown in Fig. 2. Samples were sectioned using a diamond-impregnated annular wafering saw (Isomet 5000, Buehler, Lake Bluff, IL) at 800 to 900 rpm and then polished stepwise with 6 and 1 μ m silica nanoparticles on a soft pad (Buehler), followed by 500 nm silica nanoparticles on microcloth (Buehler). The root mean square (RMS) surface roughness of all polished samples was measured using tapping mode atomic force microscopy (TM-AFM; Digital Instruments Multimode SPM IV, Veeco, Santa Barbara, CA) and found to be less than ~2 nm. After polishing, it was determined by scanning electron microscopy (SEM) of the cross sections that ~5 μ m of material was removed, leaving an ~10 μ m thick ganoine layer.

3. Scanning electron microscopy

Fractured scales were etched briefly either with 5.25% NaOCl (Sigma-Aldrich), pH balanced to 7 with HCl

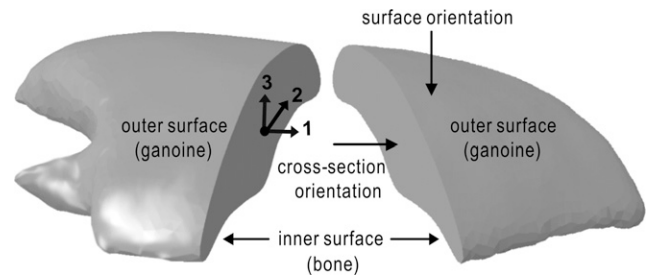


FIG. 2. Individual *P. senegalus* scale cut in half and denoting two orthogonal orientation directions of nanoindentation experiments. “Surface orientation” represents loading parallel to the outer surface normal, denoted as the 3-axis, and “cross-section orientation” represents the loading perpendicular to the outer surface normal in the direction represented by the 1 in-plane axis.

(slight deorganization), with ethylenediaminetetraacetic acid (EDTA, 0.5 M, Sigma-Aldrich) for 2 min (slight demineralization), or with phosphoric acid for 20 s (slight demineralization) to more clearly reveal their microstructure and nanostructure. Samples were fixed on a steel support using conductive tape and then sputter coated with 5 nm of gold in a Denton Vacuum Desk II (Moorestown, NJ). SEM samples were imaged in a JEOL JSM 6060 (Peabody, MA) scanning electron microscope at 10 kV acceleration voltage. Backscattered electron microscope (BSEM) images were taken with the JEOL JSM-6700F.

4. Nanoindentation

Samples were attached to a steel puck using a cyanoacrylate glue (Loctite 495, Henkel KGaA, Düsseldorf, Germany) and then tested in both the surface (load parallel to surface normal) and cross-sectional (load perpendicular to surface normal) directions. Both sets of nanomechanical experiments were carried out at a depth (parallel to the surface normal) of 5 μ m, which is prior to the depth where a decreasing gradient in mechanical properties is known to begin.²¹ Nanoindentation experiments were performed on loading and unloading in ambient conditions using a Triboindenter (Hysitron, Inc., Minneapolis, MN) with a Berkovich diamond probe tip (end radius ~300 nm). Detailed protocols and calibrations are provided in our previous work.²¹ Nanoindentation experiments using various loading rates from 50 to 200 μ N/s showed that the indentation results were similar over this range of loading rates. A constant loading/unloading rate of 100 μ N/s, a maximum load of 500 μ N, and a lateral interindent spacing of 3 μ m were used. Approximately 100 indents per sample were performed in each of the two orthogonal directions. The Oliver–Pharr analysis²⁵ was used to reduce effective material properties from the nanoindentation force–depth data into indentation stiffness and hardness.

B. Results

1. Ultrastructure of ganoine

As we have described previously,²¹ upon dehydration of the scales of *P. senegalus* microcracks are observed to occur perpendicular to the scale surface within the ganoine layer and are arrested by the underlying dentin, suggesting that the structural anisotropy of the ganoine plays a role in the mechanical functionality of the scale

[Fig. 3(a)]. Figures 3(b) to 3(e) elucidate the fine nanostructural anisotropy of the ganoine layer at higher resolutions.²¹ Elongated rodlike and well-packed HAP crystals are clearly visualized with an average diameter of ~ 50 nm and an average length of a few hundred nm, which exhibit a discontinuous and tortuous crack propagation path through the thickness of the ganoine layer [black arrows in Figs. 3(c) and 3(e)]. The long axis of the crystals is aligned approximately perpendicular to the scale surface.

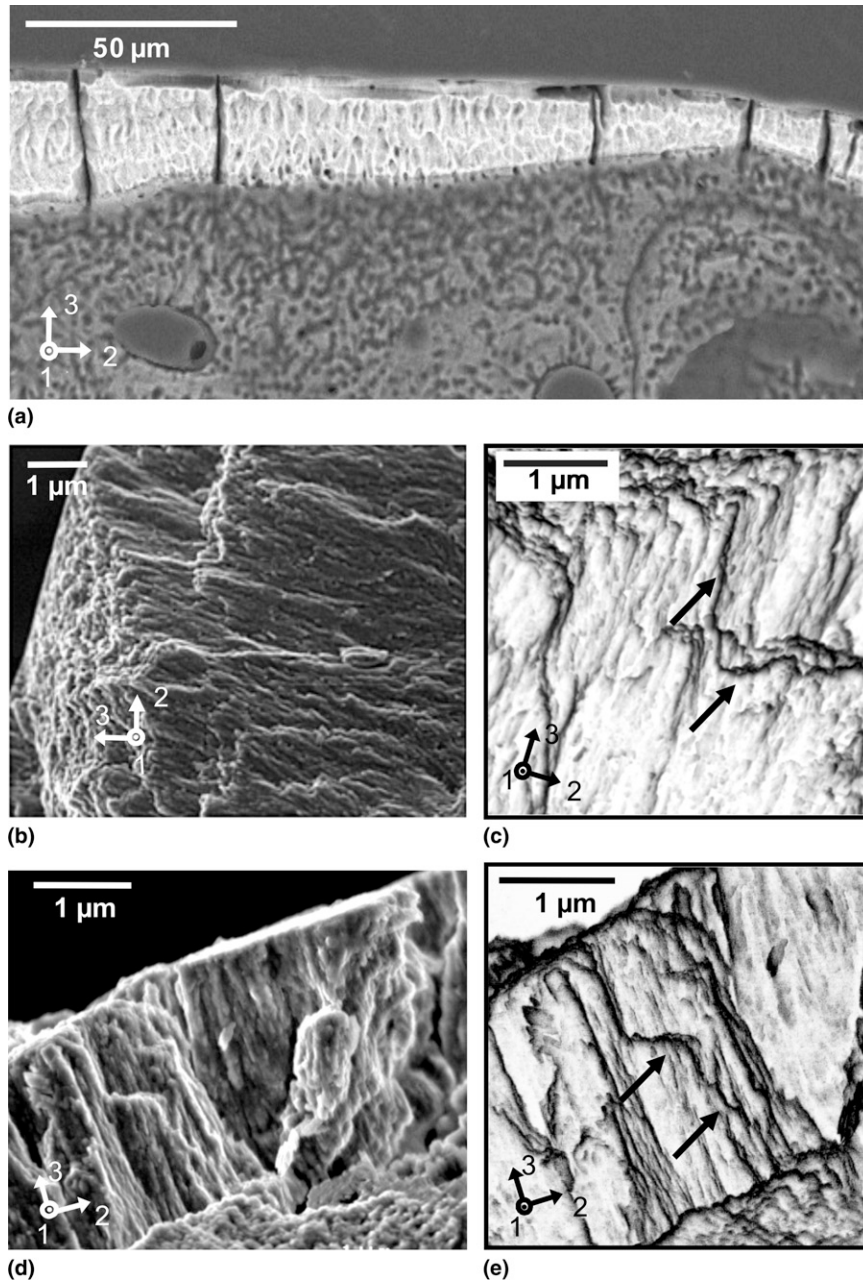


FIG. 3. (a) Backscattered electron microscopy images of the cross section of ganoine and dentin layers of the polished *P. senegalus* scale (embedded in the epoxy resin) after etching with phosphoric acid for 20 s, (b)–(d) SEM images of ganoine exposing the thin elongated hydroxyapatite nanocrystals after the scale sample was fractured and etched with (b) NaOCl for 5 min or (d) 0.5 M EDTA for 2 min. (c, e) Images modified by means of reversing contrast of (a) and (b), respectively, which show crack paths more clearly. (b)–(e) were not embedded in epoxy or polished. Arrows indicate the tortuous crack paths crossing the neighboring rods.

The ganoine nanostructure does exhibit some heterogeneity in size distribution, as well as axial orientation angle, and the surfaces of the nanocrystals do not appear smooth but corrugated.

2. Nanoindentation

The averaged nanoindentation data (force versus depth) for the surface and cross-sectional directions (Fig. 4) display an indistinguishable mechanical response. The average indentation stiffness was determined by means of the Oliver–Pharr (O–P) method²⁵ via the experimentally measured slope of the unloading force–depth curve, S , and the maximum projected contact area, $A(h_c)$, where h_c is the contact depth. S was evaluated at 95%–20% of the maximum load, $P_{\max}$. $A(h_c)$ was estimated from a sixth-order polynomial fit accounting for nonideal tip geometry on a standard fused quartz sample. Hardness values were calculated as $P_{\max}/A(h_c)$. Using this method, indentation moduli and hardness values were calculated to be 74.8 ± 5.0 GPa, 3.98 ± 0.27 GPa (surface orientation) and 75.1 ± 5.0 GPa, 3.92 ± 0.27 GPa (cross-section orientation), respectively, consistent with our previous study.²¹ Note that the O–P method assumes isotropy and is herein used only as a means for reducing effective indentation modulus and hardness for comparison purposes. Unpaired t tests confirmed that both the average indentation stiffness and hardness were not statistically different for the two orientations tested ($P > 0.05$).

III. MODELING STUDY

The mechanical behavior of ganoine was further studied using three levels of modeling. First, at the smallest length scale nanomechanical modeling of an RVE of the ganoine organic–inorganic composite nanostructure was

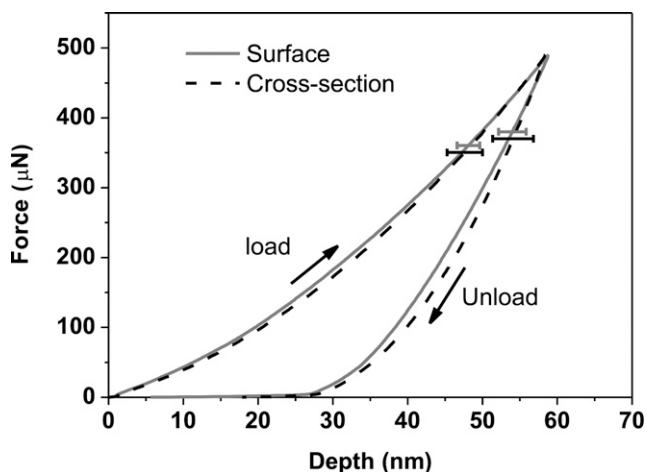


FIG. 4. Averaged experimental curves on loading and unloading (number of experiments, $n = 75$) for both surface cross-sectional directions. The horizontal bars indicate the maximum standard deviation of the curves for that particular dataset.

carried out to compute the anisotropic elastic-plastic constitutive behavior and mechanical properties. Second, these latter data were used to simulate the surface and cross-section nanoindentations of ganoine for comparison with experiments and to provide insight into the deformation and dissipation mechanisms beneath the indenter. Thirdly, larger-length scale simulations of microindentation of the ganoine/dentin/isopedine/bone multilayered structure were conducted to explore the role of ganoine anisotropy in the penetration resistance of the scales.

A. Methods

1. Nanomechanical modeling

Nanomechanical modeling was used to determine the overall anisotropic mechanical behavior of the biological composite material (ganoine) based on the constituent material mechanical properties (hydroxyapatite and organic) in conjunction with an RVE of the nanostructure. The nanomechanical RVE model construction consists of: (i) a description of the geometry of the nanostructure, (ii) a description of the mechanical behavior of the constituent materials, and (iii) a description of the periodic boundary conditions, the loading conditions, and the determination of the composite response (here, the stress–strain response) of the RVE during the different loading scenarios.

a. RVE geometry description

Based on the SEM images (Fig. 3), the ganoine nanostructure can be described as composed of well-packed HAP crystals discontinuous through the ganoine layer, interacting through their rough surfaces, and separated by thin adhesive layers of organic. This structure is here idealized as a composite of a staggered and hexagonally packed distribution of HAP crystals (cross-sectional length of ~ 40 nm and axial length of ~ 220 nm) in an organic matrix (thickness assumed to be 2 nm, based on known mineral volume fraction of $\sim 95\%$ ²⁴), as shown in Fig. 5. The periodically repeating nature of the RVE is captured by general periodic boundary conditions on the displacements.^{26,27} In the real structure, the distributed and irregular alignment of the crystallites together with the prism-to-prism interlocking surface roughness [Figs. 3(b)–3(e)] interrupts the continuity of the organic matrix and, hence, is expected to provide additional resistance to shear deformation between neighboring crystallites. To capture this effect in our idealized RVE in a simplified manner, transverse mineral elements were introduced to emulate the mechanical effect of the alignment and prism-to-prism interlocking surface roughness [Fig. 5(b)]. Simulations were conducted with and without transverse mineral elements to understand its role on effective anisotropic properties.

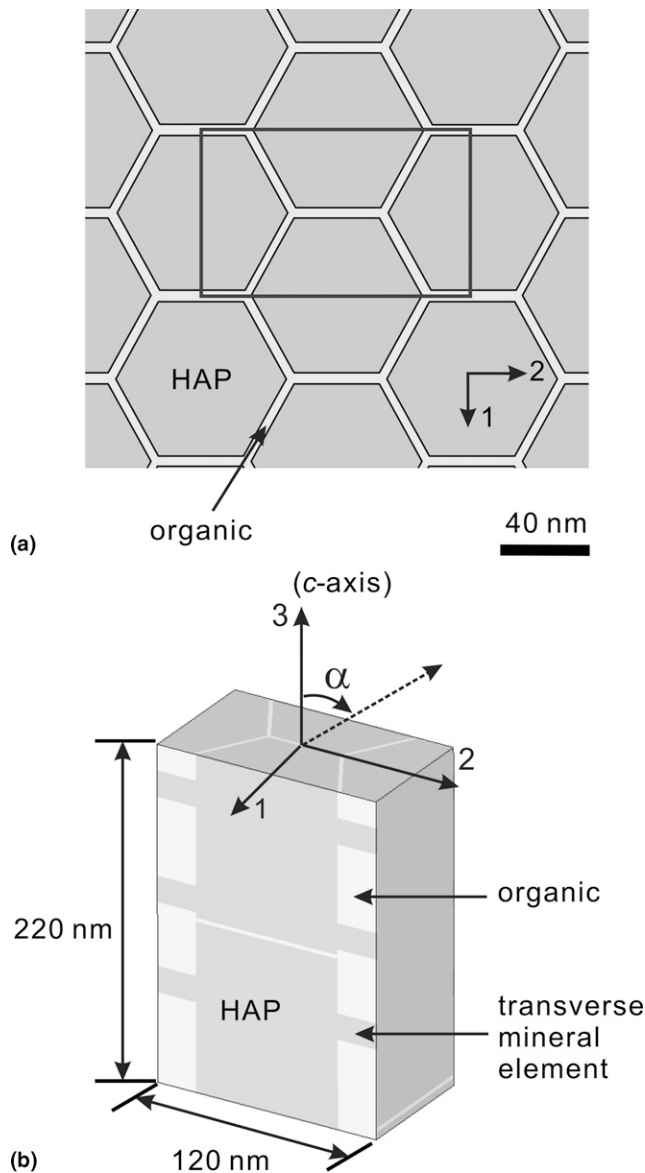


FIG. 5. RVE of ganoine nanostructure including HAP mineral crystals (dark gray) and organic matrix (light gray). (a) View of hexagonal arrangement of HAP crystals looking toward the top surface (down the 3-axis). (b) 3D view of the RVE [square region in the center of (a)] consisting of transverse mineral elements. The 3-axis (normal to the surface direction) of the scale is also coincident with the crystallographic c -axis of the HAP crystals.

b. Description of the mechanical behavior of the constituent materials

The mechanical behavior of the constituent HAP crystals and the organic matrix must be specified in the model. The anisotropic elasticity of HAP single crystals (which possess a transversely isotropic hexagonal lattice) has been obtained by using empirical potentials.²⁸ For biological composites, it is expected that the modulus values do not reach these ideal values as a result of differences in density, purity (including intercalated or-

ganic), chemical composition, and porosity of the HAP phase.^{20,29–32} Hence, we take the anisotropic base properties of the pure crystal²⁸ and scale these down, keeping the same anisotropy ratios to account for this biological reality (Table I). The anisotropic yield behavior of single crystals of HAP has been explored in hardness tests³³ that find hardness values parallel and transverse to the c -axis to be 9.7 and 8.8 GPa, respectively. Hence, the HAP elastic properties were downscaled by a factor of 1.36 and the yield stress downscaled by a factor of 1.18 (considering a hardness/yield stress ratio of 3), keeping the correct anisotropy ratios (Table I). These scaling factors used to obtain the anisotropic moduli and yield stress values were determined by iteratively fitting to experimental nanoindentation data (Fig. 4). With this said, other sources of increased compliance, such as a more compliant or thicker organic matrix, reduced degree of crystal alignment, or reduced density of transverse mineral elements, cannot be conclusively excluded. The details of such microstructural features were incorporated to the best of our knowledge into the simulations based on current experimental data. For comparison, simulations were also carried out assuming the HAP to be isotropic where best fits to nanoindentation data yielded elastic properties of $E = 80$ GPa, $\nu = 0.3$, and an isotropic yield stress of 2.6 GPa for the HAP (Table I). The organic matrix has mechanical properties much lower in magnitude than the mineral HAP crystals; a value of 4.3 GPa for the elastic modulus and a yield stress of 400 MPa of the organic were assumed, consistent with values used in other studies.^{34,35}

c. Description of the periodic boundary conditions and calculation of the effective behavior of the RVE

Periodic boundary conditions were applied to ensure the compatibility of neighboring RVEs during deformation. The RVE was subjected to various deformation histories in the FEA-based simulations (ABAQUS/standard) as described below. The averaged anisotropic elastic-plastic mechanical response of the RVE for each case was calculated through virtual work considerations using the method detailed in Refs. 26 and 27.

The highly packed arrangement of HAP in the ganoine [Fig. 5(a)] together with the symmetry of the HAP crystal structure suggests transversely isotropic elastic properties for the ganoine with the isotropy plane being orthogonal to the 3-axis, as shown in Fig. 5(b). The crystallographic c -axis was taken to be coincident with the long axis of the HAP rods, similar to that known for tooth enamel.²⁰ There are five independent properties characterizing the elastic response of a transversely isotropic material ($E_1 = E_2$, E_3 ; $G_{13} = G_{23}$, ν_{12} , ν_{13}). Two uniaxial stress loadings and two shear loadings in different directions were applied to the RVE to determine the

TABLE I. Mechanical properties of HAP crystals assumed in the nanomechanical model. The 3-axis was taken to be coincident with the crystallographic *c*-axis.

	$E_1 = E_2$ (GPa)	E_3 (GPa)	G_{13} (GPa)	ν_{12}	ν_{13}	$\sigma_{Y,1} = \sigma_{Y,2}$ (GPa)	$\sigma_{Y,3}$ (GPa)	$\tau_{Y,13} = \tau_{Y,23}$ (GPa)	$\tau_{Y,12}$ (GPa)
Isotropic	80	80	30.8	0.30	0.30	2.60	2.60	1.50	1.50
Anisotropic	86.4	73.6	24.5	0.23	0.29	2.47	2.73	1.50	1.43

effective composite anisotropic elastic constants and yield stresses. To investigate the elastic-plastic response of the structure in different orientations, the RVE was also subjected to off-axis loadings. As shown in Fig. 5(b), uniaxial compression was applied to the RVE in directions $\alpha = 0^\circ, 15^\circ, 30^\circ, 45^\circ, 60^\circ, 75^\circ$, and 90° away from the rod axis.

2. Nanoindentation model

Nanoindentations into the surface and cross section of ganoine were simulated using three-dimensional nonlinear finite element analysis. The full nanoindentation process was modeled using one-quarter of the geometry of the system together with appropriate symmetry boundary conditions. The ganoine was discretized with eight-node linear hybrid brick elements (C3D8H in ABAQUS/standard element library, Providence, RI). Mesh convergence was assessed, and a mesh of 16,704 elements was found to provide an accurate solution [Fig. 6(a)]. The Berkovich indenter was modeled as a rigid conical-like indenter; the tip cross-sectional area was the experimentally measured Berkovich tip area function (TAF). The surface-to-surface contact between the indenter surface and the ganoine surface was specified to be frictionless with no overclosure.

The constitutive behavior of the ganoine was modeled in three ways: (i) isotropic elastic-perfectly plastic with Young's modulus $E = 65$ GPa, Poisson's ratio $\nu = 0.3$, yield stress $\sigma_Y = 1.53$ GPa (data obtained from fitting results to averaged nanoindentation data Fig. 4)²¹; (ii) anisotropic-elastic/anisotropic-plastic with the properties obtained from the nanomechanical modeling (values to be reported in the Sec. III. B) and the anisotropic yield modeled using the Hill anisotropic yield condition; (iii) discrete modeling of the HAP rods and organic matrix layers in the indentation region including transverse mineral elements [Fig. 6(b)], to scale with the indenter geometry, and outer surrounding ganoine regions following the corresponding continuum level anisotropic-elastic/anisotropic-plastic constitutive representation.

In the FEA models, all of the nanoindentation simulations were conducted to a maximum load of 500 μ N in accord with the experiments; surface and cross-sectional indents were simulated for all three cases of material description described previously.

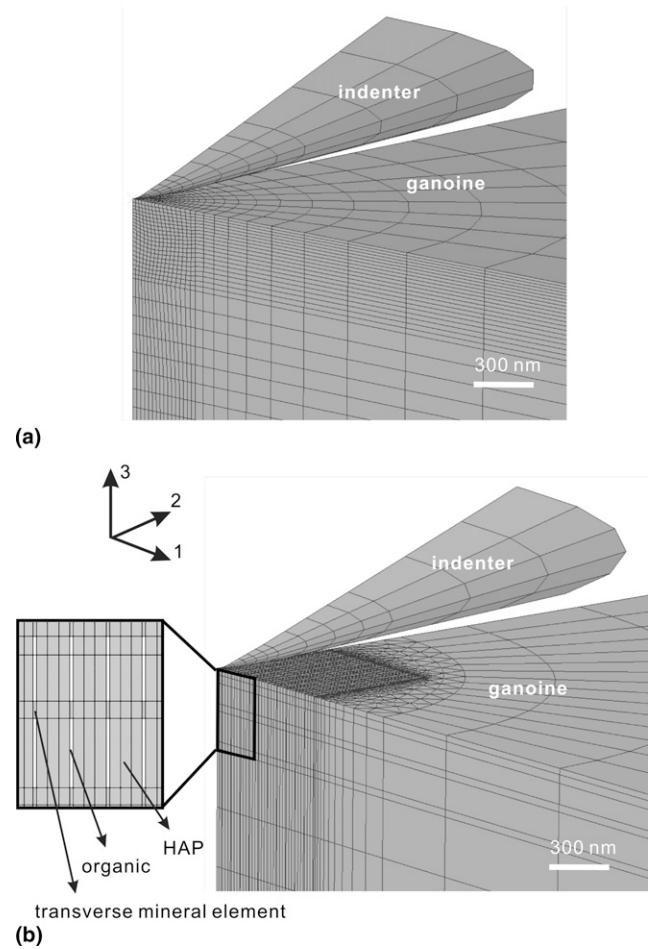


FIG. 6. (a) 3D FEA nanoindentation model of nanoindentation of outer ganoine layer showing finer mesh in region directly below the indenter. (b) Discrete nanostructured model; more than 100 nanocrystals surrounded by thin organic layers were incorporated within the region directly below the indenter. The HAP minerals are meshed with square cross sections.

3. Microindentation model

Microindentation into the multilayered ganoine/dentin/isopedine/bone scale was simulated using nonlinear FEA to/from indentation loads of 0.25 N, 0.50 N, 0.75 N, and 1.0 N. Simulation details follow those reported in our earlier work²¹ with the exception of the constitutive modeling of the ganoine layer. The indenter for the microindentation has a conical tip geometry that emulates a Vickers microhardness tip with a radius of 3.7 μ m in accord with the microhardness experiments of

Ref. 21. The effective microhardness was computed from the FEA simulations in a manner consistent with that used in the microhardness experiments, taking the indentation load and dividing it by the residual contact area (i.e., the area corresponding to the radius of the residual indent impression after unloading is complete). The role of ganoine anisotropy on the microlevel penetration resistance of the multilayered scale was explored by comparatively varying the ganoine constitutive behavior in the FEA models. In the first model, presented in our previous work,²¹ the four material layers with thicknesses corresponding to their experimentally measured values (8, 46, 45, and 300 μm from outer to inner) were each taken to possess isotropic, elastic-perfectly plastic constitutive behavior; this multilayer model is referred to as the isotropic ganoine multilayer (IGML). The material constants have been reduced from nanoindentation data on the individual layers and are provided in Table II. For the second model, the dentin, isopedine, and bone layer were taken to be isotropic, and the ganoine is taken to be anisotropic; this model is referred to as the anisotropic ganoine multilayer (AGML). In this model, the ganoine layer was represented by a continuum level constitutive model of the anisotropic-elastic/anisotropic-plastic behavior used also in the nanoindentation simulations describe previously with the properties reduced from the nanomechanical modeling simulations (described in Sec. III. B); the anisotropic plasticity was captured using the Hill anisotropic yield condition. In order to compare both multilayer models, simulations of indentation into a monolithic isotropic ganoine (to be referred to as MIG) material were conducted.

B. Results

1. Nanomechanical modeling

Figures 7(a) and 7(b) depict the ganoine stress–strain responses computed from the ganoine nanomechanical model simulations for the RVE, which considers the HAP rods to be inherently isotropic and the matrix continuity to be interrupted by transverse mineral elements [henceforth referred to as RVE (A)]. Results considering uniaxial compressions in directions aligned with, 45° off-axis from, and transverse to the long axis of the HAP rods as well as shear in the planes with normals

TABLE II. Isotropic material properties used in FEA model of microindentation into multilayered *P. senegalus* scale,²¹ obtained from isotropic elastic-plastic FEA fits to nanoindentation data for each layer.

	Thickness (μm)	Modulus (GPa)	Yield strength (GPa)
Ganoine	8	65	1.53
Dentin	46	25	0.40
Isopedine	45	14.5	0.215
Bone	300	13.5	0.18

defined by $\mathbf{n} = \mathbf{e}_3$, $\mathbf{n} = 0.707 \mathbf{e}_1 + 0.707 \mathbf{e}_3$, and $\mathbf{n} = \mathbf{e}_1$. The axial modulus and yield strength in the direction aligned with the prism axis are 15% and 26% larger than the transverse values. The off-axis axial response has the lowest modulus and strength since it samples the shearing of the matrix. The shear modulus and yield strength in the transverse plane are 13% and 100% larger than the out-of-plane values, respectively; the off-axis shear has the greatest shear modulus and shear strength as a result of the unfavorable orientation of the organic layers during this shear loading. Table III provides the full set of anisotropic material constants for the ganoine layer determined from the complete simulated response (including lateral contractions during uniaxial stress conditions) of the simulation. A second set of material properties was also deduced for the case where the HAP crystal response was taken to be anisotropic with the crystal properties previously listed in Table I.

The directional dependence of the Young's modulus and axial yield stress were computed from simulations of uniaxial loading in different directions and are shown in Figs. 7(c) and 7(d), respectively. Results for RVE (A) are compared with two other RVEs: RVE (B), which considers isotropic HAP crystals but lacks transverse mineral elements, and RVE (C), which considers anisotropic HAP crystals and includes transverse mineral elements. Values for off-axis axial modulus were also computed by transformation of the computed anisotropic stiffness tensor of each RVE case, giving the continuous curves of Fig. 7(c), which are fully consistent with the values computed via off-axis simulations. Examining the results of RVE (A), the transverse modulus and yield stress ($\sim 90^\circ$) are observed to be lower than the axial axis values ($\sim 0^\circ$, by 13% and 21%, respectively); the lowest values occur off-axis ($\sim 45^\circ$) because of the extensive shearing of the matrix. Comparing RVE (A) to RVE (B), we find the presence of transverse mineral elements to provide a modest increase in the modulus [Fig. 7(c)] in all directions as expected; the transverse mineral elements have a dramatic effect on off-axis yield (up to four times smaller) since the absence of transverse minerals enables easy shear yielding of the matrix when loading off-axis [Fig. 7(d)]. Interestingly, a “double yield” is observed for RVE (B) when loading at $\alpha = 75^\circ$ [Fig. 7(b)] where the first yield occurs at a low stress because of matrix shear. However, as deformation progresses, the HAP rods rotate (unhindered by the lack of transverse mineral elements), and the axial stress needed to provide a resolved shear stress that would yield the matrix layer monotonically increases until reaching a point where yielding of the HAP is reached. This can be interpreted as a geometrically induced self-hardening mechanism. Comparing results of RVE (A) to RVE (C) reveals the influence of the HAP crystal anisotropy on the anisotropic response of the ganoine. The modest

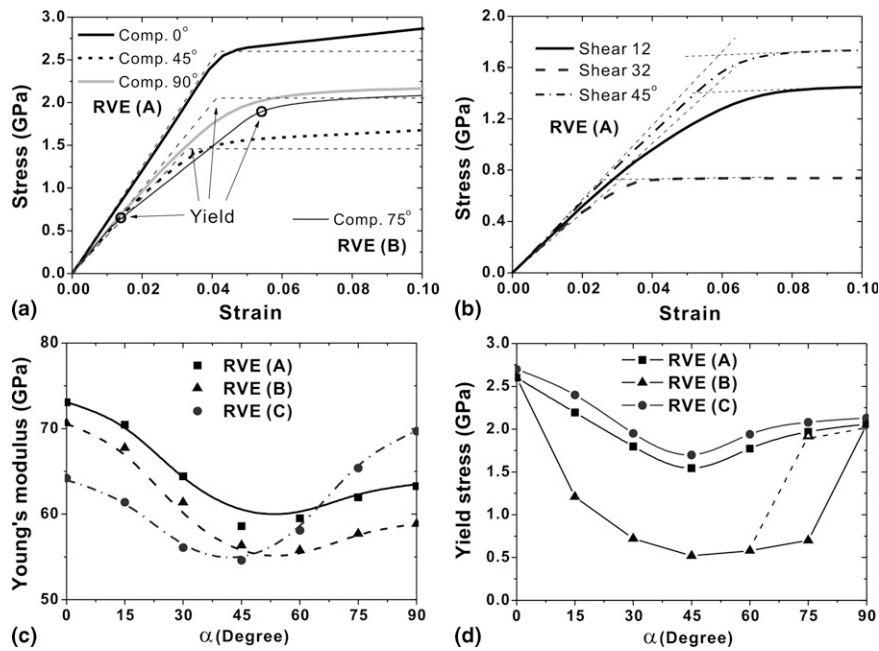


FIG. 7. (a) Predicted microscopic stress-strain response of the RVE (A) for ganoine to uniaxial compression; a stress-strain curve (showing two yield points) for RVE (B) under off-axis compression is included for comparison purpose. Thin dashed lines are simulated curves from anisotropic material assumption. (b) Predicted microscopic stress-strain response of the RVE (A) for ganoine to simple shear deformations. Thin dashed lines show the calculation of yield stress. (c) The Young's moduli and (d) yield stresses determined from the stress-strain curves as a function of orientation, as represented by the angle, α , between the surface and cross-sectional orientations [Fig. 5(b)] (a second yield point is connected by dashed line). Values for off-axis axial modulus computed by transformation of the computed anisotropic stiffness tensor of each RVE case are given by the continuous curves. The directional dependence of the Young's modulus and yield stress is shown for three different RVEs: RVE (A) considers isotropic HAP crystals and transverse mineral elements, RVE (B) considers isotropic HAP crystals but lacks transverse mineral elements, and RVE (C) considers anisotropic HAP crystals and also includes transverse mineral elements.

TABLE III. Predicted anisotropic material constants for ganoine layer from nanomechanical model of RVE with transverse mineral elements: (I) RVE consists of isotropic HAP mineral and (II) RVE consists of HAP mineral with elastic and plastic anisotropy.^{28,33}

	$E_1 = E_2$ (GPa)	E_3 (GPa)	$G_{13} = G_{23}$ (GPa)	G_{12} (GPa)	ν_{12}	$\nu_{32} = \nu_{31}$	$\sigma_{Y,1} = \sigma_{Y,2}$ (GPa)	$\sigma_{Y,3}$ (GPa)	$\tau_{Y,13} = \tau_{Y,23}$ (GPa)	$\tau_{Y,12}$ (GPa)
I (isotropic HAP crystal)	63.5	73.1	23.0	25.0	0.29	0.25	2.05	2.60	0.57	1.16
II (anisotropic HAP crystal)	69.7	64.0	20.7	28.4	0.36	0.18	2.13	2.70	0.54	1.07

inherent elastic anisotropy of the HAP crystal offsets some aspects of the geometric anisotropy, giving a transverse modulus slightly higher than the axial modulus [Table III, Fig. 7(c)]. The yield stress is slightly increased in an approximately proportional manner because of the anisotropic yielding of the HAP crystal. Although these results are based on RVEs of an idealized microstructure of ganoine, they do reveal that the anisotropic mechanical feature of primary importance is the reduced shear stiffness and yield stress relative to the axial and transverse properties.

Figure 8 depicts contours of scalar equivalent stress (von Mises) and equivalent plastic strain for RVE (A) under the macroscopic compression strain of 10% and macroscopic shear strain of 10%. These contours demonstrate the deformation mechanisms of the direction-dependent yield behavior of Fig. 7(d) described previously. Compression along the long axis requires direct axial loading of the HAP rods as best seen in the stress contours

where, as expected, off-axis loading gives extensive shearing of the matrix (as best seen in the plastic strain contours) at relatively low stress (as seen by comparing the stress contours of the different directions). Similar comparisons of contours for the simple shear loading conditions reveal how different regions and volumes of matrix govern yielding in the different loading directions.

2. Nanoindentation model

Figure 9 plots experiment and simulation results for load versus depth response of nanoindentation into the surface and the cross section of ganoine, where simulation results consider the three representations of the ganoine constitutive behavior described in Sec. III. A: isotropic ($E = 65$ GPa, $\nu = 0.3$, $\sigma_Y = 1.53$ GPa), anisotropic (with material property I in Table III, isotropic HAP with transverse mineral elements), and discrete model (with isotropic HAP and

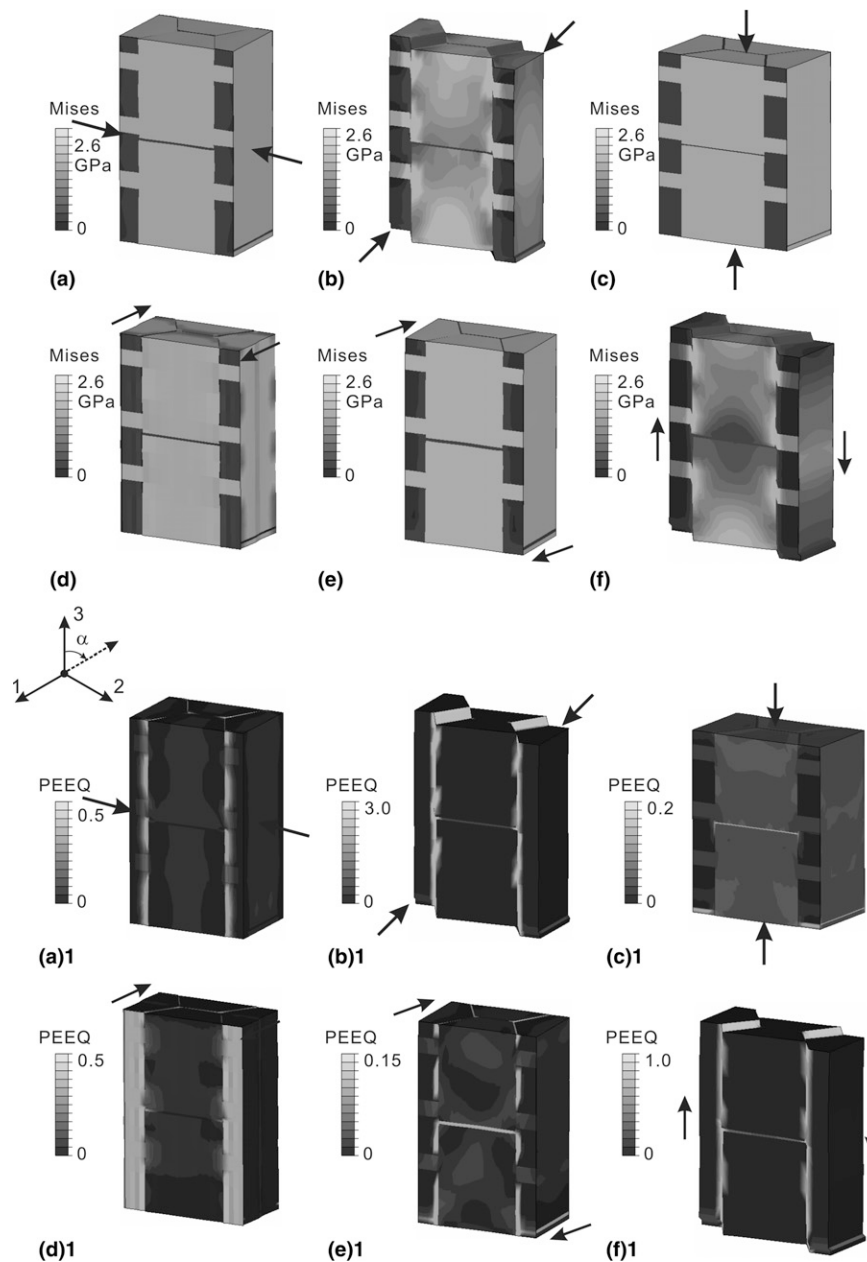


FIG. 8. Simulation contours of equivalent stress (von Mises) for RVE (A) representing ganoine, Fig. 5(b), i.e., considering isotropic HAP and including transverse mineral elements, under a macroscopic compression strain of 10% for (a) $\alpha = 90^\circ$, (b) $\alpha = 45^\circ$, and (c) $\alpha = 0^\circ$, and macroscopic shear strain of 10% in the plane with normal (d) $\mathbf{n} = \mathbf{e}_3$, (e) $\mathbf{n} = 0.707\mathbf{e}_1 + 0.707\mathbf{e}_3$, and (f) $\mathbf{n} = \mathbf{e}_1$. (a)1–(f)1 are corresponding plastic equivalent strain contours (PEEQ).

transverse mineral elements). Consistent with the experimental data, the predicted load–depth curves for all material constitutive behaviors also indicate no directional dependence that implies apparent isotropy of the indentation modulus and hardness, despite the anisotropic microstructure of the ganoine. However, interestingly, the local stress and strain distributions and underlying deformation mechanisms are found to depend on the material behavior (discussed next).

During indentation, the indenter induces inhomogeneous multiaxial stress and strain fields in the ganoine.

The stress and strain fields are governed by the constitutive response of the ganoine and the geometry of the indenter. Figures 10 and 11 show the stress and plastic strain contours for the simulated nanoindentations into the top surface and the cross section of the ganoine, respectively. The simulation results are compared for three representations of the constitutive behavior of the ganoine: isotropic ($E = 65$ GPa, $\nu = 0.3$, $\sigma_Y = 1.53$ GPa, Table II), anisotropic (with isotropic HAP, material property I in Table III), and discrete model (with isotropic HAP). Indentation is, in general, accommodated by

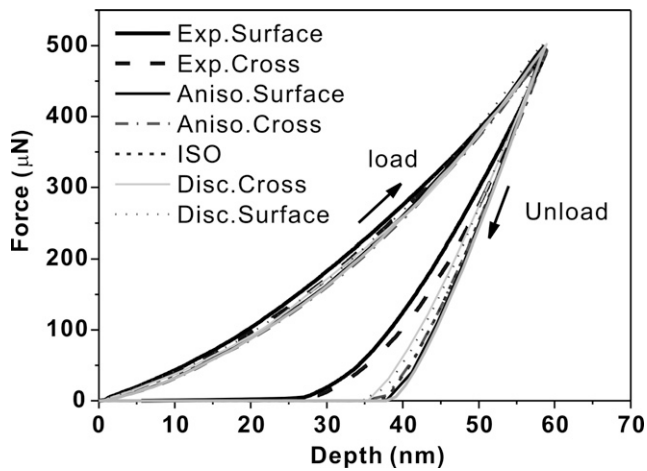


FIG. 9. Comparison of the average experimental curves to the FEA simulation curves obtained by virtual nanoindentation after the full anisotropic material set for ganoine was determined using the proper nanomechanical models. (i) Isotropic properties with $E = 65$ GPa, $\nu = 0.3$, and $\sigma_Y = 1.53$ GPa (Table II, row 1). (ii) Anisotropic properties using data obtained from the nanomechanical modeling with isotropic HAP crystals (Table III, row I) and transverse mineral elements [RVE (A)]. (iii) Discrete model assuming isotropic HAP rods [Fig. 6(b)].

compressing material beneath the indenter and shearing material away from under the indenter. Comparing the anisotropic ganoine case to the isotropic case, the compressive normal stress (S33) is seen to occur in a narrower band and to extend to deeper depths for the anisotropic cases (Fig. 10) as a consequence of the more diffuse plastic shear straining (PE13) that occurs at lower shear stress (S13) for the anisotropic case (a direct consequence of the reduced shear yield stress). This has consequences in how load and deformation are transmitted to the underlying dentin layer during microindentation of the multilayered scale (see Sec. IV). Comparison of the continuum constitutive behavior of ganoine to the discrete structural representation of ganoine reveals the role of the rodlike nanostructure during nanoindentation. Large bands of intense plastic deformation are observed in the organic matrix layers, and yielding of the HAP rods and transverse mineral elements also takes place and contributes to the total energy dissipation density (see the shear contours of Figs. 10 and 11). While the discrete model captures the separate contributions of the HAP prisms and the organic layers, comparison of the discrete model results to the

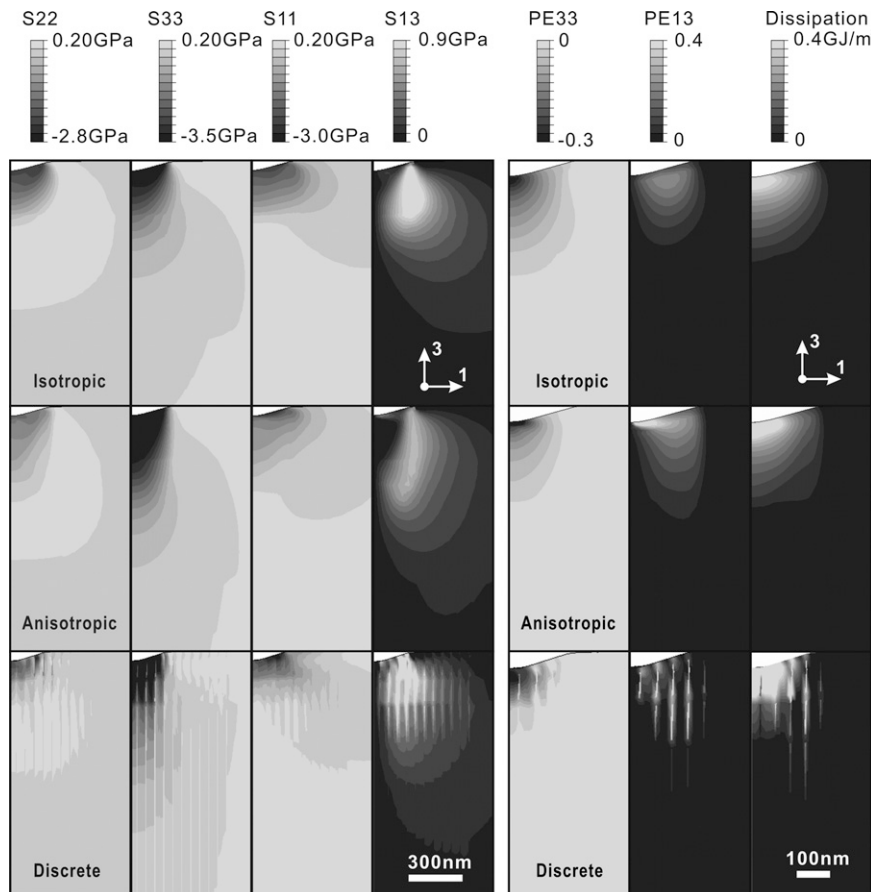


FIG. 10. Simulation contours of stress distributions, plastic strain, and energy dissipation of the ganoine layer under nanoindentation with the loading parallel to the surface normal (3-axis). A comparison is shown between isotropic (Table II, row 1), anisotropic (isotropic HAP crystals) (Table III, row I), and transverse mineral elements [RVE (A)], [Fig. 5(b)], and discrete models for 3D nanoindentation [Fig. 6(b)].

continuum anisotropic model results shows very similar overall contours even for this nanoindentation where the length scale of the rod width is of order of the depth of the indentation; hence, these results also show that use of the continuum level anisotropic model will be sufficient to capture the anisotropic behavior of the ganoine during the larger length scale microindentation simulations. It should be noted that anisotropy of HAP does not affect the stress/strain distributions in these nanoindentation models because yield stresses dominate stress and strain fields. Table IV compares O–P modulus and O–P hardness values obtained from experimental measurements and

from the simulations assuming the different material models including isotropic model, anisotropic model (with material properties I or HAP isotropic and II or HAP anisotropic, Table III), and discrete model. The modulus and hardness were reduced from the load–depth curves using the Oliver–Pharr method (which assumes the material to be homogeneous and isotropic).

3. Microindentation model

The role of ganoine anisotropy in the larger length-scale biomechanical microindentation of the multilayered

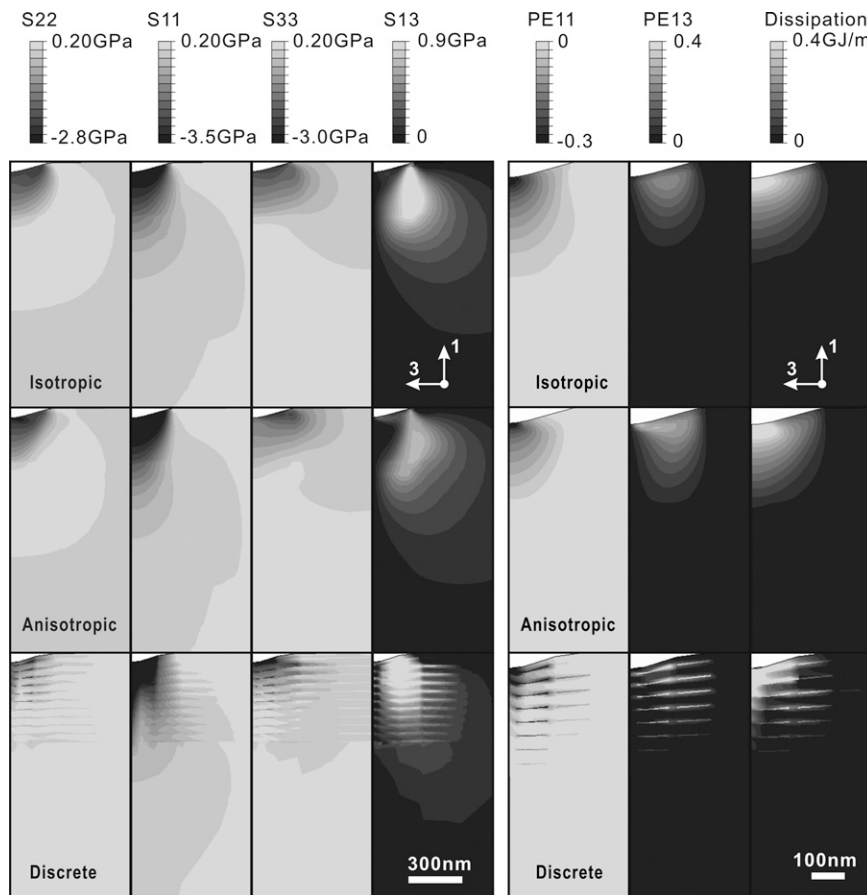


FIG. 11. Simulation contours of stress distributions, plastic strain, and energy dissipation of the ganoine layer under nanoindentation with the loading perpendicular to the surface normal (along the 1-axis). A comparison is shown between isotropic (Table II, row 1), anisotropic (isotropic HAP crystals) (Table III, row I) and transverse mineral elements [RVE (A), Fig. 5(b)], and discrete models for 3D nanoindentation [Fig. 6(b)].

TABLE IV. O–P modulus and hardness obtained from experimental measurements and various simulation models: isotropic model, anisotropic model I where the HAP crystal is isotropic, anisotropic model II where the HAP crystal is anisotropic, and the discrete model.

	O–P modulus (GPa)		O–P hardness (GPa)	
	Surface	Cross section	Surface	Cross section
Experiments	74.8 ± 5.0	75.1 ± 8.2	3.98 ± 0.27	3.92 ± 0.34
Isotropic model	75.6	75.6	3.70	3.70
Anisotropic model I (HAP isotropic)	77.2	70.8	3.86	3.86
Anisotropic model II (HAP anisotropic)	67.7	74.2	3.82	3.92
Discrete model	74.2	70.9	4.12	4.04

P. senegalus scale was explored by constructing two FEA models, the isotropic ganoine multilayer (IGML) (reported previously²¹) and the anisotropic ganoine multilayer (AGML) described in Sec. III. A. 3. We recall that, despite the clearly anisotropic microstructure of the ganoine layer, the experimental and predicted force–depth behavior and effective indentation modulus and indentation hardness were found to be independent of direction and to also be independent of constitutive representation (i.e., whether ganoine was modeled as isotropic, anisotropic, or discrete). However, the underlying deformation mechanisms, revealed in the simulations, had subtle dependencies on anisotropy. Here, the effect of ganoine anisotropy on the effective microindentation behavior of the multilayered structure is explored—examining the effect of both the effective force–depth behavior and the underlying deformation and dissipation mechanisms.

Figure 12(a) depicts the load–depth curves of the IGML and AGML multilayered structures to/from maximum loads of 0.5 and 1 N, compared with a simulation of monolithic isotropic ganoine (MIG). As also previously found in the ganoine nanoindentation cases, the IGML and AGML load–depth behaviors and corresponding reduced material properties were found to be nearly identical, giving effective modulus (~ 30 GPa),

effective microhardness (~ 1.7 to 1.8 GPa), and energy dissipation (1.6 to ~ 1.65 μ J). Indentation to/from different maximum load levels reveals the effective microhardness for both models to decrease nonlinearly with increasing indentation load [Fig. 12(b)]. The predicted microhardness values show good agreement with the experimentally measured Vickers microhardness [Fig. 12(b)]. In the 0.25 and 0.5 N microindentation experiments, most samples (~ 9 of 10 indents) were observed to deform in an elastic-plastic manner with no surface cracks observed.²¹ In the 1 N cases, $\sim 50\%$ of the samples (~ 5 of 10 indents) showed circumferential surface cracking. Circumferential cracks were observed in all of the 2 N cases (10 of 10 indents).²¹

The predicted effective total energy dissipation [area between the loading and unloading force–depth curves, e.g., Fig. 12(a)] as a function of maximum indentation load for the IGML and AGML microlayered models were found to be indistinguishable, increasing nonlinearly from close to that of the MIG model at small maximum loads to values nearly double the dissipation energy of the MIG at a maximum load of 1 N [Fig. 12(c)]. The evolution of the work of indentation during loading and unloading of the entire multilayered structure, as well as the individual contributions of the ganoine and the dentin

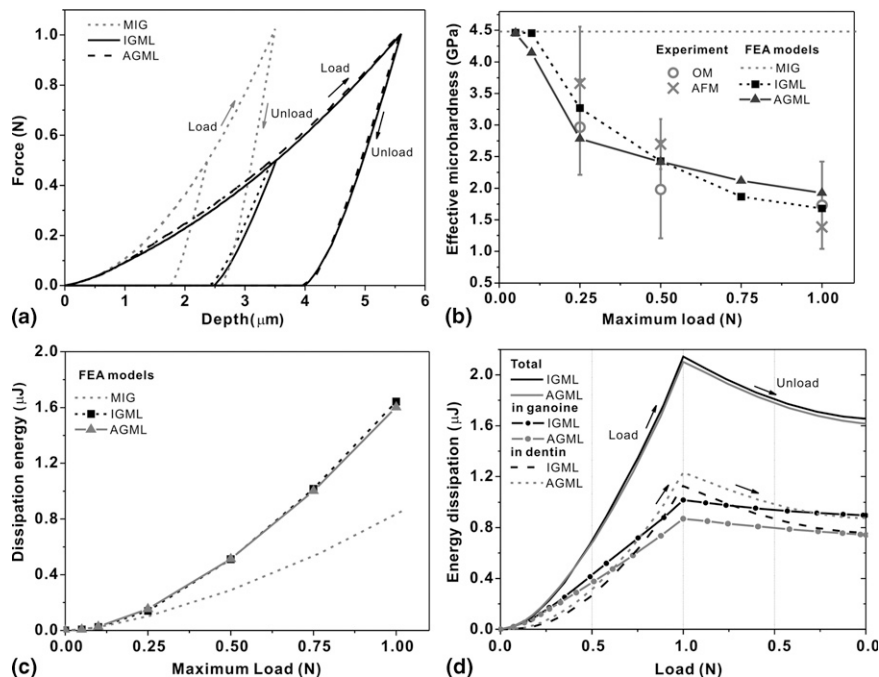


FIG. 12. Predictions of effective microindentation mechanical properties of the multilayered *P. senegalus* scale; notations include monolithic isotropic ganoine (MIG), isotropic ganoine multilayer (IGML), and anisotropic ganoine multilayer (AGML), respectively. (a) Simulated microindentation load-versus-depth curves. (b) Simulated effective microhardness compared to experimentally measured values as a function of maximum indentation load, reported by us previously.²¹ OM and AFM indicate optical microscope and atomic force microscope, respectively, which were used to measure the residual area after unloading. (c) Simulated effective energy dissipation as a function of maximum indentation load. Dissipation energy was calculated as the area between the loading and unloading portion of the load–depth curves in microindentation after unloading. (d) Simulated energy evolution (the work of indentation) of the whole multilayered structure [IGML (black lines) and AGML (gray lines)], and the individual ganoine and dentin layers as a function of applied load up to a 1 N maximum load, as well as on unloading.

layers is provided in Fig. 12(d). For both the IGML and AGML systems, a nonlinear increase in the work of indentation is observed during loading followed by the elastic recovery during unloading. Although both the IGML and AGML microlayered models exhibit a similar total energy evolution during loading and unloading, locally the ganoine and dentin layers in the IGML and the AGML structures contribute differently to the total energy evolution. During the early loading stage (<0.25 N), the ganoine and dentin layers of AGML case partition energy in a manner similar to that found in the IGML case. However, as the load is further increased, the energy-dissipation contribution of the dentin in the AGML case begins to increase more significantly than in the IGML case (because of dentin yielding earlier and, hence, greater plastic dissipation in dentin in the AGML case). At a maximum load of 1 N, the contributions to the work of indentation from ganoine and from dentin differ for the IGML and AGML models; with dentin in the

AGML case contributing 60% of the dissipation compared with 50% for the IGML case.

Contour plots of stress and plastic strain are shown in Fig. 13 when fully loaded to 1 N and after fully unloaded from 1 N. The normal stress fields, S22 and S33, of the anisotropic ganoine layer are observed to be higher in magnitude than those of the isotropic ganoine layer, whereas the shear stress (S23) magnitudes (~ 540 MPa) in the AGML are significantly lower than that of ganoine in the “IGML” model (~ 900 MPa); these contrasts are a direct consequence of the anisotropic ganoine possessing enhanced axial moduli, enhanced axial yield stresses, reduced shear moduli, and reduced shear yield stresses compared with the isotropic ganoine. Therefore, although the stress contours of the two multilayered systems show different stress fields and magnitudes in stress component levels, the two multilayered systems in the end achieve similar effective load–depth behaviors, microhardness values, and energy dissipation.

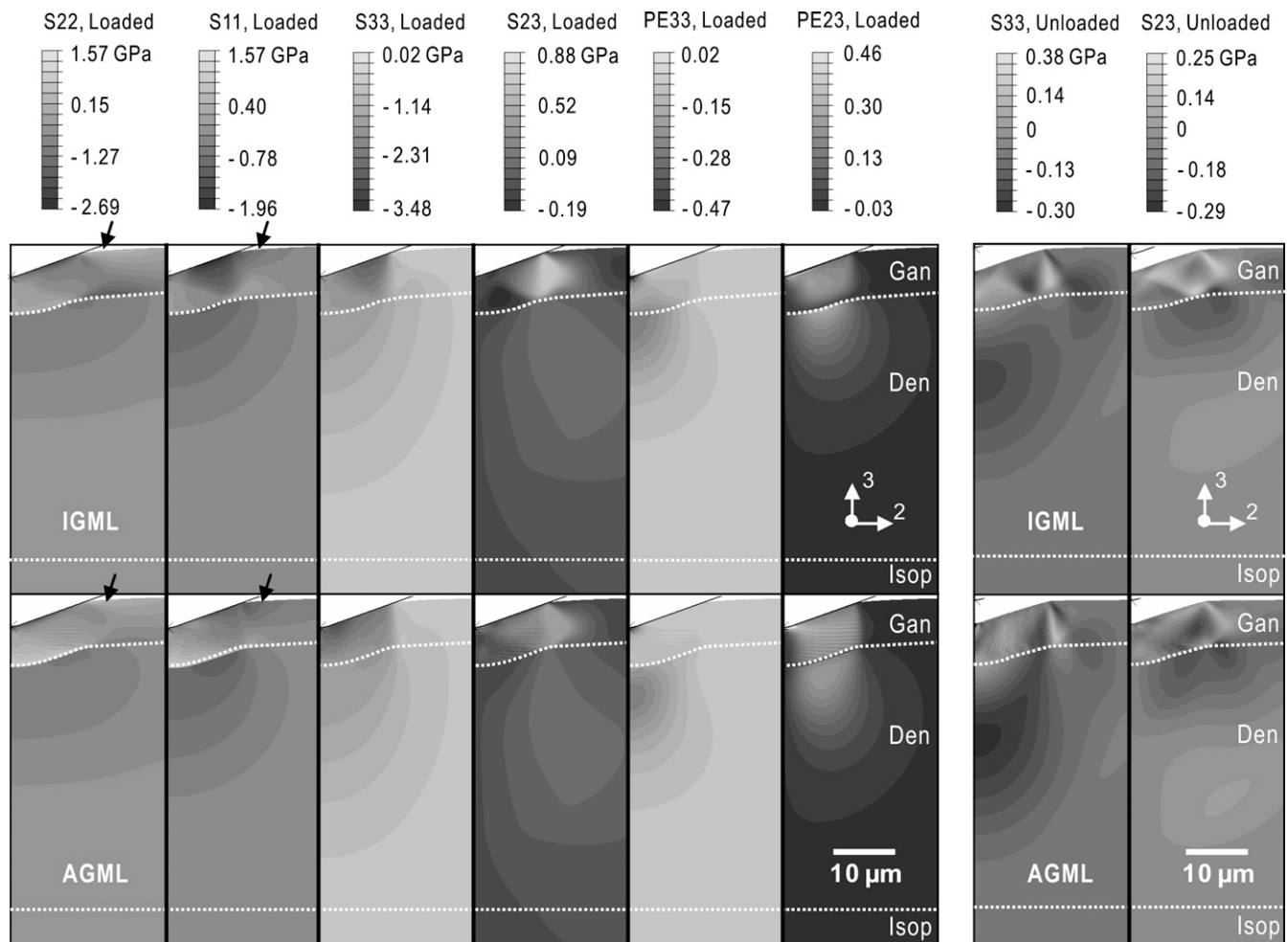


FIG. 13. Simulation contours of stress and plastic strain of *P. senegalus* scale under microindentation. FEA predictions of (a) S11, S22, S23, S33 and plastic strain, PE33 and PE23 at a maximum depth when fully loaded and (b) S23, S33 when fully unloaded for the two isotropic ganoine multilayer (IGML) and anisotropic ganoine multilayer (AGML), multilayered models. Arrows indicate the location of surface stresses on the stress contours, S11 and S22.

In our previous study,²¹ the 1 N maximum load experiments and simulations were used to estimate a maximum normal stress brittle-failure condition for the ganoine: experimentally, circumferential cracks were observed on the surface during microhardness experiments at 1 N maximum load. Using contours of the radial stress, S22, at this maximum load level gave a critical normal stress of ~ 1.1 GPa from results of our “IGML” model. Here, contours of S22 from both the “IGML” and the “AGML” multilayer models compute similar magnitudes in tensile S22 surface values whereas their circumferential stress fields (S33) exhibit compressive values at the surface, again suggesting that circumferential cracking on the surface is the preferred failure mode in these multilayered systems. These results also indicate that the anisotropy is not the primary structural property governing this aspect of the surface stress distribution, but instead it is the contrast in the ganoine and dentin behavior that gives this mechanical behavior. However, after circumferential cracking is initiated, the anisotropic rodlike structure will facilitate the propagation vertically, which is more advantageous since it can be arrested by the energy dissipating dentin layer, rather than propagating in other directions (e.g., radially), which could lead to catastrophic structural failure. Additionally, the interfacial normal and shear stresses are known to be important factors in the interfacial failure in other multilayer systems.^{21,36} The interfacial (and close to interface) shear stresses in the AGML case (S23) are found to be significantly lower during loading than those in the IGML case, a consequence of the lower shear yield stress (τ_{y23}). Upon unloading, a peak shear stress is present on the interface of both cases but is lower in the AGML case. The lower shear yield stress (τ_{y23}) of the anisotropic ganoine is seen to favor plastic deformation via plastic shear straining (comparing PE23 contours) and thus to also promote a radial spreading of plastic deformation in the ganoine layer (PE23 contours) compared with more direct compression straining (comparing PE33 contours). The different plastic straining of the ganoine layer in the two cases is observed to result in different curvatures of the ganoine/dentin interface. The plasticity in the underlying dentin layer is spatially more elongated in depth for the anisotropic ganoine compared to the isotropic case (PE23, PE33).

Contours of plastic energy dissipation density (energy dissipation per unit volume) and energy dissipation magnitude (dissipation energy accounting for the volume over which the dissipation occurs—although this is a mesh-dependent quantity, it provides a useful comparison between cases when the same mesh is used) for each layer when fully unloaded are depicted in Fig. 14. The contours of each layer are scaled differently to better reveal the contributions of each layer. Plastic energy dissipation density contours in Fig. 14(a) show the overall plastic deformation reveals less plasticity in the

ganoine layer accompanied by greater dissipation in the dentin layer for the AGML case. In the energy dissipation magnitude contours [Fig. 14(b)], taking into account the volume over which the energy dissipation occurs enables assessment of where energy dissipation is dominant within a layer as well as comparison between cases. In particular, the dissipation magnitude contours capture the increased volume of points as one moves radially outward away from the axis of indentation, revealing the dominant dissipation role around the perimeter of indentation; these contours also reveal the more prevalent dissipation role of dentin in the AGML and of ganoine in the IGML. Both forms of energy contours reflect greater energy dissipation in dentin in the AGML (Fig. 12).

IV. DISCUSSION

A major threat to *P. senegalus* is biting from its own species, which takes place during territorial fighting and feeding.³⁷ The tooth geometry and size make these penetration biting events approximately analogous to microindentation. In our previous work,²¹ we demonstrated that the micrometer-scale multilayered structure acts to provide an initially stiff and hard line of defense against low-load events, governed by the behavior of the outer ganoine layer. This behavior transitions to an increasingly dissipative response with increasing penetration load where the underlying dentin layer provides the dissipation. The goal of this study was to investigate the role of the anisotropy of the prismaticlike ganoine structure in the load-dependent penetration resistance of the entire multilayered armor. The design principles identified are expected to be relevant to a broad array of biological exoskeletons, since many different species exhibit similar nanostructures in their outermost layers,^{16–19} and to the development of improved biologically-inspired structural materials.

Nanomechanical modeling results revealed that the unique structure of ganoine does indeed result in elastic-plastic mechanical anisotropy (transverse isotropy) of the composite material. The balance between smaller length scale “inherent” anisotropy of the crystalline HAP and larger length scale “geometric” anisotropy of the HAP-organic composite nanostructure was explored, a topic relevant for most biological composites. Since the HAP crystal is modestly anisotropic, it has a minimal effect on the anisotropic elastic-plastic response of the ganoine composite, which is rather dominated by geometric anisotropy of the composite nanostructure. This minimal effect of HAP anisotropy has also been reported on the other composite systems (e.g., bone¹). Enhanced axial and transverse moduli (and yield stresses) and significantly reduced shear moduli (and yield stresses) were predicted. The shear moduli also correlate with reduced off-axis axial moduli and yield stresses, which result from shear deformation of the organic interlayers. Transverse mineral

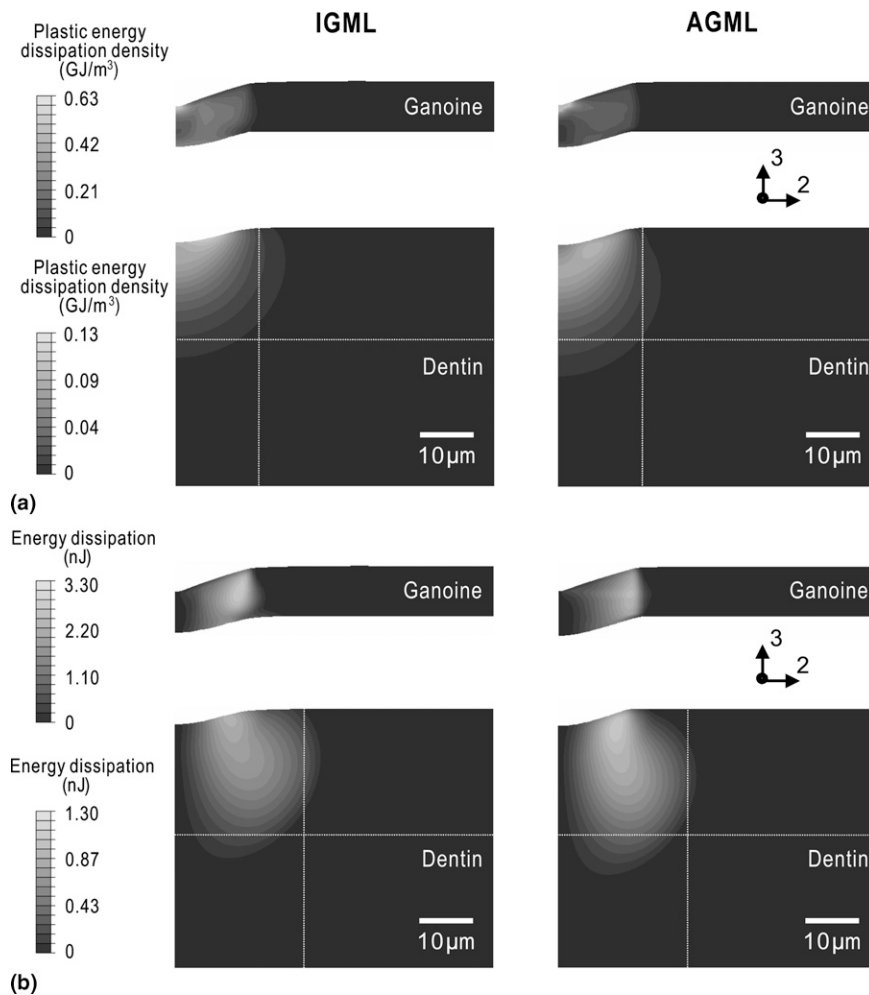


FIG. 14. Simulation contours of plastic energy dissipation density and energy dissipation of *P. senegalus* scale under microindentation at 1 N maximum load after fully unloaded. FEA predictions of (a) plastic energy dissipation density, (b) energy dissipation magnitude in separated ganoine and dentin layers with the proper scale bar for two IGML and AGML multilayered structures. The energy-dissipation magnitude contour reflects the volumetric effect on energy-dissipation density. The guidelines (white dashed lines on energy contours of dentin layers) show energy-dissipation in dentin of the AGML model covers larger area (elongated shape) with relatively higher density or magnitude than in that of the IGML model.

elements, representing mineral prism-to-prism surface roughness and adjacent rod interlocking, provided significant resistance to shear.

Even though ganoine was determined to be mechanically anisotropic, surprisingly this constitutive behavior does not result in a direction-dependent indentation modulus or hardness. However, comparison of simulations assuming an isotropic ganoine stress-strain behavior (reported previously²¹) with those assuming anisotropic stress-strain behavior did result in different local stress and strain fields accommodating the imposed indentation; in particular, greater shearing at lower shear stresses are found to accommodate indentation in the anisotropic material, and the compressive stress and strain fields directly beneath the indenter are found to extend to deeper depths. These observations suggest that the anisotropic ganoine behavior may influence the behavior of the multilayered scale during microindentation.

To understand the role of ganoine anisotropy in resisting penetrating bite attacks, the behavior of the multilayered structure during microindentation assuming an isotropic ganoine layer was compared with that obtained assuming the ganoine layer to be anisotropic. At low indentation loads (<0.15 N), the indentation modulus and hardness for the multilayered structure are close to that of monolithic ganoine in both experiments and modeling (this is the case whether ganoine is modeled as isotropic or anisotropic). The indentation modulus and hardness are found to decrease with an increase in indentation load—this is found in both experiments and in simulations; the simulation results for these properties are independent of the anisotropic versus isotropic representation of the ganoine. However, as with the nanoindentation simulations, the spatial distributions of local stress and strain differed between the anisotropic and isotropic cases. With increasing indentation load, the

anisotropic stiffness and yield strength of the ganoine were found to promote a greater level of plastic deformation in the dentin in radial and depth directions. This increasing volume of plastic deformation in the underlying dentin layer promotes the use of the dentin layer as the major dissipation element in the multilayered structure, in essence “protecting” the outer ganoine layer by limiting the strain and stress that the ganoine will experience. The anisotropic constitutive behavior of the ganoine also spreads the plastic deformation and energy dissipation to a greater volume of material overall.

Both the isotropic and anisotropic treatments of the ganoine stress–strain behavior are found to result in tensile radial stress field (S22) and compressive circumferential stress field (S11) on the surface around the indentation perimeter; the tensile radial stress promotes circumferential cracking whereas the compressive circumferential stress suppresses radial cracking. Circumferential cracking would be a favored failure event since it would be localized to the indentation region and would not be a propagating failure across the scale. Moreover, the discrete prism structure does provide the fracture pathway (normal to the scale surface between HAP rods) after initiation of circumferential cracks. Such failure pathways lead to the localized damage in the ganoine and then are easily arrested by the ductile dentin layer. These different findings of the underlying deformation, stress, and dissipation behavior when the outer layer is slightly anisotropic rather than isotropic motivates a more thorough parametric investigation into the potential of using and designing anisotropic outer (and inner) layers to promote dissipation during penetration events.

Our results on ganoine are expected to be relevant as well for human teeth. Human teeth are composed of an outer enamel layer that has a composite HAP-organic structure of similar composition to ganoine also followed by an underlying dentin layer. However, there are a number of notable differences between these two systems: (1) the ganoine thickness is $\sim 10\ \mu\text{m}$ in contrast to tooth enamel which has thickness $> \text{mm}$ presumably due to the larger length scale of the mastication process and (2) tooth enamel has an additional higher order level of anisotropic structural geometric hierarchy whereby the HAP nanocrystals self-assemble into micrometer-scale “rods” with their long axes perpendicular to the tooth surface (absent in ganoine). The anisotropic elastic and plastic properties reported in this study for ganoine are consistent with those previously reported for intrarod tooth enamel.³⁸ Larger length scale mechanical experiments involving multiple rods revealed amplified mechanical anisotropy compared with the constituent enamel composite, elucidating the effect of the second level of structural hierarchy.^{34,38} A FEA study considered different anisotropy ratios for enamel and demonstrated that an increasing anisotropic nature would more directly transfer

stresses to underlying compliant dentine and thus reduce the potential for tooth fracture,³⁹ a result consistent with those we obtain for ganoine here. Lastly, similar to ganoine, this anisotropic structure is assumed to be beneficial in directing cracks vertically down into the dentin.⁴⁰

Our study emphasizes the critical importance of the anisotropy of structural biological material at the nanoscale and the microscale in achieving macroscale mechanical properties to optimize penetration resistance and protection. The fundamental understanding of this optimization in a multilayered natural armor structure suggests an anisotropic design to improve biomimetic systems.

ACKNOWLEDGMENTS

The authors would like to acknowledge use of the MIT Department of Materials Science and Engineering Nanomechanical Testing Facility for the experiments conducted there. Thank you to A. Schwartzman for his training and assistance with the experiments. We also gratefully acknowledge the support of the National Science Foundation MIT Center for Materials Science and Engineering (DMR-0819762), the US Army through the MIT Institute for Soldier Nanotechnologies (contract No. DAAD-19-02-D0002), and the National Security Science and Engineering Faculty Fellowship (NSSEFF) Program. Discussions with Drs. Robert Jensen and Tusit Weerasooriya of the U.S. Army Research Laboratory were helpful during the course of this work, as well as Dr. Timothy Imholt (Raytheon, Inc.). The content does not necessarily reflect the position of the government and no official endorsement should be inferred.

REFERENCES

1. A. Fritsch and C. Hellmich: “Universal” microstructural patterns in cortical and trabecular, extracellular and extravascular bone materials: Micromechanics-based prediction of anisotropic elasticity. *J. Theor. Biol.* **244**, 597 (2007).
2. F. Barthelat, C.-M. Li, C. Comi, and H.D. Espinosa: Mechanical properties of nacre constituents and their impact on mechanical performance. *J. Mater. Res.* **21**, 1977 (2006).
3. V. Bucur and N.F. Declercq: The anisotropy of biological composites studied with ultrasonic technique. *Ultrasonics* **44**, e829 (2006).
4. S.P. Nicholls, L.J. Gathercole, A. Keller, and J.S. Shah: Crimping in rat tail tendon collagen: Morphology and transverse mechanical anisotropy. *Int. J. Biol. Macromol.* **5**, 283 (1983).
5. S. Vogel: *Comparative Biomechanics* (Princeton University Press, Princeton, NJ, 2003), p. 175.
6. S.L.-Y. Woo, W.H. Akeson, and G.F. Jemcott: Measurements of nonhomogeneous, directional mechanical properties of articular cartilage in tension. *J. Biomech.* **9**, 785 (1976).
7. S.N. White, W. Luo, M.L. Paine, H. Fong, M. Sarikaya, and M.L. Snead: Biological organization of hydroxyapatite crystallites into a fibrous continuum toughens and controls anisotropy in human enamel. *J. Dent. Res.* **80**, 321 (2001).
8. J.L. Katz and K. Ukraincik: On the anisotropic elastic properties of hydroxyapatite. *J. Biomech.* **4**, 221 (1971).

9. L. Ng, A.J. Grodzinsky, J.D. Sandy, A.H.K. Plaas, and C. Ortiz: Individual cartilage aggrecan macromolecules and their constituent glycosaminoglycans visualized via atomic force microscopy. *J. Struct. Biol.* **143**, 242 (2003).
10. L. Bozec, G. van der Heijden, and M. Horton: Collagen fibrils: Nanoscale ropes. *Biophys. J.* **92**, 70 (2007).
11. K.A. Dill: Dominant forces in protein folding. *Biochemistry* **29**, 7133 (1990).
12. D.A. Tirrel: *Hierarchical Structures in Biology as a Guide for New Materials Technology* (National Academic Press, Washington, DC, 1994).
13. H.A. Lowenstam and S. Weiner: *On Biomineralization* (Oxford University Press, New York, 1989).
14. S. Weiner, L. Addadi, and H.D. Wagner: Materials design in biology. *Mater. Sci. Eng., C* **11**, 1 (2000).
15. S.A. Wainwright: Stress and design in bivalved mollusc shell. *Nature* **224**, 777 (1969).
16. A. Al-Sawalmih, C.H. Li, S. Siegel, H. Fabritius, S.B. Yi, D. Raabe, P. Fratzl, and O. Paris: Microtexture and chitin/calcite orientation relationship in the mineralized exoskeleton of the American lobster. *Adv. Funct. Mater.* **18**, 3307 (2008).
17. D. Chateigner, C. Hedegaard, and H-R. Wenke: Mollusc shell microstructures and crystallographic textures. *J. Struct. Geol.* **22**, 1723 (2000).
18. A.H. Parsons: Structure of the egg shell. *Poult. Sci.* **61**, 1213 (1982).
19. A.B. Rodriguez-Navarro, C. CabraldeMelo, N. Batista, N. Morimoto, P. Alvarez-Lloret, M. Ortega-Huertas, V.M. Fuenzalida, J.I. Arias, P. Wiff, and J.L. Arias: Microstructure and crystallographic-texture of giant barnacle (*Austromegabalanus psittacus*) shell. *J. Struct. Biol.* **156**, 355 (2006).
20. F.C.M. Driessens and R.M.H. Verbeeck: *Biominerals* (CRC Press, Boca Raton, FL, 1990), p. 163.
21. B.J.F. Bruet, J.H. Song, M.C. Boyce, and C. Ortiz: Materials design principles of ancient fish armor. *Nat. Mater.* **7**, 748 (2008).
22. J. Daget, M. Gayet, F.J. Meunier, and J-Y. Sire: Major discoveries on the dermal skeleton of fossil and recent polypteriforms: A review. *Fish Fish.* **2**, 113 (2001).
23. F.J. Meunier: Histological studies of the dermal skeleton in Polypteridae. *Arch. Zool. Exp. Gén.* **122**, 279 (1980).
24. T. Ørvig: Phylogeny of tooth tissues: Evolution of some calcified tissues in early vertebrates, in *Structural and Chemical Organization of Teeth*, Vol. 1, edited by A.E.W. Miles (Academic Press, New York & London, 1967), p. 45.
25. W.C. Oliver and G.M. Pharr: An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. *J. Mater. Res.* **7**, 1564 (1992).
26. M. Danielsson, D.M. Parks, and M.C. Boyce: Three-dimensional micromechanical modeling of voided polymeric materials. *J. Mech. Phys. Solids* **50**, 351 (2002).
27. M. Danielsson, D.M. Parks, and M.C. Boyce: Micromechanics, macromechanics and constitutive modeling of the elastoviscoplastic deformation of rubber-toughened glassy polymers. *J. Mech. Phys. Solids* **55**, 533 (2007).
28. W.T. Lee, M.T. Dove, and E.K.H. Salje: Surface relaxations in hydroxyapatite. *J. Phys. Condens. Matter* **12**, 9829 (2000).
29. A.S. Posner and F. Betts: Molecular control of tissue mineralization, in *Chemistry and Biology of Mineralized Connective tissues*, edited by A. Veis (Elsevier, Amsterdam, 1981), pp. 257–266.
30. Th. Leventouri: Synthetic and biological hydroxyapatites: Crystal structure questions. *Biomaterials* **27**, 3339 (2006).
31. C. Rey, C. Combes, C. Drouet, H. Sfihi, and A. Barroug: Physicochemical properties of nanocrystalline apatites: Implications for biominerals and biomaterials. *Mater. Sci. Eng., C* **27**, 198 (2007).
32. S. Weiner: Transient precursor strategy in mineral formation of bone. *Bone* **39**, 431 (2006).
33. B. Viswannath, R. Raghavanb, U. Ramamurthyb, and N. Ravishankar: Mechanical properties and anisotropy in hydroxyapatite single crystals. *Scr. Mater.* **57**, 361 (2007).
34. I.R. Spears: A three-dimensional finite element model of prismatic enamel: A re-appraisal of the data on the Young's modulus of enamel. *J. Dent. Res.* **76**, 1690 (1997).
35. D.R. Katti, K.S. Katti, J.M. Sopp, and M. Sarikaya: 3D finite element modeling of mechanical response in nacre-based hybrid nanocomposites. *Comput. Theor. Polym. Sci.* **11**, 397 (2001).
36. R. Jayachandran, M.C. Boyce, and A.S. Argon: Design of multilayer polymeric coatings for indentation resistance. *J. Comput. Aided Mater. Des.* **2**, 155 (1995).
37. M.J. Markey, R.P. Main, and C.R. Marshall: Vivo cranial suture function and suture morphology in the extant fish Polypterus: Implications for inferring skull function in living and fossil fish. *J. Exp. Biol.* **209**, 2085 (2006).
38. S. Habelitz, S.J. Marshall, G.W. Marshall, and M. Balooch: Mechanical properties of human dental enamel on the nanometre scale. *Arch. Oral Biol.* **46**, 173 (2001).
39. I.R. Spears, R. van Noort, R.H. Crompton, G.E. Cardew, and I.C. Howard: The effects of enamel anisotropy on the distribution of stress in a tooth. *J. Dent. Res.* **72**, 1526 (1993).
40. R. Hassan, A.A. Caputo, and R.F. Bunshaw: Fracture toughness of human enamel. *J. Dent. Res.* **60**, 820 (1981).