### Ortiz Research Group Fall 2008 MRS Abstracts

<table>
<thead>
<tr>
<th>Authors</th>
<th>Title</th>
<th>Session (Type)</th>
<th>Date / Time</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsu-Yi Lee, Paul W. Kopesky, Laura Daher, Ana Mosquera Pelegrina, David Frisbie, John Kisiday, Alan J. Grodzinsky, Christine Ortiz</td>
<td>(1) Morphology of Aggrecan Produced By Equine Mesenchymal Stem Cells and Chondrocytes in Self-Assembling Peptide Hydrogels</td>
<td>DD2.2: Materials in Tissue Engineering (Oral)</td>
<td>12/01 01:45 PM to 02:00 PM</td>
<td>Hampton A/B (Sheraton)</td>
</tr>
<tr>
<td>BoBae Lee, Paul W. Kopesky, Eric J. Vanderploeg, Bodo Kurz, Christine Ortiz, Alan J Grodzinsky</td>
<td>(2) Mechanical behavior of individual bovine marrow-derived stem cells undergoing chondrogenesis</td>
<td>HH6.23/DD3.23; DD3.23/HH6.23; (Poster)</td>
<td>12/01 08:00 PM to 08:00 PM</td>
<td>Exhibition Hall D (Hynes)</td>
</tr>
<tr>
<td>Davide Carnelli, Pasquale Vena, Roberto Contro, Christine Ortiz</td>
<td>(3) Mechanical anisotropy of individual osteons in bone tissue at high spatial resolutions</td>
<td>Z1.9: Mechanics of Biological and Biomedical Materials (Oral)</td>
<td>12/02 11:00 am to 11:15 am</td>
<td>Back Bay B (Sheraton)</td>
</tr>
<tr>
<td>Haimin Yao, Ming Dao, Kuangshin Tai, Timothy Imholt, Subra Suresh, Christine Ortiz</td>
<td>(4) Size-dependent heterogeneity in plasticity promotes energy dissipation in bone</td>
<td>Z1.10: Mechanics of Biological and Biomedical Materials (Oral)</td>
<td>12/02 11:15am-11:30am</td>
<td>Back Bay B Sheraton</td>
</tr>
<tr>
<td>Christine Ortiz, Subra Suresh</td>
<td>(5) Multilayered and Graded Biological Materials</td>
<td>Z. Mechanics of Biological and Biomedical Materials (Invited Oral)</td>
<td>12/02 1:30pm-2pm</td>
<td>Back Bay B (Sheraton)</td>
</tr>
<tr>
<td>Ju Ha Song, Mary C. Boyce, and Christine Ortiz</td>
<td>(6) Parametric Modeling of the Mechanical Behavior of Multilayered Biological Exoskeletons</td>
<td>Z2.4: Mechanics of Biological and Biomedical Materials (Oral)</td>
<td>12/02 2:30pm-2:45pm</td>
<td>Back Bay B (Sheraton)</td>
</tr>
<tr>
<td>Hsu-Yi Lee, Peter J. Roughley, Alan J. Grodzinsky, Christine Ortiz</td>
<td>(7) Variations in Single Molecule Aggrecan Molecular Structure and Conformation after Removal of Selected GAG Constituents</td>
<td>Z5.7: Mechanics of Biological and Biomedical Materials (Oral)</td>
<td>12/04 10:45am-11am</td>
<td>Back Bay B (Sheraton)</td>
</tr>
<tr>
<td>Lifeng Wang, Juha Song, Christine Ortiz, Mary C. Boyce</td>
<td>(8) Anisotropic Design of Multilayered Exoskeletons in Biology</td>
<td>Z7.20: Mechanics of Biological and Biomedical Materials (Poster)</td>
<td>12/04 08:00 PM to 08:00 PM</td>
<td>Exhibition Hall D (Hynes)</td>
</tr>
<tr>
<td>Haimin Yao, Ming Dao, Timothy Imholt, Subra Suresh, Christine Ortiz</td>
<td>(9) Functionally-graded Sandwich Design of the Armor Protecting a Hot-vent Gastropod</td>
<td>EE11.10 Nano- and Microscale Materials--Mechanical Properties and Behavior under Extreme Environments (Oral)</td>
<td>12/05 11:45 AM to 12:00 PM</td>
<td>Liberty (Sheraton)</td>
</tr>
</tbody>
</table>
Abstract

TITLE: Functionally-graded sandwich design of the armor protecting a hot-vent gastropod

AUTHORS (FIRST NAME, LAST NAME): Haimin Yao, Ming Dao, Timothy Imholt, Subra Suresh, Christine Ortiz

INSTITUTIONS (ALL): 1. Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA. 2. Raytheon, Inc, Marlboro, MA, USA.

ABSTRACT BODY: Recently, a gastropod mollusk was discovered near the Kairei deep-sea hydrothermal vent field of the Central Indian Ridge, where it experiences a harsh environment characterized by extreme pressure (~250 atm), lack of light, high temperatures (~350-400°C) and temperature gradients (~350°C/cm). In addition, since this snail lives a relatively sedentary life, it can suffer attacks from predators such as Phymorhynchus snails and Brachyuran crabs. In this study, we have discovered that the microstructure of the shell of this hot-vent gastropod mollusk is an exceedingly unique multilayered, functionally graded "sandwich" structure possessing a compliant organic layer embedded in between two stiffer mineralized layers that is beneficial for both mechanical and thermal protection. Back-scattered electron microscopy, Energy Dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD) analyses indicate that the armor possesses an outer nodular mineralized layer (~20 μm thick) embedded with iron sulfide (greigite, Fe₃S₄) particulates, followed by a graded composite transition region (~10 μm thick) to an inner, relatively thick (~150 μm thick) organic layer, followed by another graded interphase region (~50 μm thick) to the inner thicker (~200 μm thick) non-nacreous, highly calcified, aragonite-based layer. Instrumented indentation across the cross-section of the shell yielded indentation moduli and hardness values as follows; the outer nodular iron-based layer, the middle organic layer, and the inner calcified layer have moduli 28.84±6.89 GPa, 8.04±0.38 GPa, and 98.89±6.63 GPa respectively and hardness values of 5.4±0.6 GPa, 0.46±0.03 GPa and 1.73±0.57 GPa, respectively. The properties of the gradient layers are intermediate to those of the neighboring homogeneous layers. Based on these experimental data, the multilayered microindentation finite element computational model of the entire shell "sandwich-like" structure was created and compared to various monolayered structures. This multilayered model was predicted to have an excellent combination of high stiffness and high energy absorptivity, suggesting superior resistance to both static and kinetic mechanical attacks. Moreover, a comparative simulation study on the thermal response to a transient, external impact of high temperature (100 °C) was carried out. Among different structures for comparison, the multilayered shell structure again stands out in terms of mitigating the impact of high temperature. The maximum internal temperature experienced by this structure is only about 64 °C, while for other control structures it reaches up to 91.7 °C. The fundamental concepts discovered here may be able to provide guidelines for the design of human synthetic engineered armors.
It was recently discovered through analysis of nanoindentation data and computational modeling that nanoscale heterogeneity in the spatial distribution of the elastic and plastic mechanical properties of cortical bone predicted increased energy dissipation compared to homogeneous controls. Here, we investigate this interesting phenomenon further by isolating the contributions of heterogeneities in elastic moduli versus yield stress to energy dissipation. Two different elastic-perfectly plastic finite element analysis (FEA) models were formulated including a 2D four-point notched beam bent by displacement-controlled loads. In this model, heterogeneity maps were assigned to a 2μm×2μm area in the vicinity of the notch and outside of this region, the material was assumed to be homogeneous. The second FEA model involved a rigid indenter (included angle of 90°, tip radius of 141nm) penetrating a 10μm×10μm sample. Likewise, heterogeneity maps were assigned to a 2μm×2μm area directly beneath the indenter and outside of this region, material was assumed to be homogeneous. The heterogeneity maps of modulus were assigned values directly measured by nanoindentation and the heterogeneity maps of yield stress were calculated from experimentally-measured hardness, by assuming that the yield stress is proportional to the hardness. Next, the heterogeneity in elasticity (modulus) or plasticity (yield stress) or both was eliminated, resulting in additional map sets with different combinations of the heterogeneity in elasticity and plasticity. The results show that, for all cases, heterogeneities of plasticity cause up to 48% promotion of energy dissipation, whereas the spatial inhomogeneity of elasticity does not lead to considerable variation in energy dissipation. Hence, heterogeneity in plasticity, rather than elasticity, plays a dominant role in promoting energy dissipation. The experimentally-measured heterogeneity maps used in these simulations have a spatial resolution of 100nm. However, heterogeneity was found to be dependent on length scale in that the larger the probe size, the lower the degree of heterogeneity. Hence, the impact of heterogeneity on energy dissipation will decrease as the length scale is increased. These findings motivated further studies on the response of energy dissipation to the variations of standard deviation and mean value of the yield stress, which are two important quantities characterizing the plasticity heterogeneity. On one hand, it was found that the energy dissipation increases monotonically as the standard deviation of the yield stress is increased. On the other hand, the response of dissipation to the variation of the mean yield stress was found to be dependent on the stress status experienced by the material. Our simulations show that the plasticity heterogeneity in bone might be the optimization result for achieving higher energy dissipation and, therefore, higher mechanical resistance in typical loading circumstances.
Mechanical behavior of individual bovine marrow-derived stem cells undergoing chondrogenesis

BoBae Lee¹, Paul W. Kopesky², Eric J. Vanderploeg³, Bodo Kurz⁴, Christine Ortiz¹
Alan J Grodzinsky²,³,⁴,⁵

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Stem cell-based tissue engineering holds great potential for the regeneration and/or replacement of damaged cartilage. Mechanical studies of individual mesenchymal stem cells (MSCs) cultured in vitro can provide valuable insights into changes in intracellular morphology that accompany differentiation to the chondrocyte phenotype, as well as the synthesis of the cartilage-like neo-tissue during chondrogenesis. Here, we probe the temporal evolution of the single cell mechanical properties of bovine MSCs cultured within 3-D alginate scaffolds and stimulated to undergo chondrogenesis using dexamethasone and TGF-β1 for up to 10 days. Quasistatic indentation and force relaxation was carried out on each cell placed in a microfabricated silicon well using a spherical colloidal probe tip (end radius~ 2.5 μm) in an atomic force microscope in DMEM culture medium. Elastic moduli were calculated using a Hertzian contact mechanical model and time-dependent mechanical properties were estimated using a viscoelastic 5-element Maxwell-Weichert model. Dimethyl methylene blue (DMMB) dye binding and hydroxyproline assays were performed to quantify glycosaminoglycan and total collagen accumulation within the beads over time, respectively. The elastic modulus of bovine MSCs was found to be 575±56 Pa on Day 0 and stayed relatively constant up to day 10, and was lower than that of bovine primary chondrocytes (1000±112 Pa). Time-dependent mechanical properties of MSCs depended significantly on culture duration (ANOVA, p<0.05). At Day 3, MSCs exhibited instantaneous and quasi-equilibrium moduli which were significantly different from MSCs at Day 0 and 10. MSCs at Day 10 showed shorter τ₁ (initial relaxation time constant) and longer τ₂ (final relaxation time constant) compared to MSCs at Day 0 and 3. Notably, the final relaxation time constant for MSCs undergoing chondrogenesis was distinctly longer than that of primary chondrocytes even by 10 days, suggesting unique differences in intracellular organization and/or PCM that have not reached the final chondrocyte-like state. Variations in elastic modulus of MSCs during chondrogenesis were not detectable up to Day 10, while biochemical assays indicated that MSCs in alginate scaffolds synthesized and accumulated GAG and collagen in amounts comparable to MSCs in agarose and self-assembling peptide scaffolds as well as primary chondrocytes in alginate. This may be due to the fact that newly developing PCM of primary chondrocytes exhibits lower stiffness at initial culture duration. Further studies are being carried out on long-term culture of MSCs in alginate scaffolds up to 1 month to include mechanical testing using dynamic oscillatory compression.
Abstract

TITLE: Variations in Single Molecule Aggrecan Molecular Structure and Conformation after Removal of Selected GAG Constituents

AUTHORS (FIRST NAME, LAST NAME): Hsu-Yi Lee¹, Peter J. Roughley¹, Alan J. Grodzinsky³,⁴, Christine Ortiz²


ABSTRACT BODY: The composition and spatial distribution of glycosaminoglycans (GAGs), for example keratan sulfate (KS) and chondroitin sulfate (CS), within the extracellular matrix proteoglycan aggrecan is thought to be an important determinant of the biomechanical function of cartilage tissue. Currently, the density and detailed location and arrangement of KS-GAGs along the aggrecan core protein for various populations, as well as the relationship to aggrecan biophysical properties is largely unknown. Knowledge of such molecular structure-property relationships, in particular at the single macromolecular level, will provide an improved understanding of aggrecan function. This study utilizes high resolution atomic force microscopy (AFM) single molecule imaging to quantify the fine structure and conformation of aggrecan before and after removal of KS or CS GAGs via enzymatic degradation. Aggrecan was extracted and purified from 29-year-old human articular cartilage with no evidence of arthritic disease or joint damage. In order to remove CS or KS, aggrecan samples were incubated with protease-free Chondroitinase ABC or Keratanase II, respectively, then deposited on 3-aminopropyltriethoxysilane functionalized mica substrates and imaged via tapping mode AFM (tip radius <10 nm, spring constant ≈ 42 N/m). The CS-digested aggrecan was observed to have a relatively long, GAG-free region of core protein, corresponding to the position of the CS1 and CS2 domains, which occupy ~70% of the total aggrecan contour length. However, shorter GAG chains were still visible, located nearer the globular G1-G2 domains, corresponding to one of the putative locations of KS. The KS-digested aggrecan appeared globally similar to the untreated aggrecan, consistent with the presence of the many CS chains which are larger than KS. The aggrecan core protein profiles of all specimens were automatically traced from the AFM height images using a custom Matlab program. Full length aggrecan from treated and untreated groups were identified by the presence of N-terminal and C-terminal globular domains (G1 and G3, respectively). The average core protein contour length of the full length untreated aggrecan was measured as 474 ± 56 nm (n=19) and the average core protein contour length of full length aggrecan decreased 14% (407 ± 112 nm, n=16) after Keratanase II treatment and 28% (339 ± 71 nm, n=16) after Chondroitinase ABC treatment. The shrinkage of the core protein after Keratanase II and Chondroitinase ABC treatments suggests that the extension of untreated aggrecan results from the presence of the GAG chains and the associated repulsive and steric forces caused by these constituent GAG chains. The effective persistence length of the core protein also changes from 172±117 nm to 136±50 nm and 139±78 nm after the removal of KS and CS GAGs, respectively. The results show that both KS and CS GAGs were responsible for the core protein extension and contribute to molecular stiffness of aggrecan.
Abstract

TITLE: Morphology of Aggrecan Produced By Equine Mesenchymal Stem Cells and Chondrocytes in Self-Assembling Peptide Hydrogels

AUTHORS (FIRST NAME, LAST NAME): Hsu-Yi Lee, Paul W. Kopesky, Laura Daher, Ana Mosquera Pelegrina, David Frisbie, John Kisiday, Alan J. Grodzinsky, Christine Ortiz


ABSTRACT BODY: Mesenchymal stem cells (MSCs) are multipotent with the potential to differentiate into cells of the chondrocyte lineage. For this reason, they are a candidate cell source for cartilage regeneration therapies. Marrow-derived stem cells undergoing chondrogenesis in vitro can synthesize aggrecan similar in structure to that synthesized by chondrocytes under the same conditions. To more accurately identify which cell sources are superior for cartilage regeneration, detailed knowledge of the molecular structure and mechanical properties of synthesized extracellular matrix molecules, such as aggrecan, is necessary. In this study, high resolution tapping mode atomic force microscopy (AFM) imaging was used to visualize the structure of individual aggrecan molecules produced by four equine cell types: adult MSCs, adult chondrocytes, foal MSCs, and foal chondrocytes seeded into self-assembling peptide hydrogel scaffolds cultured in chondrogenic media. Animal-matched chondrocytes and bone marrow-derived MSCs were harvested from foal (2-4 month old) and adult (2-4 yr old) horses. Isolated cells were seeded in (KLDL)3 self-assembling peptide hydrogel and cultured in TGF-1 and IGF-1 supplemented medium for 21 or 42 days. Aggrecan was isolated from the constructs and used in AFM imaging. Aggrecan was deposited on 3-aminopropyltriethoxysilane functionalized mica substrates. Tapping mode AFM imaging proceeded in ambient conditions with Si cantilevers (k=45 N/m, tip radius < 10 nm.) Aggrecan core protein contour length, Lc, and CS-GAG chain length, Lcs-GAG, were measured. Effective persistence length, Lp, was calculated using the worm-like-chain model. The Lc for foal MSC, adult MSC, foal chondrocyte and adult chondrocyte were 503±149 nm, 487±165 nm, 437±137 nm, and 412±166 nm (mean±SD; n=110-231), respectively. The Lcs-GAG for foal MSC, adult MSC, foal chondrocyte and adult chondrocyte were 63±11 nm, 73±24 nm, 40±8 nm, and 46±18 nm (n=28-35) respectively. MSC-produced aggrecan had significantly larger Lc and Lcs-GAG than chondrocyte-produced aggrecan (p<0.05). No effect of animal age (in the 2-month to 4-year range tested) on aggrecan monomer properties was found (two-way ANOVA, p>0.05 for cell type). All cell types were also found to have similar effective persistence length Lp (239-254 nm, p>0.05). Regardless of the animal age, MSC-produced aggrecan had not only similar morphology, but also larger molecular size than chondrocyte produced aggrecan. In contrast, recent reports showed that aggrecan extracted directly from native cartilage was larger for young animals. The similar structural dimension for aggrecan produced by MSCs and chondrocytes seeded in peptide hydrogel scaffolds, from both young and adult horses, suggests the potential use of MSCs as a cell source for mechanically-functional replacement tissue. Ongoing research aims to compare the nanomechanical properties of adult MSC produced aggrecan with that of aggrecan extracted from native cartilage.
Mechanical anisotropy of individual osteons in bone tissue at high spatial resolutions

Davide Carnelli¹, Pasquale Vena¹, Roberto Contro¹ and Christine Ortiz²

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² Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA

The structural and mechanical anisotropy of bone is critical to its macroscopic biomechanical function. Secondary osteons, the fundamental micrometer-sized building blocks of cortical bone, are multilayered cylindrical composite structures of mineralized collagen fibrils arranged circumferentially in thick and thin lamellae. The anisotropy of individual osteons is hypothesized to provide a sufficient mechanical response to physiological (largely compressive, elastic) and accidental (multiaxial plastic, fracture) loading. In this research, instrumented nanoindentation was employed on adult compact bovine femoral bone to quantify the elastic and inelastic mechanical anisotropy in the longitudinal (parallel to the long bone axis) and transverse directions (both circumferential and radial) within a single osteon. The dual indentation technique, that is the use of indenters with different sizes and geometries on the same microstructural feature (e.g. an osteon), has been extended to the study of anisotropic materials with the objective of enhancing the analysis of the indentation results to extract more precise information from the experiments. Thus, pyramidal (Berkovich and Cube Corner), conical and spherical indenters (with different apex angle and end radius sizes, respectively), have been employed to evaluate the material response when subjected to loading under differing conditions. Moreover, the residual indent topography provided by atomic force microscopy imaging has also been used to provide meaningful experimental data, additional to those deduced from the force-depth indentation curves. Since residual displacements reflect constitutive anisotropy, the use of axial-symmetric indenters results in a mapped imprint that does not exhibit axial symmetry because of specimen anisotropy. Thus, the topography of the residual indents is a source of important quantitative information on the material anisotropic properties. The coupling of these tools allows for fundamental knowledge regarding the relationship existing between osteonal bone microstructure and anisotropic mechanical properties.
Parametric Modeling of the Mechanical Behavior of Multilayered Biological Exoskeletons
Ju Ha Song1, Mary C. Boyce2, and Christine Ortiz1, 1Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; 2Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts

Natural exoskeletons are known to exhibit a huge diversity of structure and properties as they have adapted to environmental and predatory threats; typically balancing protection and mobility requirements to maximize survivability. Most exoskeletal materials are composed of different layers of materials where each layer possesses its own unique composite nanostructure, mechanical properties, and deformation mechanisms. The multilayered design of exoskeletons (i.e. number, sequence, thickness, geometry, and constitutive material of layers) and its relationship to the corresponding threats are largely unknown and are of great interest for the development of bioinspired human body and vehicle armor. Here, we focus on one model system, the quad-layered mineralized scales of the fish Polypterus senegalus, a living descendant of ancient palaeoniscoids. Computational methods (finite element analysis) were employed to predict the deformation under a penetrating load (simulating a predatory bite) for different multilayered structures. In particular, the following cases were considered: (i) the influence of the thickness of the outer enamel-like ganoine layer; (ii) the quad-layered structure compared to a simpler bilayer structure; and (iii) the sequence of the outer two layers (i.e. ganoine and dentin). To investigate the effect of the thickness of the outer ganoine layer, simulations of the microindentation of a ganoine-dentin bilayered model was carried out. The total thickness of the ganoine-dentin bilayer was fixed at 400 μm and the thickness of ganoine layer was varied from 3 to 54 μm. It was determined that when the ganoine layer was 6 - 12 μm thick (the real layer thickness observed experimentally), the tensile radial stress field (S22) exceeds the circumferential stress field (S11), thereby promoting circumferential cracking upon penetration (observed experimentally), which locally confines the deformation at the indentation site and is highly advantageous. For too thin or too thick of a ganoine layer, S11 is large which promotes radial cracking and is undesirable as this can lead to catastrophic failure of the layer. In the second set of simulations, a ganoine-dentin bilayered model was compared to the quadlayered model representing the real scale microstructure. The effective indentation modulus, microhardness, and energy dissipation for both models were extremely similar, with the four layer model achieving a weight reduction up to ~20% of the bilayered system. Lastly, we have developed a "reverse" multilayered model which consists of four material layers, but where the order of two outer layers (ganoine and dentin) are reversed so the more compliant and softer dentin layer is located at the surface followed by the harder and stiffer ganoine layer underneath. As opposed to the actual multilayered design sequence which promotes advantageous circumferential cracking on the surface, the reversed layers magnified tensile normal and shear stresses around the junction thereby producing susceptibility to interfacial failure through delamination, which is highly undesirable during a penetrating attack.
Anisotropic Design of Multilayered Exoskeletons in Biology

Lifeng Wang1, Juha Song2, Christine Ortiz2, Mary C. Boyce1

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Various organisms in biology have evolved outstanding exterior materials and structures to protect them from predatory and environmental threats, for example, mollusc shells, mineralized fish scales, and insect integument. Most exoskeletal materials exhibit a multilayered design where each layer possesses its own unique organic-inorganic nanostructure that enables it to undergo unique deformation mechanisms that contribute to its overall protective mechanical function. The goal of this study is firstly, to use microstructurally-based computational modeling to predict how the nanoscale morphology of individual layers results in mechanical anisotropy of the layer and secondly, to understand how mechanical anisotropy of individual layers contributes to the overall biomechanical properties of the multilayered exoskeleton, in particular penetration resistance. The model system chosen for this study was the outer ganoine layer of the mineralized scales of the armored fish Polypterus senegalus, which is known to undergo anisotropic fracture mechanism. Ganoine possesses a prismatic nanostructure composed of hydroxyapatite nanocrystals with 50-100 nm diameter surrounded by an organic matrix 3-5 nm thick. A micromechanical finite element analysis (FEA) model of prismatic microstructure of ganoine was created. The 3D representative volume element (RVE) considered periodic staggered arrangement of mineral nanocrystals bonded by thin adhesive layers. Several different multi-axial loading conditions were applied on the periodic RVE to determine the anisotropic elastic constants and the anisotropic yield surface and post-yield behavior and corresponding underlying deformation mechanisms. Then, a full 3D FEA nanoindentation model was carried out which compared the load-depth behavior of the anisotropic material to a corresponding isotropic material (where the effective isotropic indentation modulus and yield strength were determined from nanoindentation data as 55GPa/2.0GPa). The results showed less than 10% difference in load-depth behaviors between isotropic and anisotropic model, but anisotropy led to a more focused, localized, and deeper depth of plasticity beneath the indenter compared to the isotropic material. In addition, a 3D nanoindentation model was created which modeled the discrete prismatic nature of the ganoine layers by locally implanting nanostructured crystals surrounded by thin organic layers within the volume under the indenter. Results from this discrete model demonstrated that the effective continuum-level anisotropic model was able to rather remarkably capture the essence of the behavior of the discrete prismatic structure material. Finally, in order to explore the role of anisotropic ganoine layer in the multilayered structure, the quad-layered FEA model on the larger length scale biomechanical microindentation of the entire scale was conducted incorporating anisotropic ganoine layer. Anisotropy of ganoine layer was found to have a significant advantageous effect on increasing energy dissipation, resisting radial crack propagation, and suppressing interfacial failure in multilayered structure.
Multilayered and functionally graded materials are ubiquitous throughout biology. In addition to their biological function, their geometries and properties are designed to achieve important thermal and mechanical performance characteristics in a variety of protective and defensive exoskeletal structures. The diversity of structures and properties found in such systems is enormous presumably due to variability in the surrounding environment and predators. Here, we will discuss and compare a number of model systems in relation to their known “threats” including; 1) mineralized fish scales, 2) abalone shell, and 3) a gastropod mollusk shell from a deep-sea hydrothermal vent, and 4) teeth. The topics to be covered include; the spatial variation in mechanical properties through the cross-section from the outer to inner surfaces, the thickness and sequence of the layers, the interfacial geometry, confinement effects between the layers, structure and property gradation within and between layers, and anisotropy of the layers. Such studies provide valuable insights into rationalizing why natural materials have specific layered and graded architecture. They also help us to engineer synthetic materials with controlled gradients in composition, microstructure and properties to achieve enhancements in mechanical performance characteristics.