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A representative volume element based micromechanical analysis of a Bi-layered Ganoid Fish scale

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ABSTRACT

The Mississippi Alligator gar (*Atractosteus spatula*) possesses a flexible exoskeleton armor consisting of overlapping ganoid scales used for predatory protection. Each scale is a two-phase biomineralized composite containing bio-modified hydroxyapatite (hard) minerals and collagen (soft) fibers. The protective layer consists of a stiff outer ganoine layer, a characteristic “sawtooth” pattern at the interface with the compliant bone inner layer. The garfish scale exhibits a decreasing elastic modulus from the external to the internal layers. Scanning electron microscopy (SEM) images of the cross-section revealed a two-layered structure. Elastic moduli, measured from nanoindentation experiments, were correlated to structural changes across each layer. The “material” symmetry of this materially and geometrically nonlinear biomineralized composite is unknown. Therefore, to be able to determine the stiffness tensor requires the use of finite element analysis (FEA). The gar fish scale was computationally modeled using the representative volume element (RVE) based approach. As a result, the unknown symmetry induced by the architecture and material layering require the use of complex FEA boundary conditions. The simulation was conducted in the pure uniaxial strain regimes of tension and shear, which necessitated the mathematical determination so appropriate surface loading conditions could be applied. This paper provides the results from a highly-resolved mesoscale RVE model based on iso-strain boundary conditions (ISBC) to determine the elastic stiffness tensor for the composite system. By assuming isotropic behavior in individual elements, the results for the RVE reveal the fish scale has an “*orthotropic symmetry*” with slight local strain variations occurring at the sawtooth interface.

1. Introduction

1.1. *Biological materials for bio-inspired design*

During the last few decades, research into biological materials such as abalone shell, fish armor, turtle shell, and human bone revealed these biological composites (biocomposites) possess a carefully arranged multilayered structure (Yang et al., 2014) with unique subscale feature achieving mechanical properties which can be superior many man-made materials (Hodo, 2015). The investigation of such optimized systems provides novel insight into the future of advanced engineering materials. Over the last two decades, both design engineers and researchers have turned to nature with the hopes of understanding how biocomposites are efficiently created to have the often mutually exclusive properties of high-strength, high-toughness while maintaining a lightweight configuration (Allison et al., 2013; Song, 2011; Song et al., 2011; Yang et al., 2012; Chandler et al., 2014; Nelms, 2014).

Scientists discovered biocomposites that exhibit such desirable properties when operating under extreme conditions tend to be mineralized tissues (Chen et al., 2012; Meyers et al., 2006; Yang et al., 2012, 2013a, 2013b, 2013c, 2014). Typically these biocomposite consists of primarily two phases: a hard (inorganic minerals) matrix phase integrated with a soft polymer-like reinforcement phase (organic fiber) (Browning et al., 2013; Bruet et al., 2008; Han et al., 2011; Ikoma et al., 2003a, 2003b; Ji and Gao, 2010; Schönbörner et al., 1979; Vernerey and Barthelat 2010; Yang et al., 2014; Youn and Shin, 2009; Zhu et al., 2012), such as the superior performing examples of bone, teeth, shells and fish scales (Hodo, 2015). Designers have recently shown interest in studying fish scales for potential use in a variety of engineering applications. Fish scales were particularly attractive as an in-depth case study because of relative simplicity and involve few discrete components, yet exhibit a superior mechanical response. Since experimental measurements to determine the mesoscopic response of Mississippi alligator gar's ganoid fish scale are complex, a numerical modeling approach will be appropriate to obtain elastic properties in the present study. Therefore, the experimentally characterized biocom-

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posite was modeled through an iso-strain boundary condition (ISBC) based finite element analysis for determining the effective stiffness tensor.

2. Background

2.1. Characteristics of Mississippi Alligator gar (Atractosteus spatula)

The Mississippi alligator gar *(Atractosteus spatula)* has ganoid type scales that are a multilayered biocomposite with graded elastic modulus and hardness properties. The outermost layer having high strength that progressively decreases towards the innermost surface and showing an inverse relationship with toughness. The gar fish scales have a characteristic bi-layers construction at the mesoscale, an outer ganoine layer and inner lamellar bone layer (Allison et al., 2013; Chen et al., 2012, Hodo, 2015; Yang et al., 2013a, 2013b, 2013c). In this protective material system, the primary attribute is to understand how evolution has chemically and geometrically optimized the scales using a two component mixture, which is integrated from the nano-to-millimetric scale, to form its complex hierarchal structure. At this length scale, the bioapatite crystals are mixed with collagen fibers; varying by volume fractions, spatial proximity, and directional orientation to produce a lightweight composite exhibiting high strength and toughness (Hodo, 2015).

As a result of the hierarchal ordering, the gar fish scale exhibits strength and toughness comparable to those of most metal alloys. Uniquely, the bulk mechanical properties are several orders of magnitude higher than those found for the discrete ganoine and bone layers. The engineering properties of the individual layers have shown to be analogous to dental enamel (hard) for the ganoine and cancellous bone for the inner layer (soft). The hard enamel is lightweight, high-strength, but brittle with low fracture toughness. Whereas the sponge-like cancellous bone is also lightweight, but it is soft, low-strength and has high fracture toughness. However, when the two materials are combined, they make a lightweight composite that has high-strength with good fracture toughness.

A particular feature of interest is the distinct geometric interaction at the interface between stiff ganoine and compliant bone in gar fish scale. The interlocked interface has a distinct periodic sawtooth structure, or rasp, with the ganoine layer intruding into the interfacial region (Allison et al., 2013). In addition, the discrete layers have a modulus mismatch which researchers believe has a role in dissipating stress/energy across the interface (Ji and Gao, 2010). Until recently, research has overlooked the role of the sawtooth in efficiently transferring load across the ganoine-bone interface without debonding/delaminating (Chandler et al. 2013; Nelms, 2014).

Borrowing from the engineered composites community, the experimental characterization was used to determine an idealized volume for computational analysis (Shan and Gokhale, 2002). In order to analyze the effects of various material and structural features on elastic response, the first step is to determine the material symmetry of the fish scale through the use of high-resolution mesoscale finite element modeling (FEM) with the representative volume element (RVE) based approach. The averaging field theorems have shown to provide an adequate prediction of the effective mechanical properties for functionally graded composite plates (Cheng and Batra, 2000; Reiter et al., 1997) similar to that of gar fish scale.

The main focus of this paper is to (1) determine the material symmetry for the fish scale by applying appropriate boundary conditions to a 740 µm cubic RVE with 7.4 µm resolution, and (2) understand the effect of geometric interlocking at the ganoine-bone interface with respect to the local strain state under global iso-strain conditions. Using optical microscopy techniques, Wilson (1849) sketched the architecture of the two layer-lamina of the Needlenose gar (*Lepisosteus osseus*) and determined that continuous ridges of ganoine intrude into the bone layer, characteristically described as a sawtooth-shaped rasp. Similarly, with the advent of advanced 3-D µCT capabilities, Hodo (2015) obtained images of continuous sawtooth-like features in the Mississippi Alligator gar fish scales. The geometry of fish scale necessitates a three-dimensional (3-D) finite element analysis (FEA) to model the interface geometry and determine if the interlocking may contribute to elastic anisotropy in the gar fish scales. The local strain variations at interfaces, especially around the sawtooth features can be captured using the 3-D analysis.

In the FEA, iso-strain boundary conditions (ISBC), in terms of surface displacements, were applied to the RVE to generate pure uniaxial stress and pure shear conditions (Kelkar et al., 1992). The concept of ISBC and application are discussed in detail in Section 3. Because the fish scale exhibits unknown material symmetry, kinematic and periodic boundary conditions alone will not lead to pure uniaxial stress states. By applying proper surface loading, it is then possible to accurately determine the material symmetry of the fish scale. Knowledge of symmetry will help both scientist and engineers to better understand the mechanisms nature uses to strengthen biocomposites. Such knowledge can guide future researchers to better understand the engineering behavior of flexible, yet protective fish scale and other natural occurring biomineralized tissues.

To understand the fish scale's protective attributes, this study combined microscopy, mechanical experiments, and high fidelity computational simulations to discern what are the advantageous design principles regarding the structure and mechanical properties that appear in the protective gar fish scale. The following sections provide a) details of the preparation of fish scales for further characterization, Section 3, b) the theoretical background and application of iso-strain boundary conditions, Section 4, c) results from high-resolution scanning electron microscopy (SEM) imaging for interface geometry and assembly, Section 5.1, d) nanoindentation mechanical measurements for determining the variation of young's modulus along the layers, Section 5.2, e) details of the computational model generation, Section 5.3, and f) the RVE determined stiffness tensor for the fish scale as well as the resulting internal strain field, Section 6.

3. Materials and laboratory characterization methods

The Alligator gar fish specimens were live caught from a swamp adjacent to the Mississippi River located near Vicksburg, MS, by the biologist team at Environmental Laboratory of the US Army Engineer Research and Development Center (ERDC). The fish was euthanized, and scales were removed according to the ERDC animal care guidelines (#EL-6009-2011-5), which encompass the *NIH Guide for Care and Use of Laboratory Animals*, Fig. 1.

3.1. Scanning electron microscopy

High fidelity imaging was performed at the *U.S. Army ERDC Geotechnical and Structures Laboratory (Vicksburg, MS),* using Scanning Electron Microscopy (SEM). An FEI Nova NanoSEM 630 SEM with a low Voltage high Contrast Detector (VCD) was used to determine the interfacial geometric structure between the ganoine and bone layers.

3.2. Nanoindentation

Nanoindentation was performed to determine the elastic moduli's spatial variations by expanding on previous research from a single line scan to a uniform, high-resolution grid thus providing improved analysis on the variance present in highly heterogeneous gar fish scale. The experiments were performed with a iNano^{TM} nanoindenter provided by Nanomechanics, Inc. using a “Dynamic Indentation Constant Strain Rate” testing protocol. The experimental setup parameters are defined in Table 1. As seen in Fig. 3a, a Berkovich grid