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Micro-structure and mechanical properties of the turtle carapace as a biological composite shield

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A B S T R A C T

Turtle shell is a multi-scale bio-composite in which the components are arranged in various spatial patterns, leading to an unusually strong and durable structure. The keratin-coated dorsal shell, termed the carapace, exhibits a flat bone, sandwich-like structure made up of two exterior cortices enclosing a cancellous interior. This unique structure was developed by nature to protect the reptile from predator attacks by sustaining impact loads and dissipating energy. In the present study we attempt to correlate the micro-scale architecture with the mechanical properties of the carapace sub-regions of the red-eared slider turtle. The microscopic structural features were examined by scanning electron microscopy and micro-computed tomography. Nanoindentation tests were performed under dry and wet conditions on orthogonal anatomical planes to evaluate the elastic modulus and hardness of the various carapace sub-regions. The mineral content was also measured in the different regions of the carapace. Consequently, we discuss the influence of hydration on the carapace sub-regions and the contribution of each sub-region to the overall mechanical resistance of the assemblage.

1. Introduction

A comprehensive understanding of bio-composite structural motifs and the resulting mechanical properties is of great interest for the fabrication of novel bio-inspired engineered materials (e.g. ceramics [1], artificial nacre [2] and wood-like, fiber-reinforced polymers [3]). The formation of complex hierarchical structures, driven by nature's sophisticated integration of diverse constituents, results in outstanding mechanical properties that are often superior to their individual (or simply mixed) counterparts [4]. Therefore, quantifying and correlating the structure–mechanical property relations of these biomaterials may improve material design for particular applications. For instance, understanding the strategies by which biological composite shields are built and function may shed light on the basic principles of impact-resistant structures (e.g. fish armor [5], the crab exoskeleton [6], and the human skull [7]). In this manner, correlating the structural features and the mechanical properties of turtle shell may improve our ability to create synthetic counterparts.

Turtles belong to the Order Testudines of the Class Reptilia and are thought to have existed since the Triassic era (~200 million years ago) [8]. They possess an exoskeleton that is attached to the body and protects it from trauma caused by predator assaults, smashing against rocks, and falling. The shell is a bony organ covered by keratinous epidermal scales termed scutes.

The shell bone resembles boney tissues, which exhibit a hierarchical structure starting from organic (mostly, ~90%, collagen helices) and inorganic (hydroxyapatite nanocrystals and minor quantities of water) constituents [9]. These constituents form the bone basic building blocks, mineralized collagen fibrils. In the middle levels of the hierarchy the fibrils are assembled into fibers and fiber bundles with a typical thickness of a few micrometers. These are then organized into fibrillar structures, such as parallel fiber and woven arrays and, the most abundant, a plywood-like structure, which is found in concentric lamellae that form the osteonal unit [9]. At the macroscopic level cortical (compact) and trabecular (cancellous) bone is arranged together to form whole bone of various types adapted to different mechanical purposes (e.g. structural support, vibrational conductance [10] and impact events [11]).

The bony part of the turtle shell is coated with keratinous epidermal scutes. The relatively rigid and hard outer surface (i.e. the stratum corneum) is composed of dead cornified cells surrounded by β-pleated sheet keratin [12]. The layers underneath the stratum corneum consist of keratinocytes embedded in an amorphous keratin matrix, along with melanocytes, pigments and lipids. The epidermal scutes are attached to the bone through the dermis. The latter comprises collagenous fibers anchoring the underlying bone and the covering epidermal layers. The keratin scutes act as a waterproof barrier and also contribute to the mechanical protection, being the first line of defense enduring loading [13].

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Thus the turtle shell is a complex hierarchical composite shield evolved to protect the soft tissues against sharp predator attacks (e.g. biting and clawing) and from blunt shock due to falling or smashing against boulders [14]. The shell is composed of a dorsal part, the carapace, and a ventral one, the plastron. These are bridged by bone tissue located between the front and hind limbs. The carapace consists of endochondral (cartilage-mediated) vertebrae from which the ribs emanate laterally and are engulfed (fused) in dermal bone [8]. This leads to flat bone (sandwich-like) elements, displaying two exterior (dorsal and ventral) surfaces that enclose a cancellous interior (Fig. 1). The flat bone configuration allows a reduction of weight and thus higher stiffness, strength and toughness to weight ratios to withstand bending, compression and high strain impact loads [15,16]. It also makes the shell more buoyant [13]. In addition, these sandwich elements are attached to one another in a complex zigzag manner at soft unmineralized collagen sutures (Fig. 1). This structural feature enables deformation of the shell under minor loads, for respiration, locomotion and metabolism [17], whereas at higher loads the shell stiffens as neighboring elements become interlocked.

Recently a number of studies have appeared regarding the biomechanical behavior of the turtle carapace [13,17–20]. Balani et al. [13] and Rhee et al. [19] used nanoindentation to assess the dry state elastic modulus and hardness of the carapace rib. However, systematic characterization of the microscopic structural features and (dry and wet) mechanical properties of the carapace bony regions and horny scutes is still lacking. This is crucial in studying the adaptation of each sub-region/tissue to the overall mechanical performance of the carapace to resist (impact) loads. This is also essential in gaining a thorough understanding of the multi-scale mechanical behavior of the carapace. Since turtles are mostly found in bodies of water (lakes, seas, etc.) and their carapace is filled with fluid [8] it is important to estimate their mechanical properties under wet conditions.

In the present study we have attempted to correlate the micro-scale architecture with the corresponding mechanical properties of the carapace sub-regions (i.e. the perisuture, rib cortices and cancellous interior, and the keratin scutes) of the red-eared slider turtle. The microscopic structural features were examined by scanning electron microscopy (SEM) and micro-computed tomography (μCT). Nanoindentation tests were performed on orthogonal anatomical planes (Fig. 2) to evaluate the elastic modulus and hardness of the various carapace sub-regions under dry and wet conditions. The ash content was measured as well to evaluate the mineral fraction in the different regions of the carapace. Finally, we discuss the contribution of each region to the overall mechanical resistance. Additionally, the effect of hydration is also examined.

2. Materials and methods

2.1. Specimen preparation

Several 15–20 cm long frozen carapaces of adult red-eared slider turtles (*Trachemys scripta* subsp. *elegans*) were obtained. The red-eared slider, which is considered an invasive species in Israel, is being eradicated by environmental agencies, and were originally obtained from the Israel Nature and Parks Authority. Several carapaces were thawed and sawed into quarters. Specimens were cut from the center of the carapace with regard to the anterior–posterior (A–P) and the medial–lateral (M–L) axes (Fig. 2). Samples containing suture and rib, ~20 × 5 × ~3 mm (shell thickness) in size, were cut with an inner hole diamond-coated low speed saw (Buehler Isomet) under constant water irrigation. The cubes were then cleaned and sliced to expose the three anatomical orthogonal planes of the shell for indentation and imaging. Final cuts were smoothed with 800, 1200 and 4000 grit SiC papers, followed by

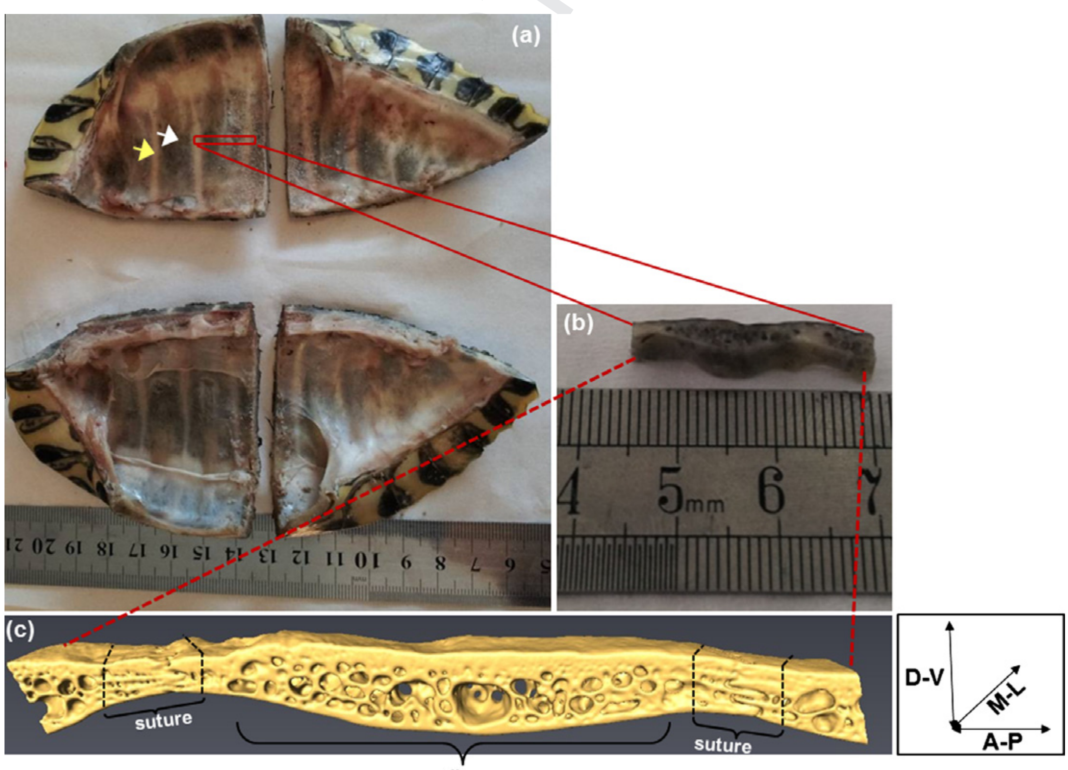

Fig. 1. (a) Ventral view of a dissected carapace. The white and yellow arrows mark an individual rib and suture, respectively. (b) A section of the rib enclosed by sutures at the edges. (c) A tomographic reconstruction of (b). The flat bone sandwich-like configuration of the rib is visible, centered on the (marked) suture regions. The anatomical orientations (A-P, anterior–posterior; M-L, medial–lateral; D-V, dorsal–ventral) are marked.

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