Colloids and Surfaces A: Physicochem. Eng. Aspects 520 (2017) 97–104

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

The hierarchical structure and mechanical performance of a natural nanocomposite material: The turtle shell

Meiling Chen[a], Narisu Hu[a,b], Chang Zhou[a], Xiankun Lin[a], Hui Xie[c,*], Qiang He[a,*]

[a] *State Key Laboratory of Advanced Welding and Joining (HIT), Micro/Nanotechnology Research Centre, Harbin Institute of Technology, 2 Yi Kuang Street, Harbin 150080, PR China*

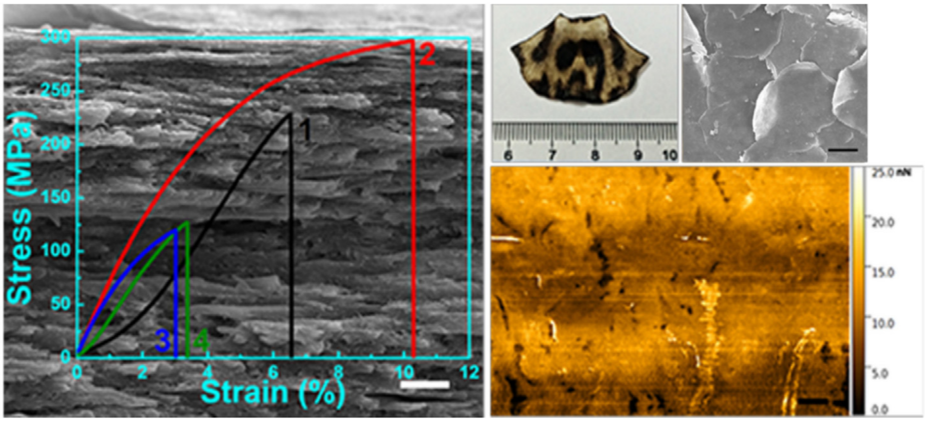
[b] *Oral Implant Center, Second Affiliated Hospital of Harbin Medical University, Harbin 150086, PR China*

[c] *State Key Laboratory of Robotics and Systems (HIT), School of Mechatronics Engineering, Harbin Institute of Technology, 2 Yi Kuang Street, Harbin 150080, PR China*

HIGHLIGHTS

- The design strategies of a natural armor: turtle shells have been investigated.
- Microplatelets stack orderly and compactly, constructing a layered structure.
- The modulus, stiffness, deformation, and adhesion are studied by AFM.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 January 2017
Received in revised form 17 January 2017
Accepted 23 January 2017
Available online 24 January 2017

Keywords:
Turtle shell
Hierarchical structure
Mechanical properties
Keratin
Biomineralization

ABSTRACT

Turtle shells protect themselves from predatorial attack, which could provide ideas and pathways for bioinspired synthetic materials. Despite with relatively weak constituents and low biomineralization, turtle shells possess unusually robust mechanical properties due to their well organized and layered structures that are accurately designed from nanoscale to macroscale in nature. Minerals (calcium phosphate and calcium sulfate polycrystal mixture) randomly disperse in the keratin, and forming organic-inorganic nanocomposite platelets. Such platelets are basic building blocks that stack orderly and compactly in the radial direction, and constructing individual platelets into a layered micro-configuration. The outstanding tensile mechanical performance of turtle shells has much relationship with rehydration and the growth orientation of the keratin cells. Compressive mechanical properties, growth texture of keratin, topography and mineral components' distribution of turtle shell are investigated by AFM experiment effectively. Such excellent mechanical properties of turtle shells, which integrated with nanocomposite ingredients and layered structure, may inspire the biomimetic strategies for advanced multi-functional materials, especially for artificial armor.

1. Introduction

The protective exterior structures of natural organisms give considerable enlightenment to synthesis materials with robust and

---

* Corresponding authors.
E-mail addresses: xiehui@hit.edu.cn (H. Xie), qianghe@hit.edu.cn (Q. He).

http://dx.doi.org/10.1016/j.colsurfa.2017.01.063

multifunctional mechanical properties [1–4]. Natural armor, such as abalone shells [5–8], gastropod shells [9], fishes [10–12], bones [13], feathers of birds [14] and other reptile epidermis (pangolin, alligators, crocodiles, lizards, turtles etc.) [15,16] are thought as biological composite shields, owing to their hierarchical structures and super mechanical performance. Hence, they have been a topic of major technological interest in diversified civilian and defense applications, and inspired high-performance materials, objects and processes that function from nanoscale to macroscale [17–27]. Methods to fabricate these materials include large scale model materials, ice templating, freeze casting, layer-by-layer deposition, thin film deposition, bone-inspired metallic nanocomposite, hierarchical alpha-helix based protein filaments and self-assembly [28–32]. Turtle shell possess considerable function of mechanical protection from predators that can induce damage from the environment. Until now, researchers have done much research on the shells from aspect of the biological evolution, but little study on detailed structural and mechanical function [33–38]. Fratzl et al. have presented mechanical performance of the complex 3-D suture joining the bony elements of the turtle shell [33]. However, investigation about the nanoscale feature, the complex interplay between organic protein and inorganic minerals, and mechanical properties for different orientations of turtle shells is still incomplete and unclear. Hence, going deep into understanding of the layered structure, chemical components and mechanical performance is of great important for the development of next-generation materials.

Here, we elaborated the elements distribution, chemical components, structure of the turtle shells, as well as the mechanical functions such as strength, flexibility, modulus, energy absorption, stiffness, deformation, and adhesion. The dermal armors of turtle are constructed with much beta-keratin polygonal microplatelets, which well-organized and compactly stacked in the radial direction. In nanoscale, minerals were identified as calcium phosphate and calcium sulfate polycrystal mixture, which disperse in the keratin layers randomly, and abundant beta-keratin are connected with bits of alfa-keratin (a kind of soft proteins). Such composite system of turtle epidermis possesses ultra-strong, flexible and anisotropic tensile mechanical properties. Compressive mechanical test was performed to analyze the compressive Young's modulus, stiffness, deformation and adhesion, and also helped to understand the distribution of organic and mineral components in individual "building block" in turtle epidermis. This analysis focus on the relationship between the composite system, orderly layered structure, and mechanical properties, of turtle shells, may provide new insights and design rules for man-made, bioinspired multiscale nanocomposites.

2. Experimental section

2.1. Preparation of samples of turtle shell

The shells were obtained from of a juvenile red-ear turtle with size of ca. 15 cm, was kindly presented as a private gift. The naturally exfoliated turtle epidermis were cut along both the longitudinal and transverse directions to be rectangular samples (15–20 mm × 3–4 mm, thickness is 30–45 μm) for the analysis of composition, structural evaluation and mechanical testing. Before further characterizations, these samples were rinsed in deionized water (18 MΩ cm) for three times and dried in circulation oven at 50 °C for 12 h. The ash of turtle shells were obtained by calcination in air at 560 °C for 40 h. The ratio between weight of ash and weight of original dried sample was quantified as mineral content.

2.2. Characterization

The morphology, energy dispersive X-ray spectrum (EDX) analysis and line scanning analysis of the outermost turtle shells were characterized by scanning electron microscope (SEM; Helios Nanolab 600i, USA). A HORIBA Raman system (LabRAM) connected to an optical microscope was used to collect Raman spectra of turtle dermis. Thermo-gravimetry (TA Q50, USA) was used to determine the thermal stability and the ratio of mineral/organic of turtle shells. Two samples were heated from 26 °C to 1000 °C at a heating rate of 10 °C min^{-1} in nitrogen gas and air atmosphere, respectively. The elemental composition of turtle shell and its ash was tested with X-ray fluorescence (XRF) analysis (Axios PW4400). A wide angle X-ray diffractometer (Empyrean, Netherlands) was employed to collect X-ray diffraction patterns of ash, operating in line scan mode, with Cu Ka radiation (1.54060 Å). The mechanical properties of the freestanding shells were measured in the tensile mode with a universal mechanical testing machine (Instron 5969, USA). The distance between the clamps was 10 mm and the load speed was 5 mm min^{-1}. Morphology and electron diffraction pattern of the mineral platelets in the ash of turtle shell were characterized by transmission electron microscopy (TEM; JEM-1400). The elemental composition of the turtle shell was identified by X-ray photoelectron spectroscopy (XPS; Escalab 250Xi, USA). A commercial AFM (Multimode SPM, Nanoscope V controller and Signal access module, Veeco Instruments) was used for testing surface topography, compressive modulus, stiffness, deformation and adhension of the turtle shell. The images recorded in this work were taken at high resolution (512 × 360 pixels, 0.5 μm per pixel) by using a force versus distance mode coupled with phase detection imaging (PDI). The work frequency, the stiffness of probe, the amplitude of cantilever and the scanning rate were 50–130 kHz, 3.2 N/m, 25 nm and 150 Hz, respectively. The Si probe was employed here with a round tip of 19 nm and data was collected in air and at room temperature.

3. Results and discussion

The red-ear turtles (chrysemys scripta elegans) are considered to be an invading species in many countries outside the Unites States. As seen the optical photo in Fig. 1a, a juvenile red-ear turtle with a body length of 20 cm and width of 15 cm was investigated here. The polygonous outermost shells (the longitudinal length is ~3.3 cm) came away from red-ear turtle naturally, which exhibits remarkable strength and flexibility. To study the microstructure of this turtle outermost armor, the sample was broke off transversally at the middle by a liquid nitrogen-cooled technique. Surface SEM image (Fig. 1b) shows that many polygonal tiles with a diameter of ca. 73 μm stack up tightly and constitute each individual shell. As seen in SEM and its high resolution images of the fracture surface (Fig. 1c, d), the turtle outermost armor is discerned a typical three-dimensional hierarchical structure with lamellas of 400–700 nm in thickness accumulating compactly. Some particles are clearly observed in each lamella that speculated as mineral here and the verification in our subsequent study (AFM experiment). Turtle shells deploy this uniform multi-layered armor system to protect themselves as the first level of defense from some impact and predators.

Besides multiscale structures, studying on chemical components is also important to understand the possible design principles of natural super-strong armor. Elemental compositions were analyzed by line scans on the SEM-EDX system. The location of scanning was selected in the center of the exposed area and line scans along the arrow from left to right (Fig. 2a). Line-scanning measurements of the carbon (C), oxygen (O), nitrogen (N), phosphorus (P), sulphur (S), calcium (Ca) and copper (Cu) contents