

Nanoindentation tests of Sulcata Tortoise's carapace

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Abstract. This paper illustrates the dependence of tortoise carapace's hardness and elastic modulus on peak-load of nanoindentation tests. Sulcata Tortoise carapace is a sandwich-like structure consisting of four submicron layers: keratin scutes, dorsal cortex, cancellous interior (porous layer) and ventral cortex. Nanoindentation test is a viable option for measuring their mechanical properties due to the size of the layers. However, preliminary results of nanoindentation tests on a carapace sample vary according to the indentation peak load. As the indentation peak load increases, both measured hardness and elastic modulus decrease. A systematic approach to determine an optimal peak load for nanoindentation test is presented.

1. Introduction

Turtle shells are natural shields optimized by years of evolution. Its protective mechanism inspires numerous studies [1-3]. African spurred tortoise or Sulcata tortoise is in the same species as turtles; therefore, its shell has the same structure as turtles and terrapins. Its bony shell is composed of a dome-shape carapace and a flat plastron. Its shell length can reach 80 cm and a weight of over 100 kg. Figure 1 shows a cross-section of the tortoise's carapace with an average thickness of 6 mm. It is consisted of four distinct layers: keratin scutes, dorsal cortex, cancellous interior and ventral cortex. Keratin scutes is the outermost layer made of Keratin, a structural protein that provides flexibility and elasticity to the shell. Beneath the keratin scutes are the bone layers. Dorsal and ventral cortices have dense or solid structures while cancellous interior has a closed-cell cellular structure. The overall Sulcata tortoise carapace resembles a sandwich-like structure with a porous core.

Nanoindentation test is a standard technique for measuring mechanical properties of both organic and inorganic materials with non-homogeneous microstructure [4-6]. Hardness and elastic modulus of the microstructures can be calculated from the load-displacement data using Oliver and Pharr method [7]. Figure 2 shows a representative load-displacement curve of elastic-plastic loading and elastic unloading with parameters for hardness and elastic modulus calculation [7]. The hardness H and the elastic modulus E can be derived by the following equation:



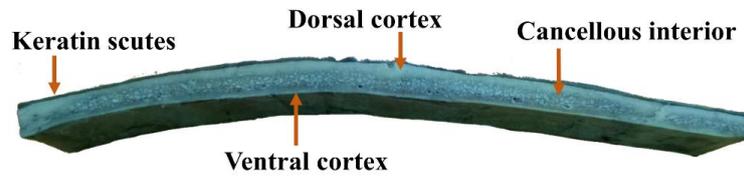


Figure 1. A cross-sectional view of carapace with four distinct layers.

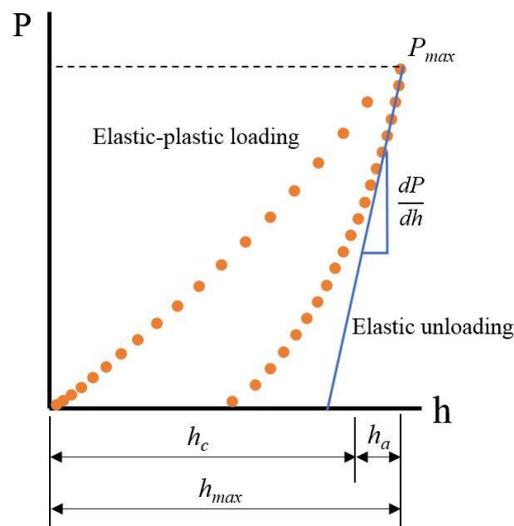


Figure 2. Analysis of load-displacement curve to determine hardness and elastic modulus.

$$H = \frac{P_{\max}}{A} = \frac{P_{\max}}{24.5h_c^2} \quad (1)$$

where P_{\max} is the peak load, A is the area function that depends on the indenter. For a Berkovich diamond indenter, $A = 24.5h_c^2$. The contact depth h_c is derived from

$$h_c = h_{\max} - 0.7 \frac{P_{\max}}{dP/dh} \quad (2)$$

where h_{\max} is the maximum depth, and $\frac{dP}{dh}$ is the slope of load-displacement curve during unloading.

The reduced modulus E^* and the material modulus E are given by equation (3) and (4), respectively.

$$E^* = \frac{dP}{dh} \frac{1}{2} \frac{\sqrt{\pi}}{\sqrt{A}} \quad (3)$$

$$E = \frac{1 - \nu_s^2}{\frac{1}{E^*} - \frac{1 - \nu_i^2}{E_i}} \quad (4)$$

where ν is the Poisson's ratio and subscript s and i are the values of sample and indenter, respectively.

Some studies [5-6] reported that hardness and elastic modulus were influenced by the peak loads. This characteristic of nanoindentation test can lead to erroneous measurements of material parameters. The non-homogeneous structure of turtle carapace further complicates the selection of correct peak loads.

Previous works that performed nanoindentation tests on turtle carapace did not clearly justify their choices of peak load or indentation depth. Rather, the selected values appeared to depend on the availability of test equipment and on the dimensions of the samples. For example, Achrai and Wagner [2] examined the red-eared slider turtle's carapace using the indent depth between 1.8-2.0 micron. Balani et al. [8] chose a load range of 0.1-2 mN with a maximum depth 50-300 nm to test freshwater snapping turtle's shell. Recommended peak load or indentation depth for testing tortoise shell has not been reported.

In addition to the peak load dependence, the nanoindentation test on turtle shell shows a time-dependent response during unloading because the layers exhibit creep behavior [2, 8]. This creep leads to an increase in the indentation depth after reaching the peak load. Figure 3 illustrates the load-displacement characteristic of creep behavior in nanoindentation test. The peak force must be maintained for a sufficiently long period to mitigate the effect of creep in the measurement of maximum depth.

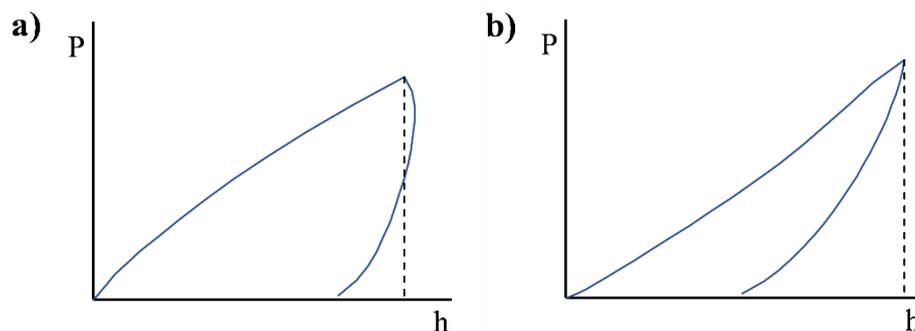


Figure 3. Load-displacement curve: a) creep during unloading b) without creep.

This paper aims to address the correlation between peak load and mechanical properties of tortoise carapace and to determine the appropriate peak load range for testing a sandwich-like structure. With an appropriate peak load, the material properties measured from nanoindentation test are validated and suitable for future reference. In addition, the systematic peak load selection procedure from this work can be followed when performing nanoindentation tests on other biological materials.

2. Experiment and method

2.1. Nanoindentation test

A Sulcata tortoise's shell was donated for this study. The shell was sun dried before cutting into strip specimens. The sides of the strips were polished using 1000 grit silicon carbide sandpaper. The hardness and elastic moduli of the carapace's four layers were examined by IBIS Nanoindentation system with a Berkovich diamond indenter. The indenter has a Young's modulus and a Poisson's ratio of 1000 GPa and 0.07, respectively. A fused silica was used for indenter tip's area function calibration [9].

In this study, the peak loads were selected from the capability of test equipment. The IBIS Nanoindentation system has two loading ranges: mid-range and high-range. The mid-range load has a peak load between 0-50 mN and an indentation depth between 0-2 micron. The high-range can supply a peak load between 0-500 mN and an indentation depth between 0-20 micron. Five values of peak loads were targeted: 10, 30, 100, 150 and 200 mN with a holding time of 10 sec at maximum load. Approximately 9-18 indents were performed for each testing condition. The values of Poisson's ratio of

each layer were taken from Achrai and Wagner [2]: the Poisson's ratio of keratin scutes layer was 0.4, while the other layers were assigned to be 0.3.

The load and displacement response were continuously measured during loading and unloading steps. The onset of the elastic unloading curve is fitted by a tangent line for the calculation of elastic modulus and hardness of the material. However, the dorsal cortex and the cancellous interior exhibit creep response when unloading after a peak load of 200 mN. The creep behavior induces a negative slope on the load-displacement curve $\frac{dP}{dh}$; therefore, the elastic modulus and the hardness cannot be determined.

3. Results and discussion

Figure 4 and figure 5 show distributions of hardness and elastic modulus versus peak loads, respectively. The average values of hardness for keratin scutes and ventral cortex tend to decrease from low to high peak load. For dorsal cortex and cancellous interior layer, the hardness slightly increases from 10 mN to 30 mN peak load. However, the average hardness values of low peak loads (10 and 30 mN) are higher than those of high peak loads (100 and 150 mN).

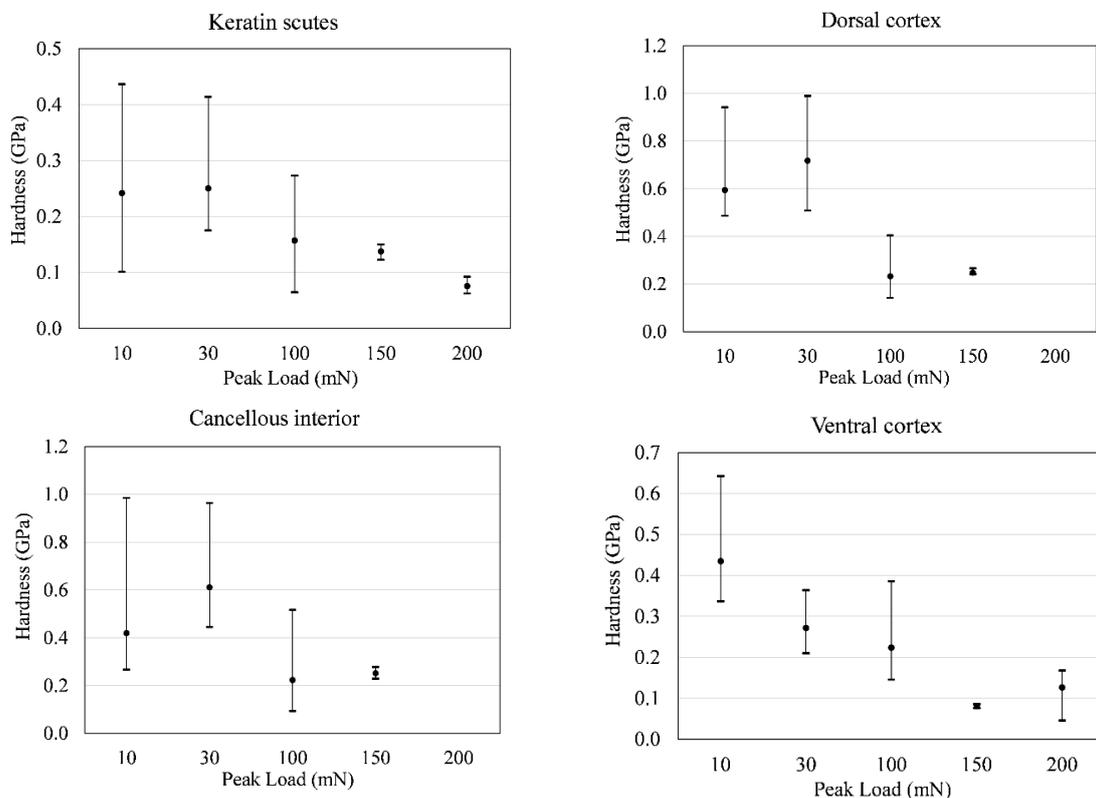


Figure 4. Hardness versus peak load of carapace layers.

The elastic moduli in figure 5 show the same characteristics for all layers of tortoise's carapace. When increasing the peak load, the elastic modulus decreases. In addition, both hardness and elastic modulus at high peak load show less variations than those at lower ones. The discrepancies at low peak loads are influenced by the local effects such as surface roughness, porosity and variations of material properties, especially nonhomogeneity in biomaterials [10].

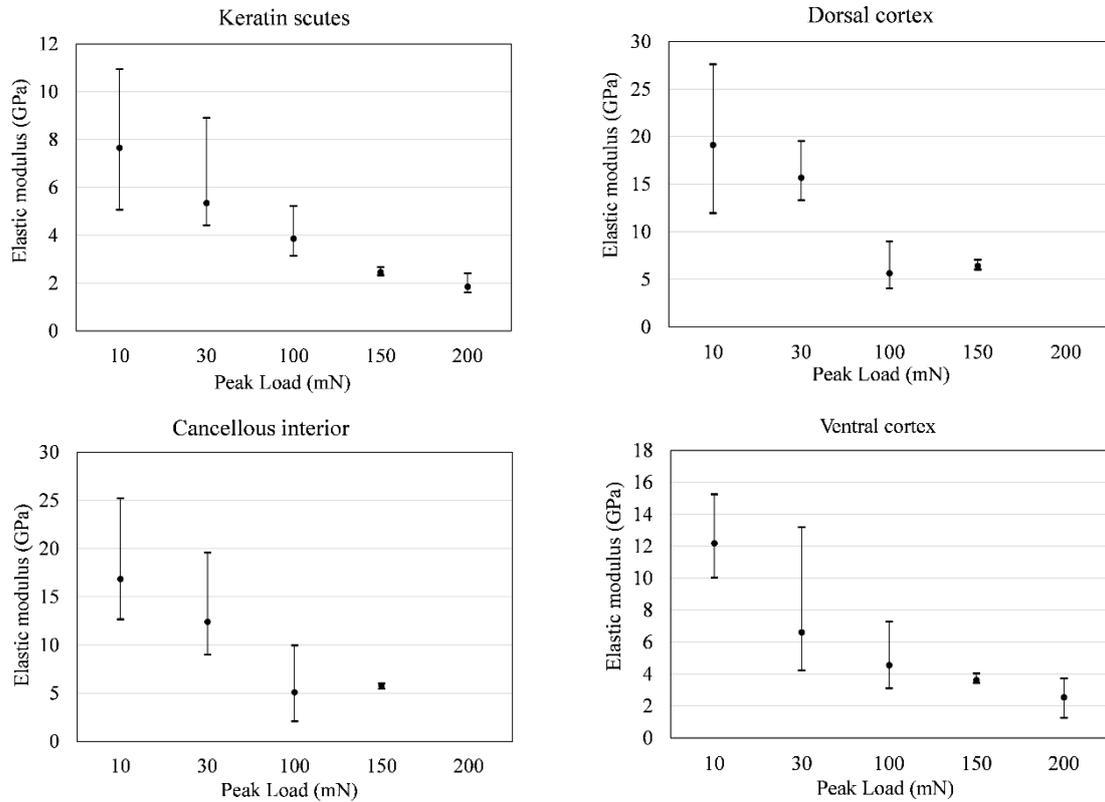


Figure 5. Elastic Modulus versus peak load of carapace layers.

However, using high peak load induces creep in the layers. When the material creeps, the indentation depth continues to sink during unloading. The negative slope of the unloading curve results in a negative modulus, which is not thermodynamically admissible. High peak load also leads to cracking of brittle layers as shown in figure 6.



Figure 6. Indentation on dorsal cortex with micro-crack at peak load of 200 mN.

The high values of hardness and modulus at low peak loads are related to the indenter shape [9], [10]; the indenter tip is idealized as a sharp cone in the calculation while in the experiment the tip is round. Figure 7 illustrates the difference between area function calculated from a round tip (A) and a sharp tip (A_i) with the same contact depth (h_c). The calculated area function A_i is less than the actual area function A . From Equation (1) and (3), the area function is inversely proportionated to the hardness and elastic modulus. Therefore, the calculated hardness and modulus are overestimated for low peak loads.

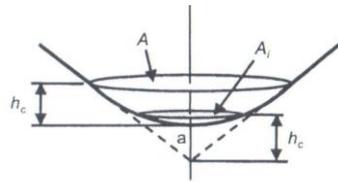


Figure 7. Area function of ideal indentation tip (A_i) and round tip (A) [9].

On the other hand, the material behavior influences the calculation of hardness and modulus at high peak loads [9]. In particular, soft materials pile-up around the indenter while brittle materials sink-in as shown in figure 8. The tortoise carapace is brittle since it is a bone-like material. The sink-in of brittle material leads to a larger calculated area function than the actual value. With this over-prediction of area function, the values of hardness and elastic modulus are underestimated when using high peak loads.

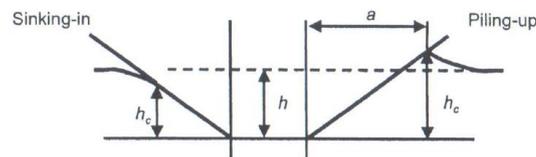


Figure 8. Material pile-up and sink-in [9].

Figure 4 and figure 5 highlight that in the mid-range (10, 30 mN) the measured data at a given peak load have large variations, except the hardness of ventral cortex. In the high-range (100, 150, 200 mN), the results have smaller variances in each peak load, which indicate an increase in data repeatability. However, at 200 mN, creeps and micro-crack occur on dorsal cortex and cancellous interior layers that result in indeterminable hardness and elastic modulus. Besides, some indentations at high peak loads have maximum depth more than the certified depth range of the indentation system. Because of these reasons, hardness and elastic modulus of tortoise carapace must be calculated from the peak load between 100 mN and 200 mN as illustrated in figure 9.

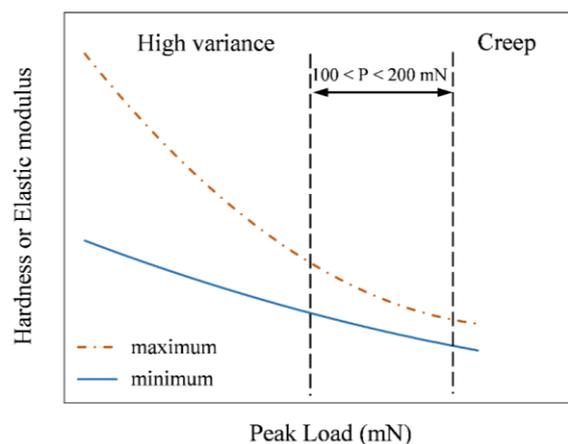


Figure 9. Peak load range for nanoindentation of Sulcata tortoise's carapace.

Table 1 presents values of hardness and elastic modulus of carapace's layers from optimal peak loads. From the data, the hardnesses of the carapace's four layers are in the same range, except the minimum value of ventral cortex. The elastic modulus of keratin scutes is the smallest value and that of dorsal cortex is the largest.

Table 1. Mechanical properties of tortoise carapace from nanoindentation tests at peak load $100 < P < 200$ mN.

	Keratin scutes	Dorsal cortex	Cancellous interior	Ventral cortex
Hardness (GPa)	0.16-0.20	0.23-0.25	0.22-0.25	0.08-0.22
Elastic modulus (GPa)	2.5-3.9	5.5-6.5	5.2-5.8	3.6-4.6

4. Conclusions

Mechanical properties measured by nanoindentation test show the peak load dependence characteristic. The assumption in the calculation, effect of microscopic measurement and material inconsistency lead to incorrect determination of mechanical properties. The results reveal that the suitable peak load for measuring tortoise's carapace is $100 < P < 200$ mN.

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