The three-cavity microstructures and mechanical properties of honeybee stingers

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Abstract
To investigate the microstructure-property relations of honeybee stingers, the cross-section microstructures were analyzed by scanning electron microscope (SEM) and the mechanical properties of honeybee stingers were tested by nanoindentation experiment in vivo in this paper. The Young’s modulus and hardness in the cross section of different segments of honeybee stingers were obtained. It is found that the honeybee stinger is of a hierarchical structure in cross section, which varies from the root to the tip and leads to quite different mechanical properties of the stingers. The natural optimized microstructure and excellent mechanical properties of the stingers effectively contribute to the biological function and self-protection performance of honeybees.

1. Introduction
In recent years, the design of painless syringes and microneedles are widely concerned in the biomedical field [1–4]. In this process, the related functions of some animals and plants in nature give people valuable inspiration. By natural selection and evolution, many plants and animals have evolved organs that have a injective function, such as mosquito mouthparts [5, 6], scorpion stinger [7, 8], caterpillar spine [9], spider fangs [10] and honeybee stinger [11]. These organs have many excellent functions which are important for their own survival. Compared with artificial needles, insect organs such as honeybee stingers are naturally optimal in geometry structures, which can pierce into the skins and tissues easily [6]. Such biological microneedle is the most advanced and sophisticated transdermal drug delivery system fabricated by nature. Therefore, it is of great significance to study the microstructures, mechanical properties and biological behaviors of animal stingers for the development, design and application of artificial microneedles [12].

Honeybee stinger has powerful penetrability. The honeybee pierces human skin with its ultra-sharp stinger and delivers the venom when it is stimulated. The sting process of honeybee can lead to a slight pain, which is mainly caused by the venom [13, 14]. The unique biological and mechanical performance of the stingers has attracted more attention in worldwide. Ling et al [15] studied the effects of honeybee stinger and its microstructural barbs on insertion and pull force. A nonlinear finite element method (FEM) [16] showed that the stress concentrations were around the stinger tip and its barbs during the insertion process, while the barbs were jammed in and torn the skin during the pull process. Wu et al [17] observed the process of honeybee stingers penetrate into four different substrates, then the morphological characteristics of the stinger cross-sections were analyzed before and after penetration by microscopy. Zhao et al [18] exhibited the process of honeybees inserting stingers into silicon substrate with a high-speed camera. Liu et al [19] indicated that the hierarchical structure and excellent mechanical properties of honeybee stingers have important implications for the design and manufacture of artificial materials.

In the mentioned above investigations, the attention is focused on the surface structures and powerful penetrability of the honeybee stingers with excellent mechanical performance. In principle, the mechanical properties of the stingers depend on their microstructures. However, limited studies on the relationship between microstructure and mechanical properties of honeybee stingers have been reported [19].

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In this paper, a typical stinger of *Apis Mellifera Ligustica Spinola* worker bees were selected to investigate the microstructure by SEM while the Young’s modulus and hardness by the nanoindentation. The microstructure-properties relation of the honeybee stingers is emphasized here to understand their biological function and mechanical performance.

### 2. Sample preparation and experimental method

In order to explore the microstructural characteristics of honeybee stingers, samples of the stingers are prepared for the observation by SEM and property test by nanoindentation. The microstructural characteristics of the stingers are observed by adjusting the polished depth of the samples. By this way, we can obtain the cross-section morphologies of different longitudinal segments of the stingers.

In this work, the adult *Apis Mellifera Ligustica Spinola* worker bees were taken as the investigative objects. In order to ensure the bioactivity of stingers, several whole alive stingers were washed within distilled water. This process does not affect the physical properties of stingers [8].

To fix the stingers for the experiment, the epoxy resin and curing agent with a mass ratio of 7:5 were poured into the mold and placed it in the fume hood. Then the honeybee stingers were fit on the surface of the resin. When the epoxy resin was completely set and the stingers were embedded into the epoxy resin, we can get the sample for the experiments, as shown in figure 1(a).

To exhibit the cross-section morphology of the stingers varying with the length of the stingers, we consider three segments of the honeybee stingers: apical, medial, basal segments, as shown in figures 1(b)–(d). For the measurement of the mechanical properties in the cross sections of the segments, the sample surface was polished with different depths. Before SEM observation, a 10 nm gold film was plated on the sample surface with ion sputtering apparatus. The prepared samples were also used to the nanoindentation test, as shown in figure 2.

Nanoindenter G200© was used to measure the quasi-static Young’s modulus and hardness in the cross-section plane of the stingers. A Berkovich tip with a triangular pyramid shape and a semi-cone Angle of 70.3° was applied to indent the cross-section plane of the stingers with the maximum depth of 250 nm.
3. Multi-cavity microstructures of stingers

The external morphologies of the stingers were shown by SEM. It is found that the tip of the stinger with one needle has quite short length and extremely small diameter, as shown in figures 3(a) and (b). Moreover, in the apical, medial and basal segments, there are three independent needles with many small barbs, as shown in figure 3(c). This three-needle composite structure can enhance the mechanical properties of the stingers. Here we only consider the apical and medial segments of the stinger in the present research.

The microstructure of the cross-section of the stingers was observed by SEM. The SEM photos show the microstructures of the cross section of the apical and medial segments of the stingers, as shown in figures 4(a)
It is also found that there are four cavities in the across sections of the stinger. One is approximately circular cavity with the dimension of about 34 microns surrounded by three needles, which nearly has no changes between apical and medial segments. And other three are semicircular cavities inside the needles. This means that the circular cavity surrounded by three needles can be used to inject the venom. Whereas, judging from the structural characteristics of the honeybee stinger in combination with figures 3(b) and 4(a), the central cavity is the only channel for injecting the venom, as only the central cavity has an opening at the tip. Simultaneously, the three semicircular cavities must not be able to inject venom because the three tips are sealed by material.

The needles are connected by protruding structures similar to the ship anchors, as illustrated in figure 4(c), which are perfectly and respectively fit to the morphologies of the needles. The tight connection of the needles can ensure the integrity and enhanced mechanical properties of the stingers.
4. Mechanical properties of stingers

Nano Indenter G200 was used in this measurement. The nanoindentation was conducted to obtain load-displacement curve, Young’s modulus and hardness. A Berkovich tip with a triangular pyramid shape and a semi-cone angle of 70.3° was selected in the test. The maximum indentation depth was set to 200 nm, the thermal drift was controlled within the range of 0.5 nm s⁻¹, and the frequency signal of harmonic force was set to 75 Hz.

The microstructures of the cross-sections of apical, medial and basal segments are very different. To understand the mechanical properties of stingers, a nanoindentation experiment in the cross-section for each segment of stingers was carried out. The load-displacement curve of apical, medial and basal are shown in Figure 5. According to the curves, the Young’s modulus and hardness can be calculated which are important mechanical parameters of the honeybee stinger.

In order to ensure the accuracy of the test results, sufficient test points were selected in the cross sections of the same samples to obtain the Young’s modulus and hardness of the apical, medial and basal segments. The experimental results are shown in Figure 6 where the load-displacement curves and values of the Young’s modulus and hardness for each segment are illustrated.

It can be found that the Young’s modulus and hardness of the stinger increase from the apical to basal segment in the longitudinal direction of the stingers. The average Young’s modulus and the average hardness is 7.133 GPa and 0.310 GPa of the apical segment, 8.181 GPa and 0.423 GPa of the medial segment; 9.853 GPa and 0.525 GPa of the basal segment, respectively. It is shown that the basal segment has the highest mechanical properties, which are about 26.5% and 17% higher than the Young’s modulus and hardness of the apical segment of honeybee stingers, respectively. The apical segment of honeybee stingers has a good ability of deformation, which can enhance the penetrating ability of stingers through rotating and bending deformation in the process of piercing. While the excellent elastic resistance ability of the basal segment can effectively enhance the stability of honeybee stingers. Besides, the Young’s modulus of the medial segment is about 13% higher than that of the apical segment of honeybee stinger. This indicates that the material properties are variable for different segments of the stingers, which can enhance the penetration abilities of the honeybee stingers.

5. Conclusion

In this paper, the microstructures in cross section of the different segments of the stingers were observed by SEM, and the mechanical properties were determined by using nanoindentation experiments. It is shown that the honeybee stinger is composed of three extremely sharp needles. The three needles are combined with a fixed sliding lock structure similar to a ship anchor to form a stinger with a central cavity. There are fillister structures on the surface of honeybee stingers, which are obvious in the range for apical to medial segments. The Young’s modulus and the average hardness of honeybee stingers were obtained for each segment. The apical segment of honeybee stingers has a good elastic deformation ability and the basal segment of stingers has a good elastic resistance and stability. In general, the unique microstructure of multi-cavities and non-uniform materials have reference value and provides a design idea of painless syringes and microneedles.

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