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Effect of honeybee stinger and its microstructured barbs on insertion and pull force

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ABSTRACT

Worker honeybee is well-known for its stinger with microscopic backward-facing barbs for self-defense. The natural geometry of the stinger enables painless penetration and adhesion in the human skin to deliver poison. In this study, Apis cerana worker honeybee stinger and acupuncture microneedle (as a barbless stinger) were characterized by Scanning Electron Microscope (SEM). The insertion and pull process of honeybee stinger into rabbit skin was performed by a self-developed mechanical loading equipment in comparison with acupuncture needle. In order to better understand the insertion and pull mechanisms of the stinger and its barbs in human multilayer skin, a nonlinear finite element method (FEM) was conducted. Experimental results showed that the average pull-out force of the stinger was 113.50 mN and the average penetration force was only 5.75 mN. The average penetration force of the stinger was about one order of magnitude smaller than that of an acupuncture microneedle while the average pull-out force was about 70 times larger than that of an acupuncture microneedle. FEM results showed that the stress concentrations were around the stinger tip and its barbs during the insertion process. The barbs were jammed in and torn the skin during the pull process. The insertion force of the stinger was greatly minimized due to its ultrasharp stinger tip and barbs while the pull force was seriously enhanced due to the mechanical interlocking of the barbs in the skin. These excellent properties are mainly a result of optimal geometry evolved by nature. Such finding may provide an inspiration for the further design of improved tissue adhesives and micro-needles for painless transdermal drug delivery and bio-signal recording.

1. Introduction

Microneedle is a microscale needle with its length ranging from 150 to 1500 µm, base diameter from 50 to 250 µm, and tip diameter from 1 to 25 µm (Arora et al., 2008). Microneedle and its array have attracted more and more attention in the biomedical fields, such as transdermal drug delivery (Dangol et al., 2016; Kim et al., 2016), cutaneous vaccine delivery(Pearton et al., 2012; Kim et al., 2014), fast drug detection(Vazquez et al., 2014), biochemical sensor (Valdés-Ramírez et al., 2014), dry electrode for bio-potential monitoring (Kim et al., 2015; Ren et al., 2016), trace amount blood collection (Aoyagi et al., 2008), tissue adhesives (Yang et al., 2013) and so on. It should be able to painlessly pierce skin without mechanical failures for the needle (Ma et al., 2011). Yang and Zahn (2004) stated that most artificial microneedles did not tolerate forces associated with insertion and intact removal. They were typically either too fragile or too ductile. In seeking a painless microneedle with minimal insertion force and high soft tissue adhesion, we looked for inspiration from the exquisite natural biomicroneedle adapted the above-mentioned functions through the course of evolution.

Through hundreds of millions of years of evolution, biomicroneedle has developed ingenious physical properties, such as low insertion force, high tissue adhesion, high stiffness and good biocompatibility. The proboscis of mosquito is a jagged shaped biomicroneedle, which can painlessly insert into skin and suck blood. Kong and Wu (2009, 2010) studied insertion behavior of the *Aedes albopictus* mosquito fascicle. Results showed that mosquito fascicle can easily cut out skins with vibration and its insertion force was 18 µN on average. It was three orders of magnitude smaller than the lowest insertion force for the reported artificial microneedle. Artificial microneedle imitated mosquito's proboscis was also fabricated and verified (Jaiswal et al., 2015). Ma et al. (2011) studied the spine of the *Parasa Consocia*

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Table 1
Mechanical properties of samples.

Characteristic	Stratum corneum	Dermis	Rabbit skin(stratum & dermis)
Young's modulus (MPa)	6–8900 (Wu et al., 2006)	0.3–20 (Hendriks et al., 2005)	1.7–2.5
Tear strength (MPa)	13–44 (Wildnauer et al., 1971)	7.3 (Mehta and Wong, 1973)	14.7–29.1
Density (Kg/m^3)	1300 (Wu et al., 2006)	1200 (Odland, 1991)	1308
Thickness (mm)	0.015–0.035 (Wu et al., 2006)	1.0–4.0 (Odland, 1991)	0.94–1.23

caterpillar. Results showed that caterpillar spine pierced mouse skin without fracture and buckling failure in a small force (about 173 μN) due to its ultra-sharpness, high hardness, and good elastic modulus of the tip end. Many nanoscale backward needle tips are distributed regularly over the spine surface and their function has not been studied. The researches of mosquito proboscis and caterpillar spine mainly focused on the insertion process while the pull process was considered little. Cho et al. (2012) studied the quills of North American porcupines. Results showed that the natural quill's geometry enabled easy penetration and high tissue adhesion and the barbs specifically contributed to adhesion and dramatically reduced the force required to penetrate tissue. But the dimension of quills of North American porcupines was in millimeter size and much larger than microneedles. Our group (Ling et al., 2016) has investigated the sting behavior of worker honeybee. The honeybee stinger directly inserted into skin without any vibration. The penetration force into artificial skin was also very small (about 1.34 mN). The stinger was elastic and it could easily recover after being bent. The sting behavior has been well described but the mechanical function of the barbs on the stinger needs to be further investigated.

In present study, the effects of the stinger and its barbs of worker honeybee on insertion and pull process were experimentally studied and numerically calculated in comparison with the acupuncture needle (as a barbless stinger). The structure of worker honeybee stinger and acupuncture needle were observed by Scanning Electron Microscope (SEM). Micro-sample mechanical loading equipment was developed to measure the forces of the stinger in the insertion and pull process with and without barbs. Insertion and pull process of finite element model of the stinger and acupuncture needle was built by ABAQUS. The experimental and simulation results were analyzed comparatively.

2. Materials and methods

2.1. Ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC), Sun Yat-sen University (Approval Number: IACUC- DD-16–0701). All efforts were made to minimize suffering.

2.2. Preparation

2.2.1. Worker honeybee stinger and acupuncture needle

The worker honeybees (Apis cerana) were raised in Guangzhou City, Southeastern China. Fine stingers were detached from adult worker honeybees. The acupuncture needles (Beijing Zhongyan Taihe medical instrument Co., Ltd, China) were purchased with a diameter of 0.16 mm and a length of 40 mm. The acupuncture needles were cut and the length of top section was about 1.5 mm. The structures of honeybee stinger and acupuncture needle were characterized by the scanning electron microscope (SEM, JSM-6380, JEOL, Japan).

2.2.2. Preparation of rabbit skin

A New Zealand rabbit (male, 3 months old, 3.0 kg) was purchased from XinHua experimental animal farms (Huadu District, Guangzhou City, China). The rabbit was mercy killed by pentobarbital through intravenous injection. Subsequently, the hair on the skin was removed and some fresh skin was cut into squares for insertion and pull test, whose size was about 20 mm×20 mm×2.5 mm. All procedures were followed the guidelines of the Institutional Animal Care and Use Committee (IACUC), Sun Yat-sen University. The mechanical properties of rabbit skin were measured with a universal material testing machine (LR10KPlus, LLOYD, UK). Some fresh skin was cut in a shape of "dog bone" with its size of 70 mm×20 mm×2.5 mm. The skin was clamped by a fixture and stretched with a velocity of 15 mm/min under the moisture degree of 90% and temperature of 25 °C. The measured mechanical properties of rabbit skin are showed in Table 1 in comparison with human skin.

2.2.3. Preparation of the stained stinger

The stinger was immersed in the 0.01% rhodamine B solution for about 30 min. The stinger was dried for about 2 h in the air. The stinger was observed by the Inverted Fluorescence Microscope (ECLIPSE Ti, Nikon) before and after mechanical test.

2.3. Mechanical test

2.3.1. Mechanical loading equipment

Mechanical loading equipment was self-developed to measure the insertion and pull process for the stinger as shown in Fig. 1. The micro-force transducer (407 A, Aurora Scientific Inc., Canada) was driven by a DC linear motor (M227-10, Physik Instrument, Germany). The force range and precision of force transducer were 1000 mN and 0.02 mN

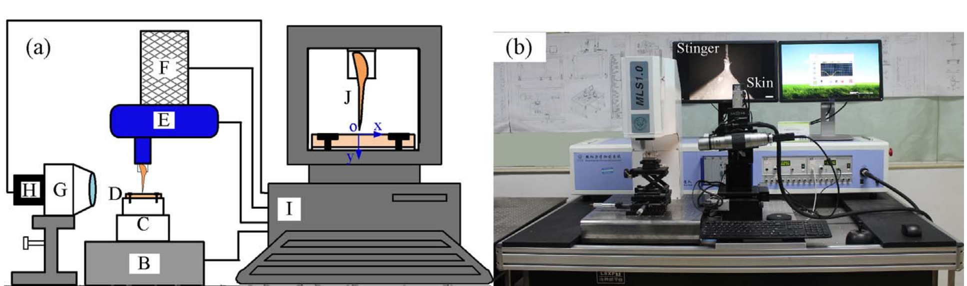

Fig. 1. Schematic illustration of mechanical loading equipment. A, vibration isolation bench; B, positioning stage; C, fixture; D, rabbit skin; E, force transducer; F, linear motor; H, CCD; G, optical microscopy; I, computer; J, honeybee stinger.