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A gecko skin micro/nano structure – A low adhesion, superhydrophobic, anti-wetting, self-cleaning, biocompatible, antibacterial surface

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ABSTRACT

Geckos, and specifically their feet, have attracted significant attention in recent times with the focus centred around their remarkable adhesional properties. Little attention however has been dedicated to the other remaining regions of the lizard body. In this paper we present preliminary investigations into a number of notable interfacial properties of the gecko skin focusing on solid and aqueous interactions. We show that the skin of the box-patterned gecko (*Lucasium* sp.) consists of dome shaped scales arranged in a hexagonal patterning. The scales comprise of spinules (hairs), from several hundred nanometres to several microns in length, with a sub-micron spacing and a small radius of curvature typically from 10 to 20 nm. This micro and nano structure of the skin exhibited ultralow adhesion with contaminating particles. The topography also provides a superhydrophobic, anti-wetting barrier which can self clean by the action of low velocity rolling or impacting droplets of various size ranges from microns to several millimetres. Water droplets which are sufficiently small (10–100 μm) can easily access valleys between the scales for efficient self-cleaning and due to their dimensions can self-propel off the surface enhancing their mobility and cleaning effect. In addition, we demonstrate that the gecko skin has an antibacterial action where Gram-negative bacteria (*Porphyromonas gingivalis*) are killed when exposed to the surface however eukaryotic cell compatibility (with human stem cells) is demonstrated. The multifunctional features of the gecko skin provide a potential natural template for man-made applications where specific control of liquid, solid and biological contacts is required.

1. Introduction

Nature has provided scientists through the process of evolution with a diverse range of micro and nanostructures which are a potentially rich blueprint for new *technologies* and *materials* [1–5]. These 'free' architectural adaptations are of particular interest in relation to surfaces as control of interfacial properties is a key aspect in many current and emerging industries. While the epidermis of many organisms (plants and animals) has been examined in numerous studies, the functions and functional efficiencies on many of these surfaces have not been investigated. The multifunctional character of these natural surfaces along with limited knowledge of the habit and behaviour of many organisms adds to the complexity in determining all the functions of these intriguing and potentially informative natural templates.

Geckos have received a considerable amount of attention in recent times, predominantly focusing on the adhesion properties of the small structures (setae) on their feet [6–11]. While the feet of some gecko species have attracted significant interest, the remaining regions of the lizard body have received little attention in relation to microstructure and particularly studies demonstrating skin functions [12,13]. This is somewhat surprising as geckos have interesting microstructuring on the dorsal and ventral regions typically consisting of small hairs (often referred to as spines, spinules or microspinules), spaced 0.2–0.7 μm apart and up to several microns in height [12,13]. The outer layer of lizard skins in general

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has been shown or speculated to exhibit a range of functions including acdysiss, coping with varying temperatures, pheromone capture, retention, and dispersal, tribological functions such as reduction of friction and wear protection and also reflection of radiation. A previous study has shown that the gecko spines can be water-repellent and suggested that they may also serve as a self-cleaning surface where rain may carry away particles and thus utilise the lotus effect to remove contaminants [13]. As well, while the evolution of the gecko microstructuring is poorly understood, the foot micro structuring may be evolutionarily linked to other regions of body skin micro-structuring (e.g., scales) [14]. In addition, almost all the studies to date have focused on a single species of gecko (*Gekko gecko*) and the degree of variation in form and function among species is not well known.

Geckos live in a diverse, and at times, hostile environment where contact with liquid and solid surfaces and particles is unavoidable; for example water droplets, condensation, contaminants such as plant material, soil particles and micro-organisms. Continuous exposure of the skin to these environmental contaminants can potentially inhibit or degrade the functioning of the skin as a protective mechanical barrier. For example as the growth of many micro-organisms is enhanced by increased water availability, proliferation may result from wetting property changes of the skin. Studies have shown that some lizards are susceptible to various external contaminants that can cause serious skin problems and diseases, thus the epidermis plays an important role in particle and microbial resistance [15]. Some environmental conditions (e.g., high humidity conditions and low temperatures) have been reported to be potential factors in the development of reptile bacterial infections [16]. In addition, contaminants remaining on the surface (especially those with hydrophilic surface properties) may act as nucleation points for further solid particle contamination (e.g., soil fragments, bacteria, fungi). Thus, *structuring* and '*technologies*' on the gecko skin that limit water exposure and contact, or contact times, with solid bodies may enhance the ability of the lizard to maintain the integrity, health and functioning of their epidermal layering.

A previous study has suggested that the skin of the gecko may be able to facilitate self-cleaning as high contact angles (CA)s were shown [13]. In this present study we have investigated the gecko (*Lucasium steindachneri*) that will typically encounter contamination conditions (ground dwelling habit) and which lives typically in a semi-arid habitat. This environment will however present a range of varied conditions from relatively brief periods of heavy rain (water covering expansive regions of the ground where the gecko inhabits) to high humidity and light rain or fog conditions and intermittent (and sometimes cyclic) exposure to particles such as soil particles (e.g., silica), fungi, bacteria and plant material such as pollens.

2. Materials and methods

2.1. Gecko capture and preparation

Three box-patterned geckos (*L. steindachneri*) were captured at night by hand from the Mingela Ranges (S 20 08′ 06″ E 146 52′ 32″) Queensland (QLD). The Mingela Ranges are semi-arid with a long-term (50-year) median of 62.5 rain days per year on average. Only healthy adult lizards were returned to the laboratory with a heat source for thermoregulation and suitable plant foliage and water. They were fed domestic European crickets (*Acheta domestica*) three times a week. Geckos were allowed to shed twice before experiments were conducted. Lizards were euthanised and experiments performed after shedding to ensure the skin's surface was intact and undamaged. Both the lizard skins and their shed skins were used in experiments. The lizard skins were surgically separated by scalpel, cut into smaller sections and attached to glass slides for experiments.

This work was conducted under Ethics Approval A1676, and QNP permit WITK05209908.

2.2. Adhesion experiments with contaminants

The adhesion measurements were carried out with an atomic force microscope (AFM); ThermoMicroscope TMX-2000 Explorer. The instrument is based on detection of tip-to-surface forces through the monitoring of the optical deflection of a laser beam incident on a force-sensing/imposing lever. The analyses were carried out under air-ambient conditions (temperature of 24–26 °C and 70–75% relative humidity (RH)).

Tipless beam-shaped levers (diving board in shape – NT-MDT Ultrasharp) were used throughout the work. The attachment procedure of the silica and pollen particles to the lever has been described in the literature [17]. The particle and lever are collectively termed the 'probe' in our study. The pollen grains and silica particles were characterised by optical microscopy and scanning electron microscopy (SEM) while the roughness of silica beads were determined by AFM. Only pollen grains with no observable damage upon fixing to a lever were used for adhesion measurements. Force versus distance (*f*–*d*) analysis was used to obtain adhesion data. The probe was held stationary at an *x*–*y* (sample plane) location and was ramped along the *z*-axis, first in the direction of approach and contact with the surface, and then in the reverse direction. *F*–*d* curves were acquired at rates of translation in the *z*-direction in the range 5–10 m s^{-1}. Each *f*–*d* curve consisted of 600 data points.

Fifty measurements per particle were acquired for each general location. A total of 4 particles were attached to cantilevers for each particle type e.g., four silica beads and four pollens were used for adhesion measurements each yielding 50 measurements for each sample). The normal force constant of the probe was determined by using resonance methods and the scanners were calibrated using atomically flat surfaces [18].

Adhesion was measured under the conditions of the two surfaces coming into contact with no applied loading force (i.e., adhesion represented the force of attraction that the particle–surface would experience where deformation of structures is minimised and where the main contributing force involved is simply that of the adhesion of the particle to the surface). The preparation procedures of insect samples for adhesion experiments were the same as used in the literature [17].

2.3. Scanning electron microscopy and X-ray photoelectron spectroscopy

In the case of scanning electron microscope (SEM) imaging (Figs. 1 and 2), a small section of lizard skin (approx. 3×5 mm^2) was excised and mounted on an aluminium pin-type stub with carbon-impregnated double-sided adhesive, then sputter coated with 7–10 nm of platinum, before being imaged using a JEOL 6460 or 7001 Scanning Electron Microscope (SEM) at 8 kV. The same conditions were used for insect cuticle examination.

The X-ray photoelectron spectrometer (XPS) was assembled from components from many manufacturers and included a hemispherical electron energy analyser, multichannel detection and a Mg K_{alpha} X-ray gun at a source power of 300 W. The UHV envelope was oil-free being based on turbomolecular and ion pumping. Survey and detailed scans were obtained at resolutions of 1.0 and 0.2 eV, respectively. The analyses were carried out at a base vacuum of 10^{-9} torr.

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