Tendril, adhesive disc and super adhesive effect of climbing plant

Wenli Deng

College of Materials Science and Engineering

South China University of Technology

Guangzhou 510640, China.

Tel: 86-20-22236708

Fax: 86-20-22236706

wldeng@scut.edu.cn

Abstract

Understanding the super adhesion mechanism of biological system is of great scientific interest and a prerequisite for bioinspired design of adhesive systems. To investigate the versatile climbing plant requires the development of new methods. Here I present strategies to fully study the super adhesive effect of climbing plant — *Parthenocissus tricuspidata* by the first time measuring the mass and the attached area of single adhesive disc, by further determining the microscopic structure and the adhesive strength of single adhesive disc and by finally elucidating the adhesion mechanism in cellular and molecular level using the classical theories and the proposed new hypothesis and model. I have measured that a single mature adhesive disc has an average mass of only about 0.0005 g, an average attached area of about 1.22 mm^2 and an adhesive force of about 13.7 N. I have found that a single adhesive disc can on average support a weight produced together by the stem, leaf, branchlet and tendril which is 260 times greater than its own weight during the growing, and can sustain the maximum pulling force which is 2,800,000 times higher than that produced by its own weight. Microscopic experiments show some new microstructures which have never been reported and reveal that the adhesive disc has super adhesive property which inspires us to fabricate a lot of mimic adhesives that have potential applications in biomedical science, bioelectronics and space science and technology.

There have been some animals and plants having the amazing climbing ability that have attracted the interests of philosophers and scientists for centuries[1~3]. Geckos have evolved into one of the most versatile animals that can adhere to and freely move along vertical walls and even ceilings[4]. Microscopy shows that there are nearly five hundred thousand keratinized setae on a gecko's foot and each seta contains hundreds of spatulae[5]. Despite more than centuries of research on seta attachment systems, there is still a sharp debate on the dominant mechanism of a gecko walking on smooth walls or ceilings. Different hypotheses have been proposed to explain the mechanism of attachment: suction[6], friction[7], intermolecular forces[8], electrostatic forces[9], capillary forces[10, 11], van der Waals forces[5, 8, 12, 13], skin gland glue[9, 14] etc. Moreover, there have been several theoretical models to describe the mechanism of gecko foot adhesion[15]. One conclusive evidence is that the adhesion of gecko setae is caused by van der Waals forces[12, 13] rather than by capillary forces[10], which is not convincible in view of the conclusions reached from a large number of studies[6~11, 14]. Anyhow, the further study in the correlation between the microstructures of setae and spatulae and the adhesion mechanism is of great scientific significance for artificial material design and functional device fabrication in microelectronics, robotics and space technologies[16~19].

Like the evolution of geckos, *Parthenocissus tricuspidata* has also evolved into one of the most versatile plants that can stick to vertical walls and even precipitous cliffs[2, 3], for example, stone mountains, roadside stone banks, house outside walls and expressway piers with a height of a few meters to twenty meters or more shown in Figure1. *P. tricuspidata* is also called *Virginia creeper*, *Boston ivy*, *Japanese creeper*, *Chinese Pashanfu* and so on, which grows in Japan, Korea, North Korea, China, North America and Europe[2, 3, 20~27], which has exceptionally tenacious drought-, heat-, cold-, insect pest- and disease-resistant vitality and whose leaves, branches and stems are not edibles for plant-eating animals, such as cattle, sheep and rabbits. All the year round, it can outlive the gale blowing and the storm beating and firmly climb on the various substrates no matter whether it is a smooth or a rough surface, a vertical or a tilted location. It has been widely used for solidifying sand and soil, for protecting slope, for making environment green and beautification, and for healing diseases[20~27]. Although the sticking ability of *P. tricuspidata* has attracted great interests for hundreds years[2,3], it's distinctive essentiality still inspires our enthusiasm in further understanding the structure of adhesive disc and the mechanism of adhesion, as well as it's potential applications. Here I report the first direct experiments on measuring the mass and attached area of a single adhesive disc, the microstructure and adhesion force of the adhesive disc. Furthermore, I use the theory of *chemistry*, *biology* and *material* to carefully elucidate some new findings in detail. Especially, I use a few classical theories to explain the adhesion of adhesive disc in cellular and molecular levels. Meanwhile, I also propose a new hypothesis and model to describe the tendril and adhesive disc adhesion. I finally bring forward an exciting prognostication and prospect.

P. tricuspidata is of important value in pharmacology. An antitumor alkaloid alstonie[20] and quercetin 3-monoglucoside, quercetin 3-diglucoside and cyaniding 3, 5-diglucoside are discovered in it. Quercetin 3-O-β-D-glucuronopyranoside, quercetin 3-O-β-D-glucopyranoside and quercetin 3-O-(6′′-n-butyl)β-D-glucurono- pyranoside are isolated from the leaves and used as folk remedies for the treatment of arthritis, jaundice, toothache, neuralgia[21]. Free sterols, esterified sterols, steryl glycosides and acylated steryl glycosides are also isolated from the leaves. Resveratrol, a phytoalexin (3, 5, 4'-trihydroxy-trans-stilbene) and it's two dimmers: 2R, 2'R-(4-hydroxyphenyl), 3R, 3'R-(3, 5-dihydroxyphenyl)-tetrahydrofuran and isoampelopsin F are isolated from the stem wood described in different reports[22], which is proved to be of cancer chemopreventive activity in assays representing three major stages of carcinogenesis[23]. Palmitoleic acid and cis-vaccenic acid are isolated from seeds, antiplasmodial stilbene derivatives from leaves[24], and ethyl 1, 2-dihydro-2-oxoquinoline-4-carboxylate and methyl 1, 2-dihydro-2-oxoquinoline-4-carboxylate from fruits. The leaves, stems and branches contain various substances[20~24] that can be used as drugs, but they taste terrible, thus leading to the fact that they are not eaten by plant-eating animals, and they are insect-pest- and disease-resistant.

P. tricuspidata plays an inimitable role in environment protection and climate improvement. In the urban area, it relieves the airborne dust load and helps to improve climate and to keep atmospheric-hygiene[25]. It can be used as a passive accumulation indicator plant for monitoring the heavy metal aerosol pollution caused by traffic[26], thus benefiting the air pollution control and surroundings improvement[27]. It can cool the wall surface by more than 5 ℃ at midnoon in a burning-hot summer, which not only creates a cool and comfortable living environment but also reduces a light pollution caused by the hot wall. In recent years, the climate change around the world has been a common trouble to all of us. Natural disasters, such as hurricane, cyclone, typhoon, rainstorm, snowstorm, flood, drought, happened frequently, which caused disastrous casualties and huge wealth losses. Obviously, the climate change has affinity with the environment protection.

The mass of the adhesive discs, tendrils, branchlets, leaves and stems of *P. tricuspidata* is directly measured using an electronic balance. Experiments firstly find that the average mass of a single mature adhesive disc is only about 0.0005 g, that a single mature adhesive disc can on average support a weight produced together by the stem, leaf, branchlet and tendril that is 260 times greater than its own weight during the developing and growing. Furthermore, it is measured that the minimum pulling force produced by the mature adhesive disc when it falls off the attached substrate is about 13.7 N, about 3.1 *lb*. An average attached area of a single mature adhesive disc measures about 1.22 mm^2. Measurement results show that a single mature adhesive disc can sustain the maximum pulling force which is 2,800,000 times higher than that produced by its own weight. The mature adhesive disc can support an average stress of 1124.6 N/cm^2 (Fig. 2 B), which is 112 times greater than that the gecko foot can sustain[12], 52 times greater than that the live gecko toes can sustain on GaAs and Si[13], 374 times greater than that the polyimide hairs can sustain[17], 96 times greater than that unpatterned carbon nanotube patches can sustain on silicon[18] and 31 times greater than that carbon nanotube-based synthetic gecko tape can sustain[19] (Fig. 2 A). The actual values may be greater, because it is unlikely that an equivalent area of adhesive disc completely adhere simultaneously to the substrate due to a sponge-like micro-hole mesh disc attached to the substrate.

According to the stress of 1124.6 N/cm^2 of the adhesive disc and the attached area of about 1 cm^2 of a fingertip of palm, I approximately estimate that a mimic palm made of adhesive disc material and attached only with a fingertip can firmly sustain a body weight of about 114 kg (Fig. 2 C). Considering the adhesive property of the natural material such as the gecko foot seta[12] and the adhesive disc[2, 28] of *P. tricuspidata* and the optimized mimetic material such as the polyimide gecko hair[17], the unpatterned carbon nanotube gecko patche[18]and the carbon nanotube-based synthetic gecko tape[19] and the total area of 200 cm^2 of human palm[17], I can approximately estimate that a single mimic palm made of various material and attached fully on the substrate can firmly sustain a weight equal to an animal's body weight respectively including a 61kg of chimp, a 204 kg of bear, a 238 kg of lion, a 734kg of urus, a 14,885 kg of shark and a 22,951 kg of cachalot (Fig. 2 D, E). As early as in 1875, Darwin[2] found that a 10-year-old branchlet with only one adhesive disc left could support a weight of 2 *lb* without the disc detaching from the wall[28]. My findings (~ 3.1 *lb*) are higher than the unique one (2 *lb*) discovered by Darwin[2, 28], which might result from an evolution in the past 130 years. Experimentally, I clearly reveal that the adhesion of adhesive disc is very strong, which helps us to easily understand why the *P. tricuspidata* climbing on the vertical substrate can outlive the gale blowing and the storm beating forever. The possible reasons will be further discussed in detail later. Since the super adhesive property of the adhesive disc, I assert that it is absolutely possible in the near future to have an excellent synthetic option of designing and fabricating a few kinds of adhesives with a reversible physiological role in biomedical science and with a reversible conductive role in bioelectronics and bionics.

Tendrils of *P. tricuspidata* show a spectacular thigmotropic reaction leading to a permanent attachment to the stimulating substrate, the tendril either coiling around it or developing an adhesive disc for its attachment[28]. As early as 320 years ago, Malpighi first accurately described the tendril of the *Virginia creeper* in the chapter 'de capreolis et consimilibus vinculis' from his 'opera omnia'[3], whose record was later extended macroscopically by Darwin[2] and Ewart[29] and microscopically by Mohl[30], Lengerken[31] and others[32]. Until 30 years ago, two groups reported scanning electron microscopic studies on *P. tricuspidata* describing the surface structures of the tendril stalks and the developing adhesive discs[28, 33], which confirmed and extended previous findings on the morphology and development of the tendrils as well as the synthetic site of an adhesive fluid, mode of secretion, and nature of the adhesive substance[34]. Meanwhile, the cytochemical studies on the developing adhesive disc of tendrils and adhesion of tendrils were reported [33].

Each tendril is made up of a main axis with five to nine branchlets alternately attached (Fig. 1 e arrows), each of which has a minute stipule-like scale located at the base[28]. At the tip of the branchlets there is a small swelling, which after the stimulus of contact develops into an adhesive disc (Figs. 3 a and 4 a). The epidermal surfaces of the stalk cells appear a wrinkled structure of longitudinal corrugations called microplicae (Fig. 4 a, b) and they even show on the tip of the finger-like cells terminating the scale. However, epidermal cells of the young branchlets are found lacking these ruffled structures and appear to be rather smooth (Fig. 4 b), thus indicating that microplicae is a structure of only fully developed epidermal cells. Near the distal end of the scale a few epidermal cells lack microplicae. A few stomata instead of hairs are seen on the tendril stalk (Fig. 6 a arrow). The branchlet appears to be a warty protrusion protected by a scale and finally bursts. The preformed swollen tip, i. e. the young adhesive disc, is respectively uncovered and covered (Figs. 3 a and 4 a). During the subsequent development, the branchlet elongates and the epidermal cells of the adhesive disc begin to swell and finally develop into a round shape, leading to the compact appearance of the hemispherical cells (Fig. 4 c, d)[28].

Prior to contact stimulation, the tip of the tendril is bulbous and is composed of a central area of largely parenchymatous cells encircled by a peripheral area with three to six layers of cells which in the early development are approximately isodiametric in shape [33]. When stimulated by contact, most of the epidermal surface of the adhesive disc is found covered by an adhesive fluid (Figs. 3 a, b and 4 a, b, c), secreted by the epidermal cells and indirectly confirmed by contaminated stains (Fig. 3 b arrow) sticking to the surface after withdrawal of a tendril from a substrate. The fluid seems to harden when exposed to air, as indicated by cracks in the covering layer (Fig. 4 b, d). Around the regions of contact with the substrate the peculiar morphology of the hemispherical epidermal cells is seen. The cuticle is folded (Fig. 3 e); at high magnifications these spheres are found to be swelling of the cuticle rather than objects sticking to the surface (Fig. 3 e arrow). Exudation of the sticky fluid is affected by a micro-hole on each cuticular bleb (Fig. 3 d arrow). The tendril tip expands greatly in size (Figs. 3 a and 4 a), and when firmly attached to the substrate, the tendril stalk contracts spirally (Fig. 1 e arrows). During the development of the adhesive discs, a striking epidermal activity is examined. Epidermal cells in contact with the substrate become extremely elongated (Fig. 3 f arrows), and sometimes periclinal cell divisions are seen. These elongated cells thus form a bursh-like pattern and force themselves into all the depressions of the surface of the substrate; in this way they tie up the whole branch. The remaining compactly distributed epidermal cells swell and divide anticlinally, thus forming a pattern like a cluster of balloons (Fig. 3 f). Most of experimental results are well agreement with those reported thirty years ago[28]. However, to the best of my knowledge, the sponge-like micro-holes with sizes of 5 ~ 40 μm seen around the central area (Fig. 3 c) and some micro-holes sealed by the wrinkled epidermal cells (Fig. 4 e arrow) were never reported in the previous studies[28, 33~35].

Cell walls of mature tendril branchlets of *P. tricuspidata* are reported to possess the same complex wrinkled structure as the previous descriptions[28]. It should be pointed out that very young epidermal cells on the branchlets do not possess this ruffled surface, but appear to be smooth, indicating that microplicae occur only at cell maturity. Furthermore, the morphology of the surface structure of epidermal cells of the adhesive disc is quite different. The tendril branchlet shows the main stalk as a warty protrusion covered by a minute stipule, which finally bursts. Only a few stomata and no hairs are shown on the tendril branchlets. The adhesive disc can be seen as a preformed ovoid swelling with highly folded epidermal cells, bearing no resemblance to the epidermal structure of the branchlets. Subsequently, the development of the branchlet through cell divisions and elongating of the stalk cells, or epidermal cells of the adhesive disc are seen to swell, and the adhesive disc finally appears with smooth hemispheres densely packing the whole surface. During this stage, the tendril becomes mature, perceive and respond to a contacting stimulation. Several hours after contact, debris from the substrate adheres to the adhesive disc, making eminent researchers[2, 3, 31, 36] suggest that an adhesive fluid, named 'therebinthina' by Malpighi[3], be secreted. The existence of such a viscous fluid was indirectly confirmed by Darwin[2], indicating that attached debris were loosened partially by a day immersion on sulphuric ether, and completely detached in warmed essential oils of thyme and peppermint. A light microscopy experiment suggested that the content of epidermal as well as subepidermal cells of non-attached adhesive discs stained with aniline glycerine gave an intense mucus reaction, and concluded that both of these cell layers contained great amounts of dextrin[31]. Furthermore, it was confirmed that, after a certain time of contact between adhesive disc and substrate, the mucus was secreted from the cell lumen into the space between the cell wall and the cuticle, resulting in the loss of staining of cell lumen and the positive dark-red reaction between the cell wall and the swollen cuticle[28]. Finally, the cuticle burst and the mucus exuded. Positive staining reaction of glass rods to adhesive discs adhered offered further evidence to the secretion of a fluid, which enables the adhesive disc to stick to a substrate[31]. These findings were demonstrated with the secretion of mucus into the space between the cell wall and cuticle appeared prior to contact and the exudation of mucus only after stimulation of contact.

Most of the surfaces of adhesive discs that have been in contact with a substrate for a few hours were covered with a substance, which seemed to have floated on the surface like a viscous layer and finally hardened, as visualized by the crackled surface (Fig. 4 b, e). At a beginning of contact, epidermal cells around the region of contact with the substrate were seen to have a peculiarly folded cuticle, which clearly suggested the prior presence of a substance had expanded the cuticle. Within the same area, a distinct area of blebbing epidermal cells could be seen. The number of blebs varied from fifteen to thirty per cell, and examinations at high magnifications indicated that the blebs were protrusions from the cuticle instead of foreign bodies attached to the surface (Fig. 3 e arrow)[28]. Exudation of fluid from the bleb is influenced by a hole, looking like a rupture of the cuticle rather than a pore (Fig. 3 d arrow). This assumption is made according to the reports[31], although a rupture of the entire cuticle is examined. Since the adhesive fluid hardens when excreted, it is tempting to suggest that the dark patches scattered on epidermal cells of young adhesive disc are premature excretions of mucus. In addition to the exudation of an adhesive fluid as the response to a stimulation of contact, the adhesive disc expands drastically, and the extensive swelling of the epidermal cells is seen, thus resulting in anticlinal cell divisions and a cushion-like adhesive disc being composed by numerous 'clusters of balloons' (Fig. 3 f). Cells in contact with the substrate become extremely elongated in the vertical direction and finally lead to periclinal cell divisions. This side of the cushion develops the appearance of a worn brush, more or less with the elongated rows of cells pointing outwards[28]. The early appearance of viscous fluid on the surface of adhesive discs stimulated by contact implies that the adhesion of the organ is first occasioned with this sticky sap, but the firm attachment to the substrate is possible to result mainly from the elongated epidermal cells, forcing themselves into all the depressions in the surface of the substrate and moulding the adhesive disc to the shape of the substrate. When firmly attached, the tendril coils and subsequently becomes thick and woody, leading to a considerable power of tension of the tendril and the adhesive disc. The tension is measured to be about 13.7 N (~ 3.1 *lb*), being greater 8.9 N (2 *lb*) discovered by Darwin[2], which will possibly open a spectacular study field to find the correlation between the adhesion of adhesive disc and the evolution of centuries of vines. To fully understand the relationship between the adhesion and the evolution of adhesive disc, more carefully designed experiments and scientific theories are obviously needed.

Scanning electron microscopic experiment of the tendrils reveals that the adhesive secretion occurs from the peripheral cells on the contact surface of the adhesive discs[33]. Moreover, ultrastructural and cytochemical studies on the developing adhesive disc and adhesion of tendrils suggest that the adhesives were possibly tanniniferous and mucopolysaccharide, with little or no protein or lipid. However, the dramatic expansion of the adhesive disc after a contact stimulus is presumably auxin-mediated[34]. Previous studies indicated that the adhesive substance moved through porous regions within the walls[31]; but, later investigations indicated that the development of a type pocket and the separation of the walls provided the route for those movements. Furthermore, the separation of the walls indicated the activity of hydrolytic enzymes, such as pectinases or cellulases. We experimentally found the sponge-like micro-hole regions (Figs. 3 c and 4 e arrow and 5) similar to those porous regions within the walls[31], the microtubules (Fig. 5 a, b, c, d) as the route for movements[33] and the stomata through the tendril stalk (Fig. 6 a arrow), all of which are useful for the auxin transportations and the adhesives movements elucidated in detail later.

SEM images of the mature and dry adhesive discs (Fig. 5 a) indicate a large number of microtubules with an average diameter of about 5 μm and sponge-like micro-holes with sizes of 5 ~ 15 μm (Fig. 5 b, c, d, e, f). The microtubules pass through the tendril stalk and stretch out to the micro-holes around the central area. The gap-wall of micro-hole is folded, which is similar to the wall of the spring-tube (Fig. 5 e). There have been common walls not only between the microtubules and microtubules but also between the micro-holes and micro-holes. In some microtubules the inner walls appear relatively smooth, but in other microtubules the inner walls appear bamboo junctions (Fig. 5 d). In addition, a lot of particles with diameters of 1 ~ 4 μm are found on the inner walls of micro-holes (Fig. 5 f). It is first found from a striking example that the extension paths of the microtubules seem like the expressways which extend respectively on the left, right and straight down (Fig. 5 c with *L*, *R* and *S* arrows). It is also found that the connections between the microtubules seem like the complicated expressway nets built in large cities (Fig. 5 c), which imply that the beauty and miracle can be created by a natural power. These results have never been reported in the previous papers[28, 33, 34, 37]. SEM images of the back side of mature and dry adhesive disc suggest that the wizen surface is very corrugated and rugged, whose surface seems like the drought farmland which forms crevices (Fig. 6) and bowl pits (Fig. 6 b). Very recent studies revealed that a cortical band of fiber cells originated in tendrils of vines when these convert from straight, supple young filaments to stiffened coiled structures in response to contact stimulation. The fiber cell wall is made up of a primary cell wall, two lignified secondary wall layers and a less lignified gelatinous layer proximal to the plasmalemma. The fiber cell walls are highly enriched in cellulose, callose and xylan but they have no homogalacturonan, either esterified or de-esterified. Lignin is concentrated on the secondary wall layers of the fiber and the compound middle lamellae primary cell wall, but it is absent from the gelationous layer. Therefore, researchers recently proposed a differential elongation theory (DET) that the fibers play a major role in tendril and adhesive disc function, which is further substantiated by the absence of gelatinous layers in the fibers of the rare tendrils of vines that fail to coil[37].

The examinations of accumulation of adhesive throughout the intercellular spaces and along the contact interface indicate that the adhesive is capable of flowing. However, it is unlikely that the very strong adhesion of the tendril can result from a fluid adhesive [33]. Further, the adhesive disc senesces soon after attachment and continuous production of the adhesive is not probable. It is believed that after the secretion of the adhesive substance, a chemical modification of this material is performed, i.e., it ‘sets’, ultimately producing a stationary adhesive binding the cells together and binding the tendril to a substrate. However, our recent findings indicate that the adhesion of the adhesive disc is very strong, which is in agreement with Darwin's discovery[2], rejecting the only other one untested speculation[33], supporting the proposal of a considerable power of tension of both the tendril and the adhesive disc[28]. The adhesive contains a large number of available acidic groups[34]. Further, these reagents are also reported to react with mucopolysaccharides and to have the possibility that the adhesive is a realistic mucopolysaccharide since such compounds apparently have an adhesive capacity in other systems similar to the zoospores of the alga. The chemical theory underlying the adhesive interactions has attracted intensive interests[33, 34, 38].

The geometrical positional array of the adhesive discs alternately attached along the tendril main axis is similar to the fingers of palm (Fig. 1 e arrows), which is helpful for the adhesive discs tightly grasping the substrate in view of the mechanics. For the thigmotropism of tendrils, a series of challenging problems have been perfectly investigated for nearly forty years[34]. The nature of most of these problems reveals a possible role for hormones. The specificity of the auxin transport system of suspension-cultured crown gall cells from *P. tricuspidata* has been examined with regard to indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2, 4-D), 1-naphthylacetic acid (NAA) and benzoic acid (BA)[35, 38]. IAA is a molecule synthesized by plants and a few microbes and plays a key role in root shoot development in plants. The hormone moves from one part of the plant to another by a designated importer and efflux pumps. In fact, as early as in 1875, Darwin described the rapid development of adhesive discs following stimulus as one of the most remarkable peculiarities possessed by any tendrils[2], which had been confirmed by Reinhold and his co-workers in 1970[34]. They finally proposed the mechanism that one of the events in the chain following tactile stimulus was a sharp increase in free auxin concentration in the tissue, that the increase in auxin concentration was responsible for the observed coiling responses, and that the coiling normally taking place along the entire length of the tendril and leading the stem closer to the substrate involves a supply of auxin translocated basipetally from the site of contact[34]. The study of *Saccharomyces cerevisiae* perceived the phytohormone IAA which at higher concentration grew but at lower concentration induced filamentation and adhesion. These responses are mediated by a family of transporters and the fungal transcription factor[39]. Boresh confirmed that the lateral displacement of IAA resulted from contact stimulus, which was analogous to the transverse transport of auxin examined after phototropic and geotropic stimulation. However, Bunning made no mention of hormones at all in the article on thigmotropism for the Encyclopaedia of Plant Physiology[40].

A striking example is found in the genus *P. tricuspidata* where the contact causes expansion, at the tips of thin thread-like tendrils, of adhesive discs which attach firmly to the substrate. It is well-known from a variety of systems that auxin induces the formation of a lignified xylem[41] and that tendrils in contact with a substrate become lignified[40]. Coiling has been shown by a few careful investigators to be the result of unequal growth on the two sides of the tendril[40]. It has been confirmed that contraction of the concave side appears mediated by a contractile protein during the initial stage. The anatomical examinations have revealed another asymmetric aspect of the response. This asymmetry is due to the appearance of lignin, which appears sooner on the concave side in tendrils stimulated by either contact or auxin[34]. Regarding the coiling, lignification and development of adhesive discs on *P. tricuspidata* tendrils, Reinhold and his co-workers have afforded strong support for a rise in auxin concentration as part of the thigmotropic response. Based on the known properties of mechanoreceptors in the animal kingdom, Reinhold confirmed that the contact stimulus rose from an action potential. Umrath reported the development of such a potential in tendrils after contact nearly a century ago. However, this does not seem to have been demonstrated in the climbing plants or other plants[34].

Although Darwin has devoted an entire monograph to the coiling and twining behavior of vines and tendrils for centuries[2]. When a tendril contacts a substrate, it rapidly wraps around the substrate, securely attaching to it as a support and guying the vine close to the substrate. A differential elongation theory (DET) proposed for the winding behavior of tendrils[37]. In the case of the redvine tendrils, the fibers act as a cylinder of tissue with the lignin distributed preferentially nearest to the contacted area, which ensure a greater ability to twist on the outer surface than the inner one, much as it is required of the net movement of the tendril. In the case of the *P. tricuspidata* tendrils, my experimental investigations are good agreement with those reports[42]. In addition, more subtle variations in wall composition across the cells in the fiber band, distribution and timing of lignification might allow for more subtle nuances and positioning of the coiling. These changes might allow for an enhanced flexibility, allowing the tendril tremendous latitude to successfully twine about substrates of diverse size and shape. Furthermore, it is well-known that gelatinous fibers increase the tendril, especially increase the adhesive disc strength of wood after drying [37, 42], which is supported by a conclusive evidence of a tension between the adhesive disc and the substrate obtained from our direct experimental measurements.

A classical embryological analysis has framed the concept of a developmental unit called the field, including the primary and the secondary fields[43]. It's all the same as a prediction made thirty years ago, the model should make it possible to assess its applicability to other developing systems, including not only animals but also plants, allowing the investigation of the cellular mechanisms involved. Considering the positional information theory[43], the attachment of adhesive discs alternately along the tendril main axis of *P. tricuspidata* is advantageous to the adhesion between the discs and the substrate, which is not only in accord with a symmetry-asymmetry rule in architectonics but also in agreement with a stable adsorption principle in surface physical-chemistry. Furthermore, the geometrical positional array of the attachment of adhesive discs alternately along the tendril main axis is a classical example controlled by an effect of structural mechanics. The geometrical correlations among the adhesive discs, tendrils and stems of *P. tricuspidata* are amazingly similar to that among the water faucets, branch water pipe and main water pipes in daily life. Based on our first direct measurements of the attachment, we propose a principium and audacity hypothesis as follows: the development of tendril cells is modulated and controlled by the primary field; after a contacting stimulation, the development of adhesive disc cells is modulated and controlled by the secondary fields[43]. In the adhesive discs, the constructions of netlike microtubules (Fig. 5 a, b, c) and sponge-like micro-holes (Figs. 3 c, 4 e and 5) are dominated by the cell development and cell division. The microtubules and micro-holes further hasten the mucus secreting, transporting and accumulating, with mucus containing auxins[34], lipis, proteins and carbohydrates[33]. The secreted auxins stimulate the cell to develop and grow, which accelerates the formation of the netlike microtubule and the sponge-like micro-hole. A complete circle system is set up in the tendril and adhesive disc of *P. tricuspidata*. Obviously, it is wondrously existence that the comparability of molecular mechanism can be used to explain cellular behavior between the animals and plants in terms of the positional information theory[43].

A century ago, Wilson discovered that the cells and cell clusters obtained by squeezing a sponge through the meshes of fine, silk and bolting cloth could reunite, and that aggregates obtained by this way could reconstitute themselves into functional sponges[44]. In 1939, Holtfreter found that tissue fragments from young amphibian embryos showed marked preference in their adhesive properties. He framed the concept of tissue affinities to describe these associative preferences closely related to normal morphogenetic events[44]. By subjecting a fragment of an amphibian gastrula to an environmental pH of about 10, he could cause the individual cells to separate and fall away from one another. Moscona opened the way for investigations with the cells of older avian embryos, and discovered that trypsin was effective in dissociating their tissues [44]. Our findings reveal that cells and cell clusters constructed functional sponges with micro-holes and microtubules (Figs. 3 c, 4 e and 5), micro-holes taking charge of auxin secretions and accumulations and microtubules taking charge of auxin transmissions and transportations. In addition, the sponge structures of the adhesive disc cells and cell clusters enhance the adhesive strength between the adhesive discs and the substrate in the view of structural mechanics, which is substantiated by our experimental investigations on the adhesive disc tension.

A few theories have been proposed to account for cell adhesion. The first one ascribes the celluar binding and recognizes events within the structures of a single tissue different in surface marker molecules. Chemoaffinity hypothesis is originally proposed to account for the exquisite mappings of neural cells in the central nervous system[45]. In spite of the attractions and the considerable supporting data, there has always been a number of persisting objections and gaps in the evidence to prevent their accepting the hypothesis completely. By contrast, modulation theories show that tissues will have only a few cell-cell adhesion molecules (CAM's), the corresponding number is perhaps equal only to that of major classes of cells and tissues[45]. Modulation theories are compatible with more noncommunal phenomenological or thermodynamic theories of adhesion but are not coextensive with them. For a especial adhesion system regarding the geck foot setae and spatulae[4, 5], large numbers of theories have been proposed to describe the mechanism of adhesion, including suction[6], friction[7], intermolecular forces[8], electrostatic forces[9], capillary forces[10, 11], van der Waals forces[5, 8, 12, 13] and skin gland glue[9, 14], among which, up to date, only the theories of van der Waals forces and the capillary forces are quite popular. To fully understand the cited theories is necessary for us to describe the adhesions of the adhesive discs and tendrils of *P. tricuspidata* in cellular and molecular level. Indeed, the evidence has already been afforded for three separate kinds of supramolecular systems mediating cellular interactions, including cell-cell adhesion mediated by CAM's, cell-substrate adhesion mediated by SAM's and cell contacts via intercellular junctions, i. e. gap and tight junctions and desmosomes. Each system consists of different gene products, but certain portions of each of these systems may act heterarchically with the others and mutually modulate their functions. So far, no example of the three systems has been examined within a single tissue in terms of formation, temporal appearance, and molecular interactions. However, a striking example of CAM-SAM interaction is provided by the conjugate relation between N-CAM expression and fibronectin appearance in the development of neural crest cells[45].

In the case of the tendril and adhesive disc, I think that chemoaffinity theories and modulation theories may basically give an interpretation on adhesion mechanism in cellular and molecular level. Moreover, I also assert that adhesion has an assistant effect via weak interactions such as suction force[6], intermolecular forces[8], surface electrostatic forces[9], capillary forces [10, 11] and van der Waals forces[5, 8, 12, 13]. After contact stimulation, at the beginning of adhesion, a series of experimental studies provide chemoaffinity theories with lots of support evidence[33, 34, 38]. When tendril firmly contacts substrate during the growing and developing of the adhesive disc, two of three separate kinds of supramolecular systems mediating cellular interactions, such as cell-cell adhesion mediated by CAM's and cell-substrate adhesion mediated by SAM's, are mainly cellular behavior. It is not difficult to find the mutual homeotic effect between modulation and chemoaffinity theories. In order to distinguish from chemoaffinity theories, I particularly propose a hypothesis based on a fact that the chemical reaction between the adhesive substances secreted and the substrate attached is supported by a direct microanalysis study[35]. It is well-known that most of auxins secreted are all weak acids[34, 38]. A very slow chemical reaction occurs at the interface between the adhesive disc and the substrate, which is quite hard to recognize by our naked eyes and common analyses. The chemical products of interface reaction acting as a micro-stuffing in molecular level can greatly enhance the adhesion between the adhesive discs and the substrate. Such an interface chemical reaction leads to an adhesive disc 'anchor' on the surface of substrate. Furthermore, I propose another model to describe the adhesion of adhesive disc. The tendril tip stimulated by a consistent contact with the adhesive discs growth and development and the secretions produced continuously, which leads to air encapsulate into the adhesive disc. During the growth and development, photosynthesis depletes the nitrogen encapsulated in the adhesive discs. Meanwhile, oxidation reaction of some reductive secretions depletes other oxygen encapsulated in it. Both the photosynthesis and oxidation reaction nearly uses up all air in the adhesive discs, resulting in a negative pressure in it and enhancing the adhesion strength between the discs and the substrate. This model is substantiated by my experimental studies on the microstructural examinations of the adhesive disc and tendril stalk finding the sponge-like micro-holes (Figs. 3 c, 4 e arrow and 5), microtubules (Fig. 5 a, b, c, d) and stoma (Fig. 6 a arrow), as well as the folded micro-hole walls and microtubule walls (Fig. 5 e). To the best of my knowledge, both hypotheses of an interface reaction leading to an adhesive disc 'anchor' and a nitrogen-oxygen suction resulting in a negative pressure have never been reported in the literatures[6~14, 45].

A simple dynamical model can generate reliably known sequence and geometries of compartmental boundaries in *Drosophila*, and can induce the proper developmental program in each region[46]. This model might be feasible to describe the control of sequential micro-hole formation in *P. tricuspidata* adhesive disc. During the development of adhesive disc, sequential commitment to alternative developmental programs appears in neighboring groups of cells, and is probably reflected by the formation of micro-hole boundaries which progressively subdivide the early tendril and the later adhesive disc. The successful chemical pattern model in predicting the locations and temporal order of micro-hole boundaries may result in other projects which show that a uniform mechanism may act throughout the development to determine the locations of successive developmental commitments. The successful binary combinatorial code forming via the chemical patterns considering transdetermination and homeotic mutants not only underscores the possibility that the logic of developmental commitments in *P. tricuspidata* is written in the binary code but also yields a new view of sequential commitments in early cell tissues, which is an open experimental examinations. This simple chemical dynamics model is supported by my direct measurements of sponge-like micro-hole structures[46].

Jones has developed a conception of cell adhesion based on the mechanochemical principle (MCP) involved in the contraction and relaxation of actomyosin-like protein with ATPase activity located at the cell surface[47]. It is found that the splitting of the exogenous ATP by a surface ATPase in the existence of calcium leading to an actomyosin at the cell contract surface to increases the rigidity of the cell surface, leading to little adhesives. A relaxing factor presents at the cell surface, and the effect of this factor on the contractile system at the cell surface may well govern the ability of the cell to send out probes, which initiates adhesions between cells. The cell surface will be relaxed sufficiently for the linkage sites to be exposed and the favour adhesion in the presence of calcium ions. The proposed hypothesis proves to be rational for the inhibitory effect of exogenous ATP on cellular adhesiveness and illuminates such debatable substance as the role of the cell-binging material obtained from the surfaces of sponge cells. Adhesion between surfaces ultimately depends on the balance between the attractive and repulsive force, and meanwhile the adhesion between cells has similar properties, inasmuch as the non-adhesive cell in contrast to its adhesive counterpart carries a high negative charge[48]. Curtis first revealed that the cell surface showed such discontinuous properties, and he developed a rheological theory of cell adhesion. According to the rheological theory, the surface expansion expected to increase adhesion by reduction the surface charge density, van der Waals forces being unaffected. However, the rheological theory was only explicable in terms of shear viscosity, which did not fully explain the driving forces of expansion and contraction. As for the adhesive system of adhesive disc of *P. tricuspidata*, the secretion and transmission of auxin is the greatest driving forces of expansion and contraction in terms of the rheological theory. Goldacre has proposed a contractile protein mechanism for amoeboid movement. Cell adhesion has an inverse correlation with surface negativity, and it is therefore possible to propose such contractile protein models for cell adhesion, including direct and indirect models for cell adhesion. Although Goldacre's theory proposed half a century, is only used for describing the amoeboid movement, the contractile protein mechanism is still used for the elucidating of the cell adhesion of the adhesive disc and tendril of the climbing plant, such as *P. tricuspidata*, redvine and other vines[37].

Protein tyrosine phosphatases (PTPs) is implicated in the control of normal and neoplastic cell growth and proliferation[49]. Receptor-like protein tyrosine phosphatases (R-PTPs) offer a novel family of transmembrane proteins that can transducer external signals by dephosphorylating phosphotyrosyl residues on intracellular substrates. Epidermal growth factor (EGF) is a small soluble protein that can bind to its receptor EGF-R, stimulates the latent tyrosine kinase activity in the cytoplasmic domain of the receptor and sets off a cascade of biochemical events, eventually leading to mitogenesis and cell proliferation[50]. The mechanism of Golgi apparatus reoriente inside the bound secretory involves the well-recognized association of the Golgi apparatus with the microtubule organizing center

(MTOC) inside interphase cells. Upon the receipt of the appropriate signals emanating from the region of cell-cell contact, it is possible that some of the microtubules, with one end already connected into the MTOC, become attached by the other end to the cell membrane at the cell-cell contact region. These microtubules can be stabilized by such dual attachment, compared to the other microtubules in the cell that are in rapid polymerization-depolymerization equilibrium. In the tendril and adhesive disc system, on receiving the appropriate signals emanating from the region of cell-cell contact in tendril, some microtubules pass through the tendril stalk with one end connected to the MTOC and the other end attached the cell membrane at the cell-cell contact region in adhesive disc. These microtubules become quite stable with such dual attachment, and then exert a tension on the MTOC. These forces can thereby reorient and perhaps pull the MTOC along with its associated Golgi apparatus towards the cell contact. In this process the cell nucleus surrounded by the microtubular cytoskeleton can re-set their position within the cell. Secretory vesicles derive from the Golgi apparatus can be tracked along the stabilized microtubules attached to the cell membrane at the cell-cell contact, and fuse with only that region on the membrane[50].

In summary, it should be an interesting to understand *P. tricuspidata*, including its' amazing climbing ability, important pharmacology role, tenacious vitality and inimitable environment protection function. To fully understand a secretion, transmission, accumulation and metabolism of auxin may open a recency study on the metabolism, transportation and transformation of nanoscale druggery in the system of plant and find the application of nanotechnology in the biological genetics and evolution. Particularly, I affirm the existence of a signal tunneling effect that is divided into a few kinds dealing with chemical, biological, mechanical, electrical and so on during the organ growing and developing in climbing plant. Using *P. tricuspidata* as a raw and processed material to carefully determine its microstructure and function of adhesive disc organ will prompt a wide and deep study on biomimetic and bioinformational materials and biomimetic device. More experimental and theoretical work is obviously needed to fully re-understand not only the microstructure and adhesion mechanism of adhesive disc but also the correlation between the adhesion strength and the adhesive disc structure. So, I believe that this paper is quite useful for future studies.

Methods

Scanning electron microscopy

Adhesive discs of *Parthenocissus tricuspidata* were collected from building walls in Guangzhou, China. Samples were prepared completely according to a standard method described in literatures[28, 33, 35, 37]. The dried specimens were mounted on specimen stubs with conducting silverglue, set in a vacuum evaporator with a rotating stage and coated with carbon and then with gold. Scanning electron microscopy experiments were performed at 10 kV with a Philips FEI XL-30 ESEM.

Mass and attached area estimation of a single adhesive disc

Mass measurements were performed with a homemade electronic-balance Acculab SG (Beijing, China). A group of 5 specimens with stem, leaf, branchlet, tendril and adhesive disc were collected together from representative region, and their total mass were respectively measured. Each specimen included 5~14 tendrils and 22~85 adhesive discs respectively. The mass of both tendril and adhesive disc was measured, followed by the measurement of the mass of the adhesive discs. The average mass of a single adhesive disc was finally calculated from the measured data of the 5 groups including more than 250 adhesive discs. The average attached area of single adhesive discs was measured from SEM images regarding the attached region. Reported data were calculated from 13 adhesive discs observed.

Force estimation of a single adhesive disc

A homemade resiliometer Zhun Zi M-6B (Guangzhou, China) was used to measure the adhesion force of a single adhesive disc. Breaking or detaching force was defined as the minimum pulling force or as the maximal force an adhesive disc could exert attach to a substrate immediately before it released. We determined this value for individual adhesive disc by measuring the force. In all trials, detachment force was calculated from the maximum displacement of the adhesive disc by the resiliometer and the readings were recorded. In this measurement we chose 13 representative sites and then collected 13 adhesive disc specimens for SEM observation and attached area determination.

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Figure Legends

Figure 1 *Parthenocissus tricuspidata* climb along various substrates.

Figure 2 **A** and **B**, Shear stress of natural gecko, mimic gecko foot tapes and *P. tricuspidata*. **a**, natural gecko foot[12]; **b**, gecko toes on GaAs[13]; **c**, gecko toes on Si[13]; **d**, polyimide gecko tape[17]; **e**, carbon nanotube gecko patch[18]; **f**, carbon nanotube gecko tape[19]; **g**, *P. tricuspidata* adhesive disc[2, 28]; **h**, *P.tricuspidata* adhesive disc (this work). **C**, Based on the shear stress (1124.6 N/cm^2) of the adhesive disc (**h**) and the attached area (~1 cm^2) of a fingertip of palm, a mimic palm made of adhesive disc material and attached only with a fingertip can sustain a body weight of about 114 kg. **D** and **E**, A mimic palm made of various materials fully attached to substrate can sustain a weight equal to animal's body weight under it. **i**, polyimide gecko tape[17], 61 kg chimp; **j**, natural gecko foot setae[12], 204 kg bear ; **k**, carbon nanotube gecko patch[18], 238 kg lion; **l**, carbon nanotube gecko tape[19], 734 kg urus; **m**, *P. tricuspidata* adhesive disc[2, 28], 14,885 kg shark; **n**, *P. tricuspidata* adhesive disc (this work), 22,951 kg cachalot.

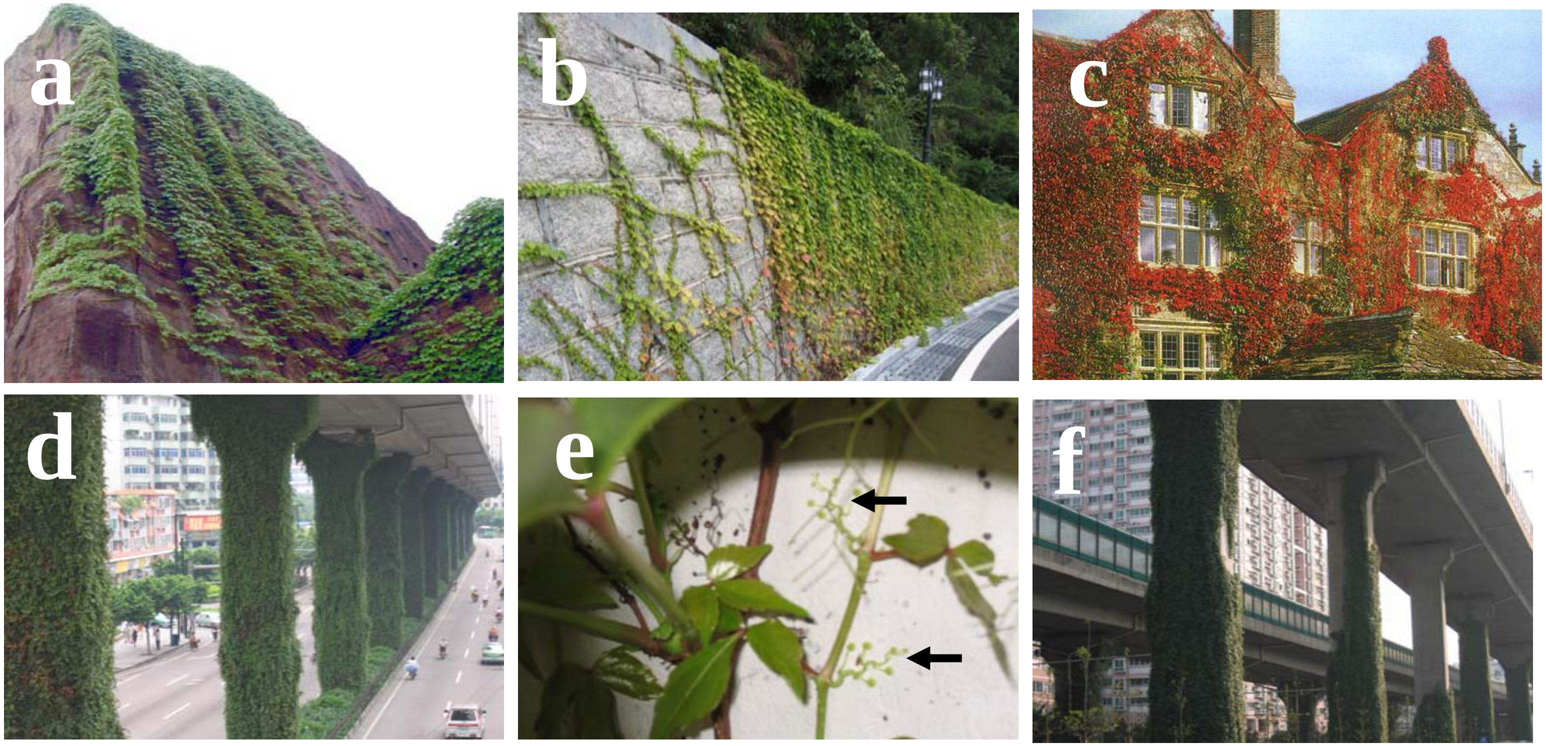
Figure 3 SEM images of immature adhesive discs of *P. tricuspidata*.

Figure 4 SEM images of immature adhesive discs (backside) of *P. tricuspidata*.

Figure 5 SEM images of mature and dry adhesive discs of *P. tricuspidata*.

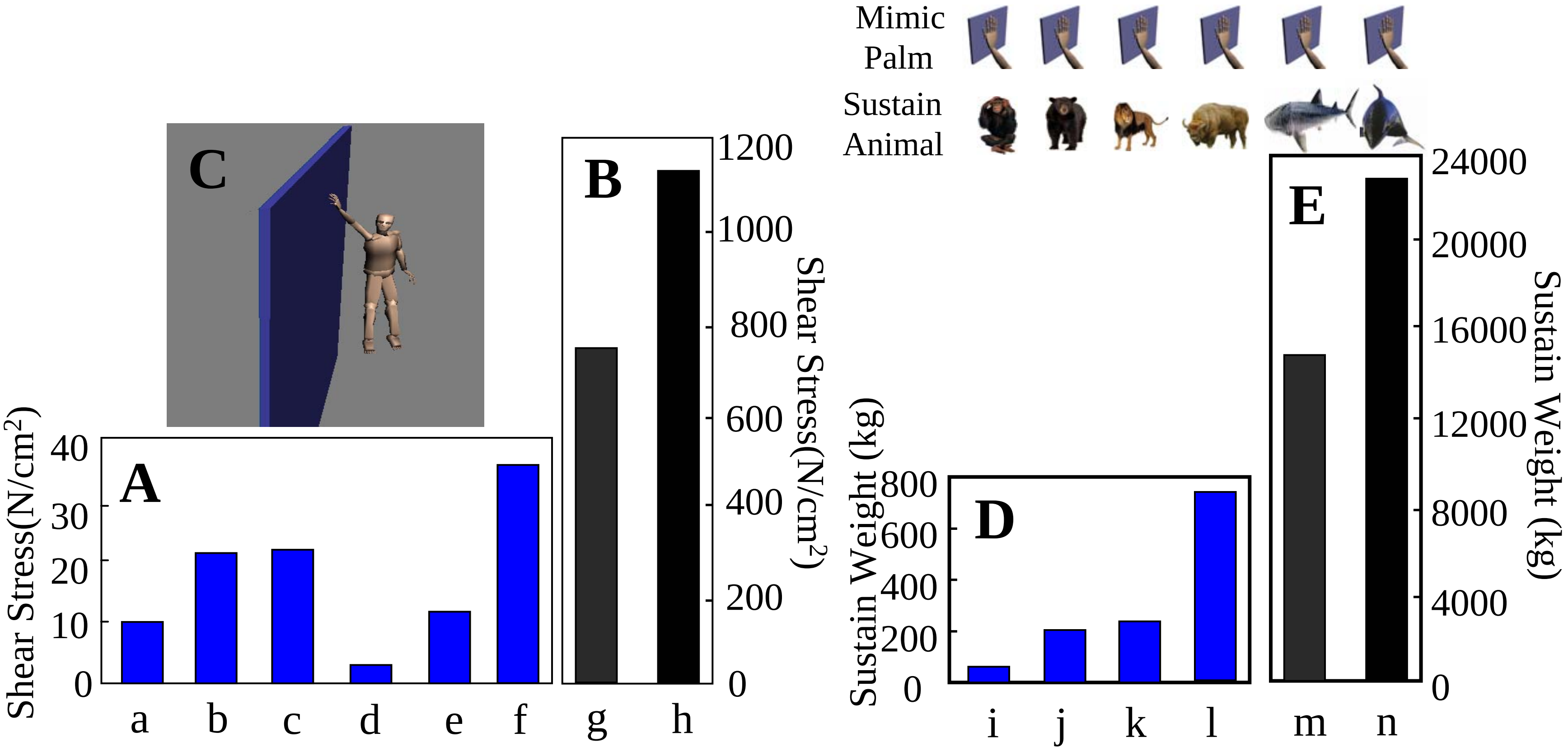
Figure 6 SEM images of mature and dry adhesive discs (backside) of *P. tricuspidata*.

Figure 1



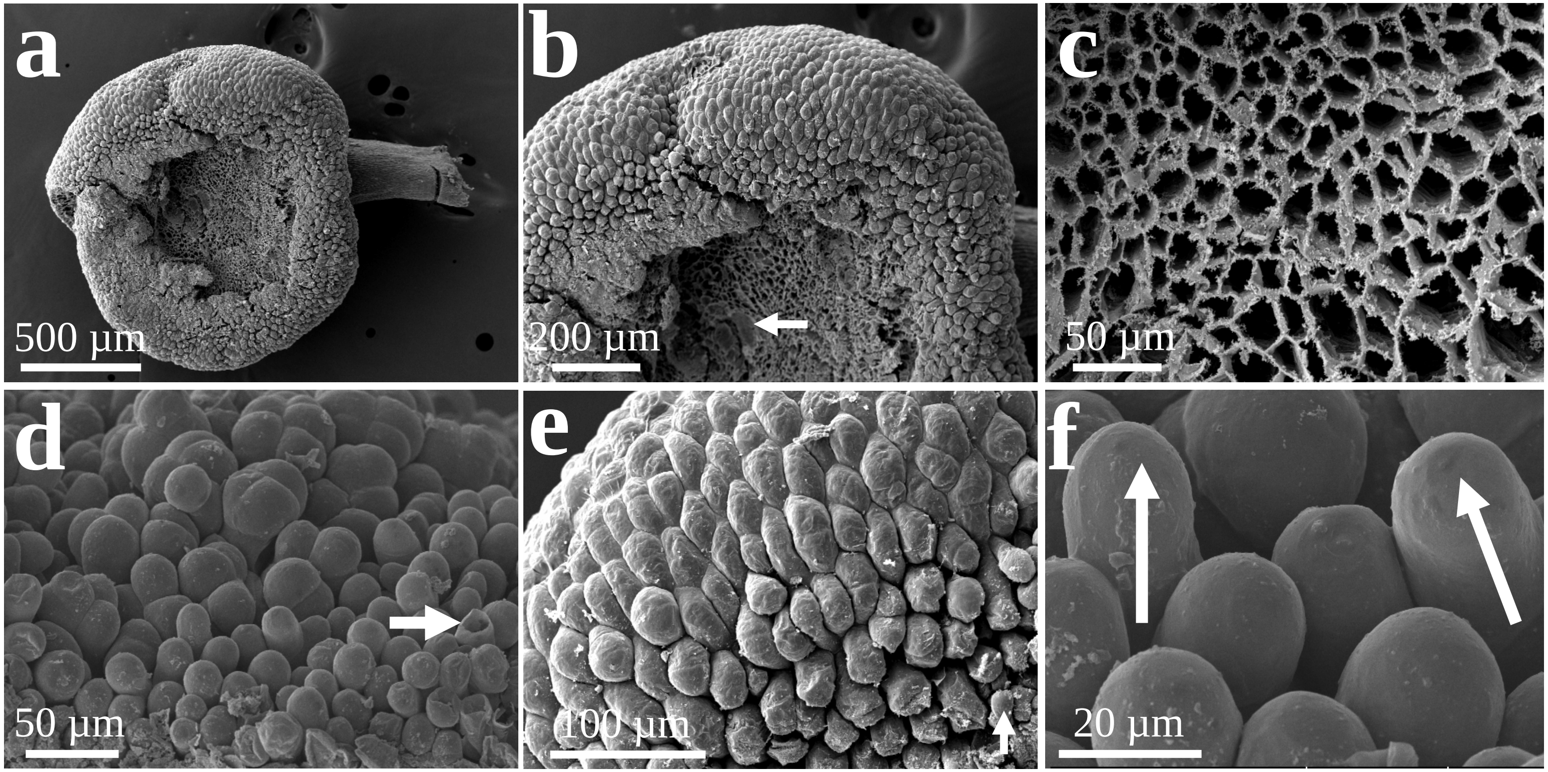
Wenli Deng

Figure 2



Wenli Deng

Figure 3



Wenli Deng

Figure 4

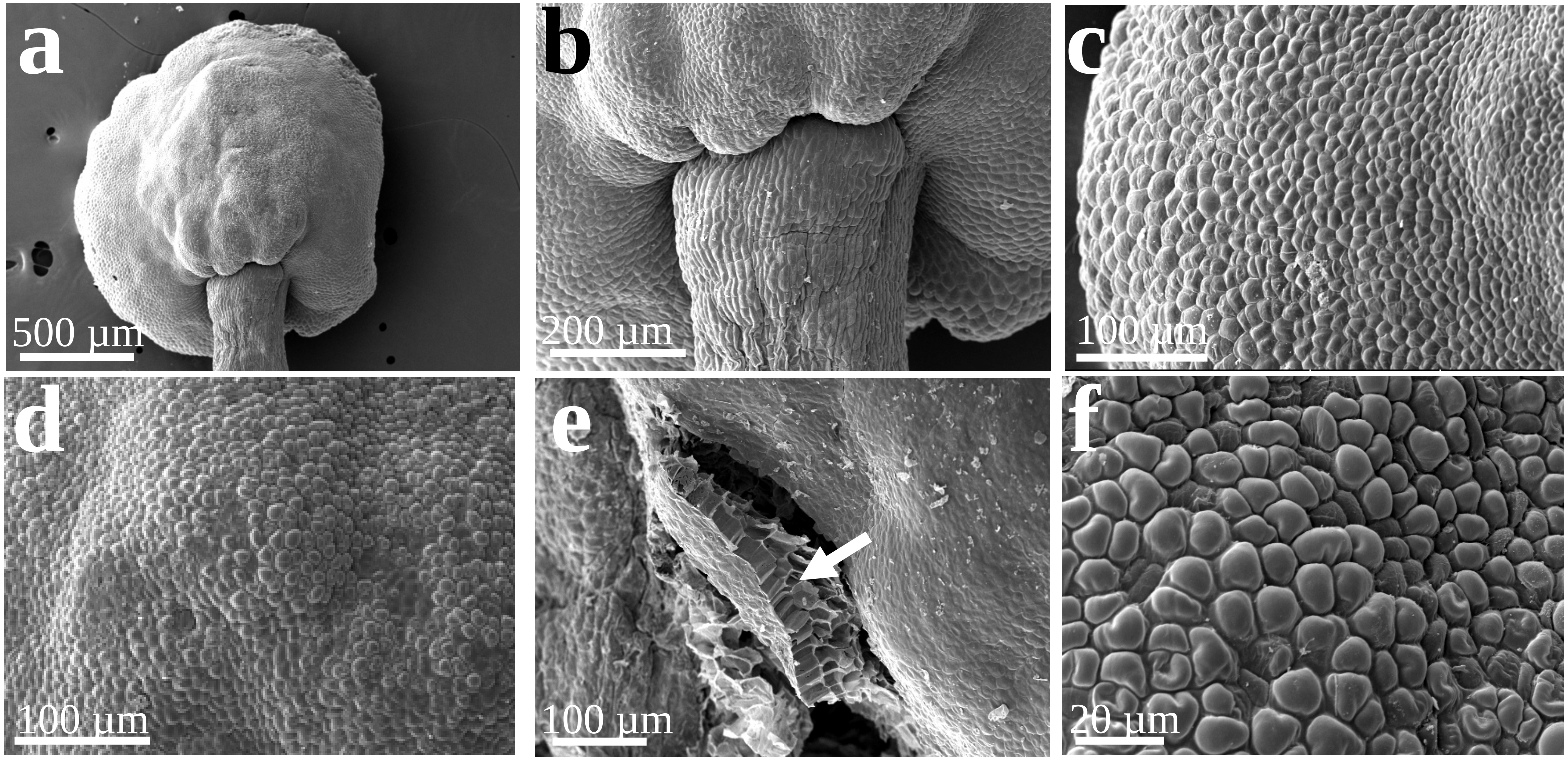


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Figure 5

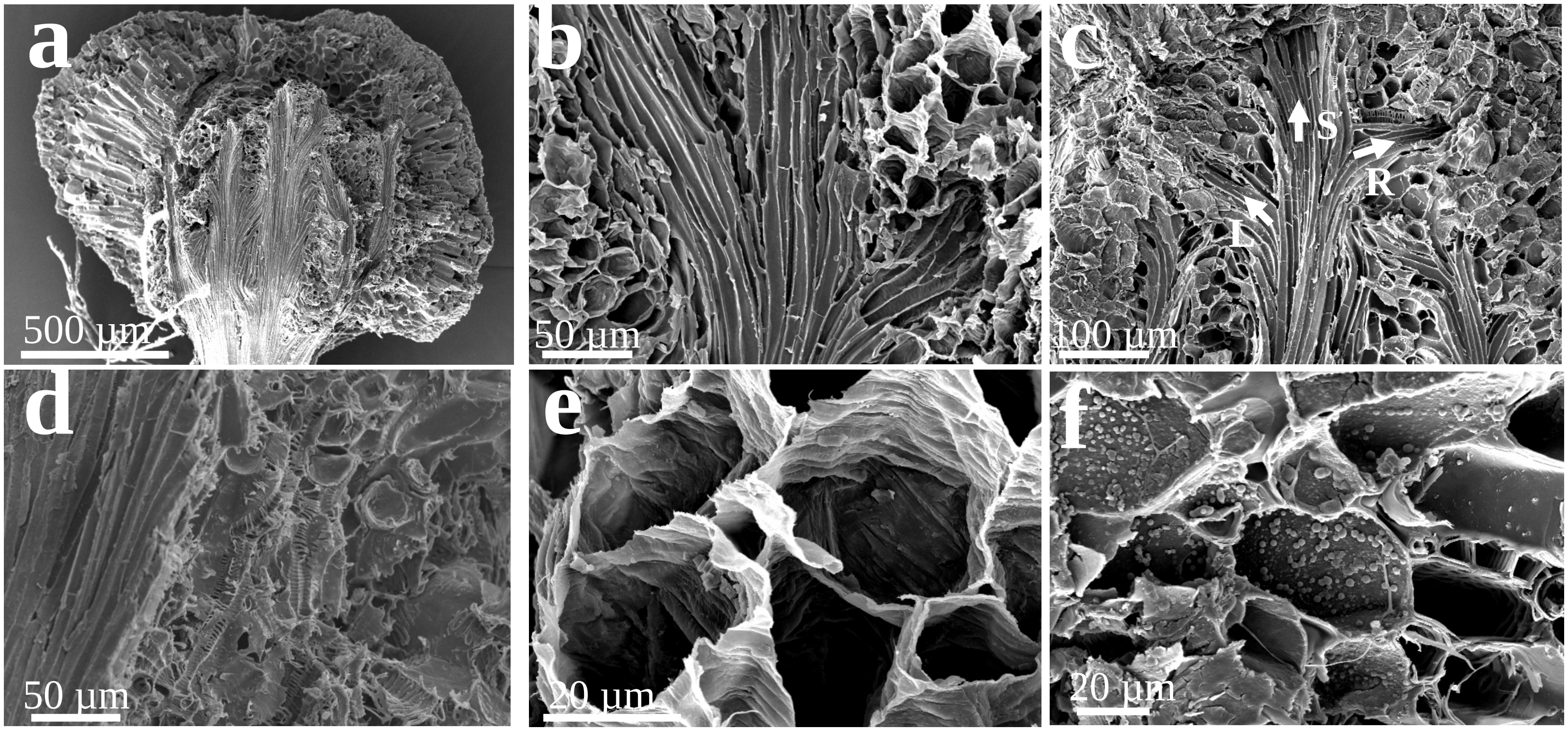

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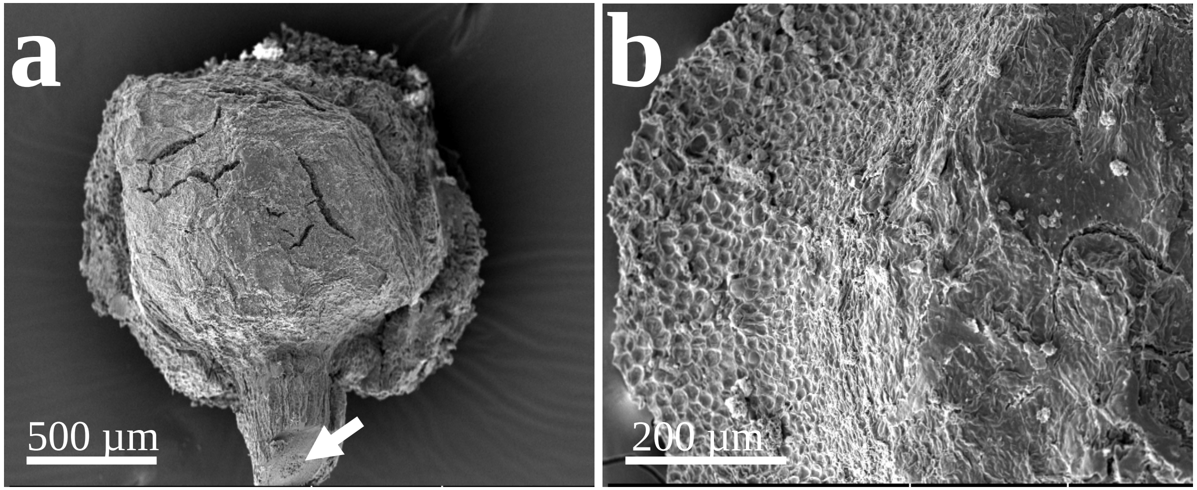
Figure 6



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