Short Communication

# Investigation of the microstructure and mechanical properties of the domestic cat's paw pads

**Ye Mei[1*]**

### *Abstract*

Domestic cats have a biological function such as cushioning when they are jumping or walking which is mainly due to the biological coupling system formed in the long-term evolution process of nature, including the trunk-leg-paw pad structure. Among them, the paw pad, as the only part of the body that directly contacts with the ground in the process of movement, plays a key role in its biological function. Hence, in order to clarify the micro structure, mechanical properties and functional characteristics of the paw pad and reveal the relationship between it and the domestic cat's biological function, this paper has carried out an in-depth research on the histological structure and mechanical properties of the domestic cat paw pad, including observation by tissue staining, scanning electron microscopy and quasi-static compression test. The results show that: the histological structure of the paw pad presents a three-layer coupling structure and has different material mechanical properties, such as the rigid elasticity of the stratified epithelial layer, the viscoelasticity of the dermis layer and the hyperelasticity of the subcutaneous tissue layer. At the same time, the paw pad of the domestic cat has the characteristics of variable stiffness and the stiffness value is low in the early stage of contact, which is conducive to the dissipation of impact energy. It can be seen that the microstructure and mechanical properties of the domestic cat paw pad are of great help in the realization of its function.

**Keywords:** domestic cats, paw pads, microstructure, mechanical properties

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## Introduction

After a long process of natural optimal selection, cats have formed their unique excellent sports ability to survive and reproduce, which makes them more adaptable to a harsh and complex living environment (Brad JF *et al*., 2015). The max-peak grounding reaction of their limbs after jumping from a height to the ground is often more than 6 times their body weight (Matijevich E *et al*., 2019). However, the limbs of domestic cats are rarely injured. Besides, compared with dogs of the same size, the impact noise of a domestic cat when it comes into contact with the ground is much smaller (Huaibin M *et al*., 2017; Motoshi K *et al*., 2006). It can be seen that domestic cats have strong biological function characteristics of cushioning when jumping and walking, which is mainly due to the efficient coupling biological cushioning system including the trunk-leg-paw pad structure formed in the long-term evolutionary process of nature. Among them, the paw pad of the domestic cat, as the only part of the body that directly contacts with the ground in the process of movement, plays a key role in its biological function (Gustas P *et al*., 2001 ; Honert EC *et al*., 2019). The paw pad has the following different mechanical characteristics when it contacts with the ground in the process of movement: on the one hand, in the early stage of contact with the ground, flexible deformation is used to effectively store or dissipate the impact energy from the ground to achieve the purpose of buffering and damping; On the other hand, in the later stage of contact with the ground, rigid deformation is used to fit closely with the ground to support the body and at the same time, the power needed to move forward to the ground is generated to maintain movement. When the cat's paw pad contacts the ground, it will produce a significant impact force and the resulting shock wave will enter the limbs, which may cause limb or body injury in the case of severe exercise. Therefore, in order to absorb shock and reduce shock wave strength, it is necessary to perform joint flexion and extension and other soft tissue deformation. In this process, paw pads play an important role in energy dissipation and the protection of foot bones and other tissues (Huang G *et al*., 2000). The function of the paw pad to transfer the ground support force and absorb impact energy has attracted the attention of researchers. Alexander (Mirela RV *et al*., 2013) carried out experiments on the compression characteristics of various mammalian paw pads in vitro. It was concluded that paw pads should have a variable mechanical properties to prevent the phenomenon of instantaneous foot off the ground caused by excessive peak ground reaction and shock vibration, because this phenomenon may make the animal's movement extremely unstable. Chi KJ (Chi KJ, 2005)has done a lot of research on animal paw pads and human heel parts and has concluded that paw pads should have different mechanical properties for different movements of animals and the human body: in order to effectively reduce the impact force, the paw pads should have a certain degree of flexibility; In order to transfer the ground supporting force, it should have a certain stiffness; in order to contact with the ground stably, it should have a certain degree of damping; and, in order not to consume too much energy in the process of touchdown, it should have good elasticity. The realization of different biomechanical properties of the domestic cat paw pad is closely related to its microstructure composition, layout and material mechanical properties. However, there have been few related studies and reports and previous studies have not given quantitative results on the impact of the relative size relationship of the microstructure and mechanical parameters of the paw pad on the buffering characteristics of cats.

The purpose of this study is to clarify the micro structure, material composition and functional characteristics of domestic cat paw pad, and reveal the relationship between the micro structure, mechanical properties and biological functional characteristics of domestic cat paw pad; the in-depth study on the tissue structure and mechanical properties of domestic cat paw pad will be carried out, including observation by tissue staining, scanning electron microscope and quasi-static compression test.

## Materials and Methods

***Ethical statement:*** All procedures used in the animal experiments were approved by the Faculty of Veterinary Science Animal Ethics Committee of the Jiangsu University (No.2019-0016).

***Domestic cats included for the experiments:*** Four domestic cats were used in this study, among them, two adult cats that had died of cardiovascular disease were used for histological experiment including the observation of tissue staining and scanning electron microscope and other two healthy adult cats were used for quasi-static compression experiment. In addition, none had recorded histories of musculoskeletal disorders and their feet were intact and healthy.

***Experimental content and procedures***

***The histological experiment:*** The histological experiment on the tissue structure of the domestic cat paw pad mainly includes the following two aspects: one is observation of and research on the tissue staining of domestic cat paw pad, the other is observation of and research on the tissue scanning electron microscope of the domestic cat paw pad. The fine structure of the internal tissue of the cat paw pad can be obtained by the observation of tissue staining, and the internal fine structure can be further magnified by the observation of tissue scanning electron microscope of cat paw pad.

***(1) Observation of tissue staining:*** Observing and studying the tissue staining of the domestic cat paw pad can help to clarify the fine structure of its internal tissue from the microscopic point of view. In this section, the tissue sections of the domestic cat paw pad are stained by the HE staining method and then observed by biological microscope. The specific operation process is as follows:

①Tissue section of paw pad: After cutting the front and back paw pads from the limbs of domestic cats and depilating treatment (as shown in Fig.1a), they are soaked in 4% paraformaldehyde solution at room temperature for 12 hours to achieve the purpose of fixing the original shape of paw pad tissue cells and tissues, Then, several 1 mm thick slices were cut from the four digital pads and palm pad of the cat paw pad (as shown in Fig.1b);

②Dehydration and transparency of slices: In order to facilitate the penetration and observation of the staining agent, the slices needed to be dehydrated and transparent. The obtained slices were soaked in 80%, 90%, 95% ethanol solution and anhydrous ethanol solution for 1-2 hours respectively to achieve the purpose of dehydration and then soaked in xylene solution for about 30 minutes to make the slices transparent.

③Paraffin impregnation, embedding, slicing and baking: The dehydrated and transparent slices were immersed in the heated paraffin and placed in the incubator at 60 ℃. After about 2 hours, the paraffin was completely immersed in the slices, and then the slices were embedded by an automatic tissue embedding machine and then cooled and solidified into blocks. Among them, the purpose of paraffin section was to use paraffin to fill the gap caused by the dissolution of lipid substances in the process of tissue dehydration, so as to play a supporting role and ensure that there was no deformation in the tissue during subsequent cutting. Then, the solidified slices were fixed on the slicer and 5 pieces cut out and the thin sheet was hot water ironed and dried in a 45 ℃ incubator.

④HE staining: Firstly, paraffin in 5μm thick sections was removed with xylene solution and then washed with a high concentration to low concentration alcohol and distilled water in turn, i.e. staining agent could be used for staining. Hematoxylin and eosin staining solution were used as the staining agent. The washed slices were dyed in hematoxylin aqueous solution for about 2 minutes and then separated by acid water and ammonia solution. Then rinsed with running water for 10 minutes, distilled water added for a moment and then dye with eosin staining solution for about 5 minutes. Finally, the section repeated after staining ② after the section had been dehydrated and become transparent, the section was sealed on the glass plate with gum and the paw pad section sample for tissue staining observation and research could be obtained (as shown in Fig.1c).

⑤ Observation of section samples: The sections of paw pad were observed by stereomicroscope and optical microscope respectively. Among them, the stereomicroscope was used for low-power observation to obtain the whole picture of the sliced sample and the optical microscope was used for high-power observation to obtain the local appearance of the sliced sample, as shown in Fig.1d.

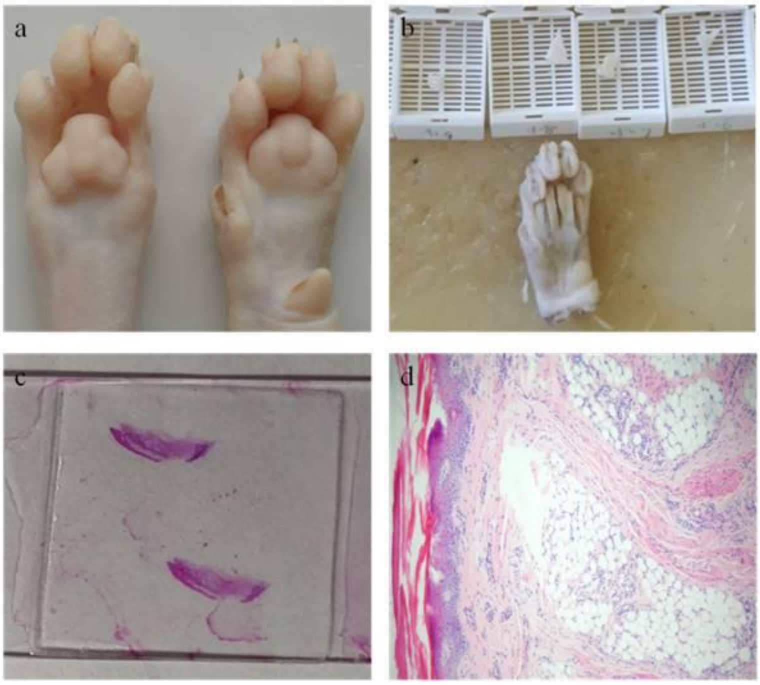


**Figure 1**

***(2) Study by scanning electron microscopy of tissue:*** The observation and study of cat paw pad with a scanning electron microscope can help us to understand the characteristics of its internal tissue structure. It is a deeper exploration based on the observation and study of cat paw pad tissue staining (Lascelles BD., 2007). The specific operation process is as follows:

①Acquisition of paw pad tissue section: With the help of a blade, the sections to be observed were cut from the digital pad and palm pad of cat paw pad and the shape of the section was a cylinder of 10 mm * 10 mm * 5 mm (as shown in Fig.2a). After that, the slices were washed with sodium chloride solution to remove the adherent blood and other impurities for scanning electron microscopy.

②Fixed, dehydrated and transparent slices: The slices washed with sodium chloride were immersed in 2% glutaraldehyde solution for about 1 hour, then washed with 0.1mol/l phosphate buffer for about 2 minutes and then immersed in 1% osmic acid solution for 1 hour; Finally, the slices were immersed in 80%, 90%, 95% ethanol solution and anhydrous ethanol solution for 1-2 hours respectively and xylene solution for about 30 minutes to complete the dehydration and transparency treatment.

③Slice drying: The fixed, dehydrated and transparent slices were immersed in hexamethyldisilane for 10 minutes to complete the drying treatment.

④Slice gold spraying processing and observation: the dried slice was put into the ion sputtering instrument and spray gold used to enhance the conductivity of the slice and improve the clarity of the image (as shown in Fig.2b); Then the sections could be observed by scanning electron microscope.

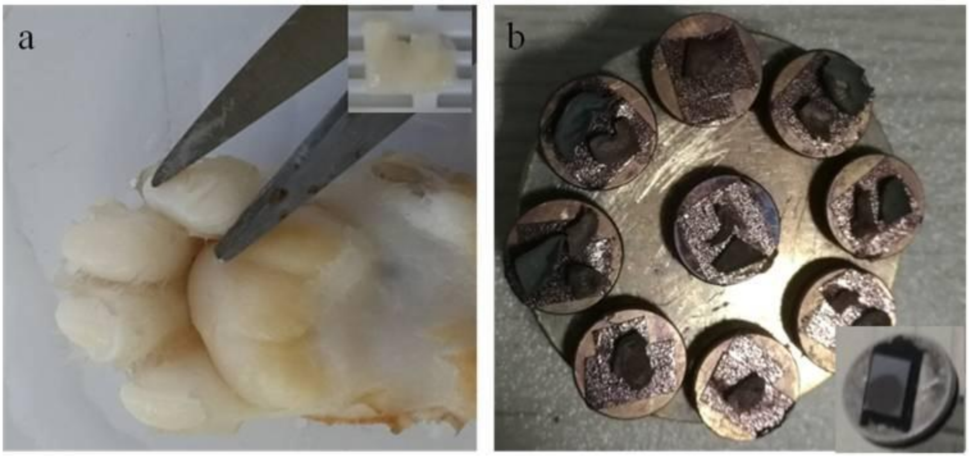


**Figure 2**

***The quasi-static compression experiment:*** In order to study the mechanical properties of the domestic cat paw pad, quasi-static compression tests were carried out on each meat pad of paw pad to obtain the mechanical response of meat pad under dynamic load. The WDW-20 micro control electronic universal testing machine was used for quasi-static compression test and the real-time recording and acquisition of test data was realized with the help of smart test, the supporting software of the testing machine. The test process is shown in Fig.3.

During the experiment, in order to ensure the validity of the quasi-static compression test data, two domestic cats were anesthetized for the experiment; During the test, a flat head indenter was selected to dynamically load the meat pad parts of the domestic cat paw pad, as shown in Fig.3 and the loading speed was set at 5mm/min; Based on the peak vertical force of the paw pad of domestic cat and the force of four digital pads and palm pad, the test machine was set up to automatically load the palm pad of paw pad to 20N, the digital pad to 10N and the force stopped. Each meat pad of the domestic cat was measured five times and then the data were statistically processed.

**Figure 3**

## *Results and Discussion*

***The histological experiment***

***(1) Observation of tissue staining:*** After observing the tissue staining of the paw pad section of the domestic cat, the tissue images of the metacarpal pad area and digital pad area of No. 1 and No. 2 domestic cat paw pad were obtained respectively as shown in Fig.4 and Fig.5. Preliminary comparison showed that the histological structure of the metacarpal pad and digital pad of two domestic cats were basically similar, showing multi-layer tissue coupling structure. Specifically, the outside to the inside of the paw pad can be divided into three layers: stratified epithelial layer, dermis layer and subcutaneous tissue layer. Among them, as the outermost layer of the paw pad, the stratified epithelial layer is relatively hard and can be divided into two parts: the keratinocytes (the red part of area ① in Fig. 4a1) and the non-keratinocytes (the purple part of area ① in Fig.4a1), which have the dual functions of bearing the friction and impact with the ground during movement and protecting the inner tissue structure; The dermis, as the middle layer (the pink area of area ② in Fig.4a1), is composed of dense collagen fibers arranged layer by layer, with high strength viscoelastic mechanical properties; The subcutaneous tissue layer (area 3 in Fig.4a1) is the most medial layer, which is mainly composed of a large amount of adipose tissue and presents a reticular fibrous layer structure. It is the most important energy storage and absorption layer in the paw pad tissue layer. Among them, the adipose tissue is composed of adipocytes, which are divided into circular chambers through collagen fiber membrane. They are closely connected with each other and have strong elastic properties. The mechanical properties can be regarded as equivalent to the static pressure damping system filled with incompressible fluid (Kim J *et al*., 2011； Pantall A *et al*., 2012).

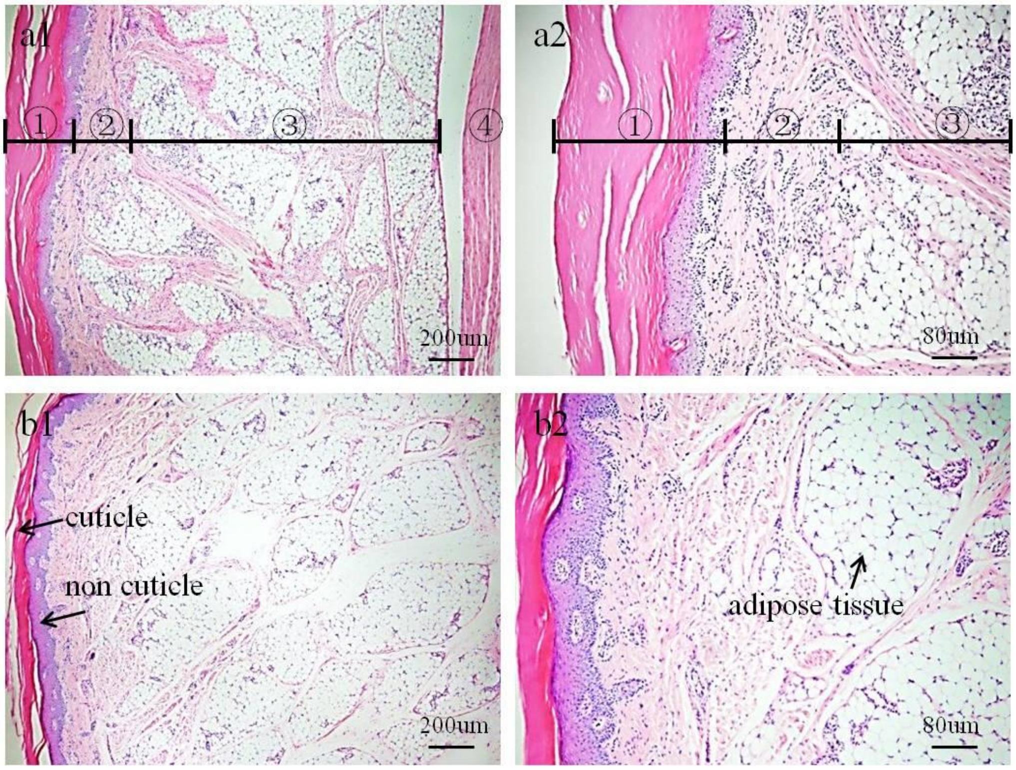


**Figure 4**

***(2) Study by scanning electron microscopy of tissue:*** In order to further study the characteristics of the three-layer coupling structure of domestic cat paw pad, the section images of different positions of the domestic cat paw pad were obtained as shown in Fig.6 by scanning electron microscope.

Fig.6a and Fig.6b show that the thickness of the dermis is thinner than that of stratified epithelium and subcutaneous tissue in the three-layer coupling structure of cat paw pad. It can be regarded as an intermediary layer to connect the rigid elastic stratified epithelium and the super elastic subcutaneous tissue. Thus, the gradient elastic mechanical properties of the cat paw pad were realized. For the stratum corneum in the stratified epithelial tissue layer, combined with the observation in Fig.6b and Fig.6c, it was found that the stratum corneum was mainly composed of layers of uneven cuticles, and the layers supported each other; According to the observation of Fig. 6b and Fig.6d, the tissue structure of the non-cuticle is characterized by hollow out geometric polygon solid column. At the same time, as the contact layer connecting with the dermis, the hollowed out part is filled by the mastoid of the dermis, thus forming a compound polygon solid structure with hard outside and soft inside, From observation of the results of tissue staining in Fig.4b2 and Fig.5b4, it can also be found that the pink dermis is embedded in the purple cuticle in various mastoid forms, forming a compact connected whole; As for the tissue structure of the dermis, as shown in Fig.6e, collagen fibers, as the main component of dermis, present an interlaced network structure, thus enhancing the tensile deformation characteristics of tissue; As the innermost layer of the histological structure of the cat paw pad, the subcutaneous tissue layer shown in Fig.6f presents a large number of fat chambers with fat cells as the matrix, which has strong compression deformation characteristics and can absorb and store a lot of energy.

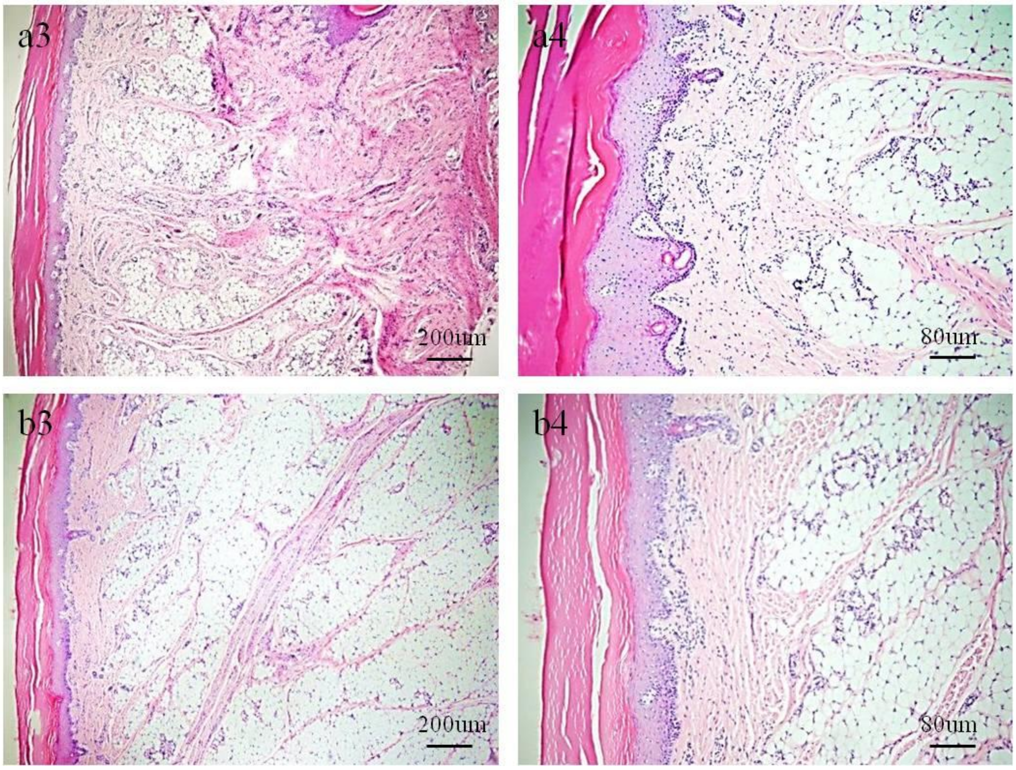


**Figure 5**

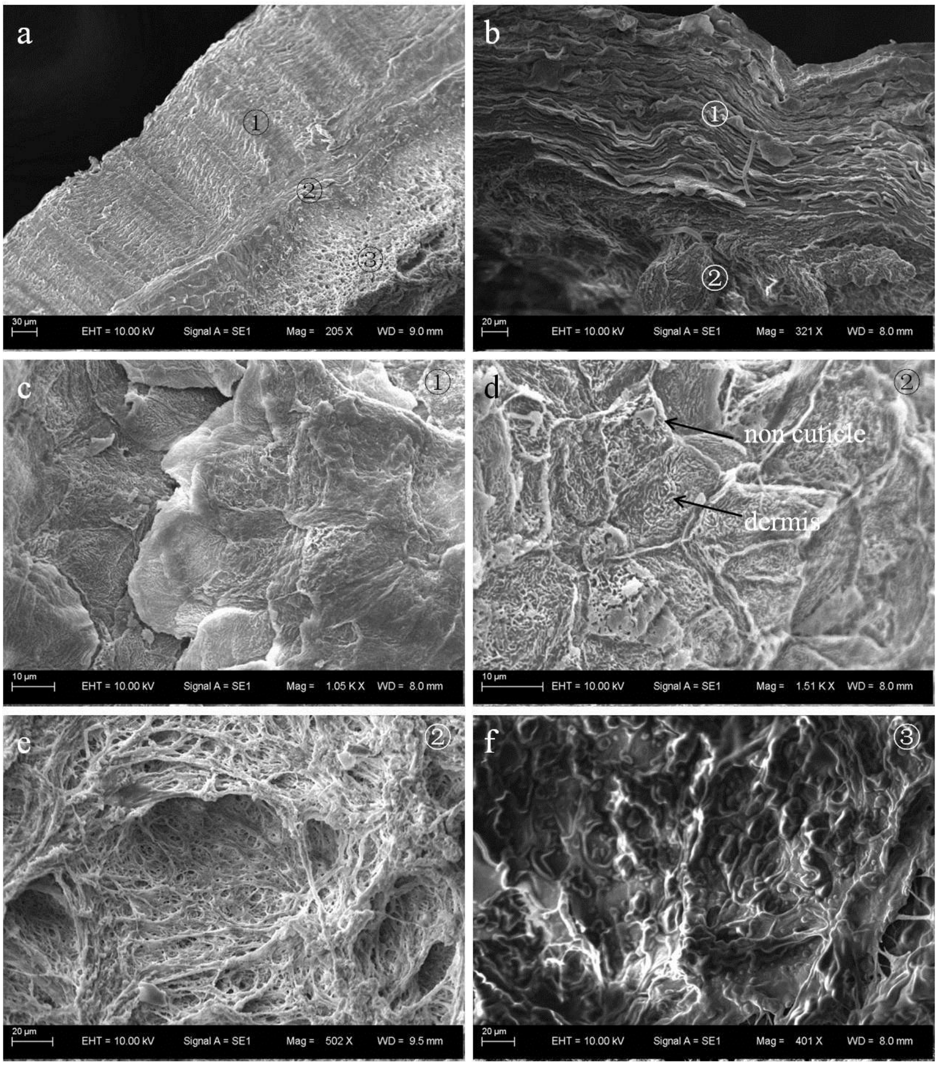


**Figure 6**

***The quasi-static compression experiment:*** Through the quasi-static compression tests on the palm pad and digital pad of the domestic cat, the load displacement curves of the meat pad are obtained as shown in Fig.7 and 8, in which the ordinate is the load, which is expressed by the actual value of the test, the abscissa is the displacement and the average value of multiple groups of test data is used ± Standard deviation.

It can be seen from the Fig.7 and 8 that the load displacement curve of domestic cat paw pad is characterized by nonlinear variation, that is, with the increase of the load on the paw pad, the displacement deformation decreases gradually, indicating that with the deformation of the paw pad, the stiffness value increases gradually and the domestic cat paw pad has variable stiffness characteristics. In order to further characterize the change of stiffness value of the domestic cat paw pad, it is found that the fourth-order polynomial function fitting method is more ideal to express the evaluation parameters of domestic cat paw pad displacement curve. The formulas are shown in (1) and (2), and the $R^2$ values are 0.99946 and 0.99968 respectively. Then, the first derivative of the fitting curve can be used to obtain the stiffness value of the face paw pad under a certain displacement value. The results are shown in Fig.9 and Fig.10.

$$y_1 = 0.276 - 0.83x_1 + 0.856x_1^2 - 0.268x_1^3 + 0.029x_1^4 \quad (1)$$

$$y_2 = 0.04 + 0.159x_2 - 0.325x_2^2 + 0.246x_2^3 - 0.019x_2^4 \quad (2)$$

According to the stiffness displacement curve of cat paw pad, the stiffness values of palm pad and digital pads show a nonlinear trend, which can be divided into two parts: the low stiffness value under small deformation and the high stiffness value under large deformation. For the palm pad and digital pad, when the displacement value is less than 5mm and 2mm respectively, the stiffness value is smaller. At this moment, the meat pad is elastic, which is conducive to the consumption of load impact through deformation, so as to play the role of vibration reduction and energy storage; when the displacement value is higher than 5mm and 2mm respectively, the stiffness value shows a trend of rapid increase. At this moment, the meat pad is full of strength, which is conducive to bearing the following heavy load, so as to support the body and firmly adhere to the ground.

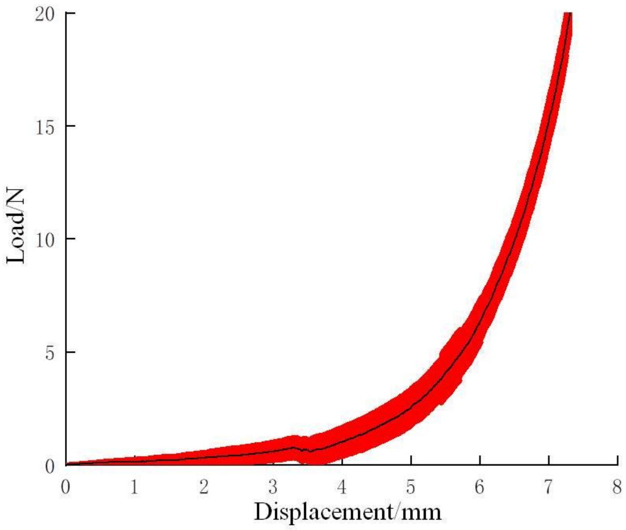


**Figure 7**

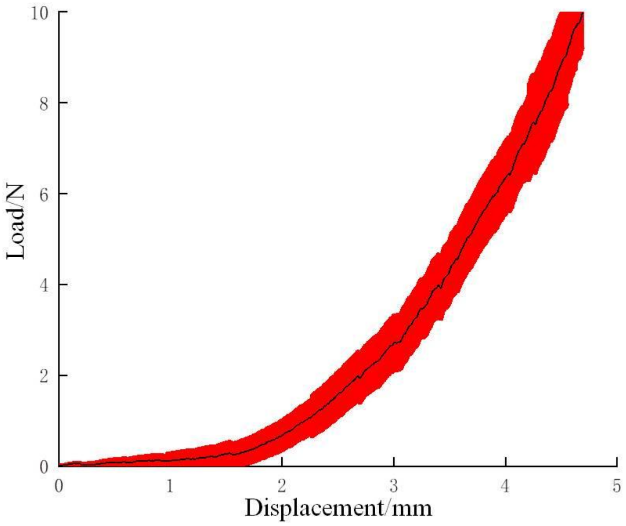


**Figure 8**

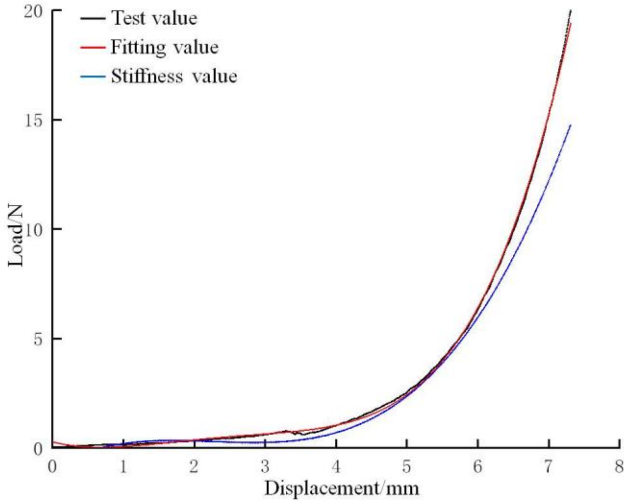


**Figure 9**

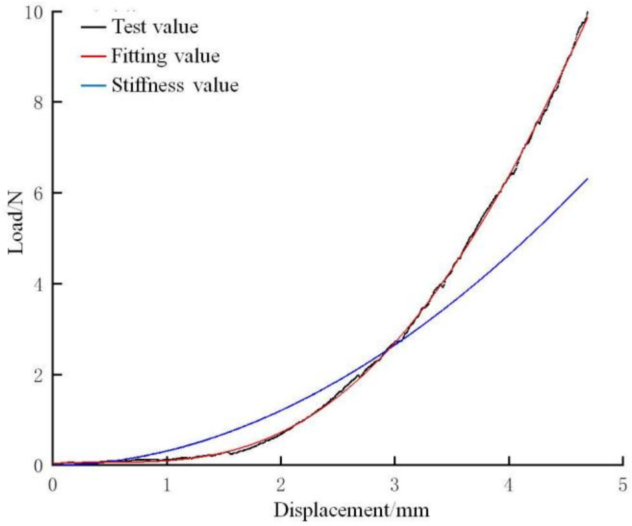


**Figure 10**

In summary, as the most terminal part of the cat's motion system, the foot pad is affected by the ground reaction in the process of movement. The mechanical properties of the foot pad will inevitably affect the impact characteristics between the foot pad and the ground impact. Specifically, the function of the foot pad is mainly manifested through three aspects: shock absorption, energy absorption and prevention of local stress concentration. The multi-layer composite structure of foot pad makes it show viscoelastic characteristics in compression state. When they suddenly deform and the deformation remains unchanged, the corresponding load decreases with time and the stress relaxation phenomenon occurs. When the viscoelastic material is loaded and the load remains unchanged, these structures continue to deform and creep phenomenon occurs, under cyclic loading, the force-strain relationship under loading is different from that under unloading, which leads to hysteresis. The area between the loading curve and unloading curve represents the dissipated energy, which can be used to characterize the vibration reduction characteristics of paw pad (McLaughlin RM. 2001; Nunamaker DM., 1985).

In conclusion, through the relevant experiments, the tissue staining observation, scanning electron microscope observation and quasi-static compression test of domestic cat paw pad were realized. The three-layer coupling structure of the domestic cat paw pad and the variable stiffness mechanical characteristics of the domestic cat paw pad under load were found. The tissue structure and mechanical properties of the domestic cat paw pad are conducive to the effective exertion of its biological function of cushioning and vibration reduction.

***Conflict of Interest*:** All authors declare no competing interests.

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