Measuring the micromechanical properties of oesophageal mucosa with atomic force microscopy

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Abstract: The micromechanical properties of soft tissue can be used as markers for the physiological state and function of the tissue. Deep understanding of the micromechanics of soft tissue, such as the oesophagus, is of great significance to the design of artificial oesophagi, endoscope materials and coatings for medical devices. Here, the micromechanical properties of oesophageal mucosa were studied under different loading rates, deflections, and dwell time by using atomic force microscopy. The micromechanical properties of soft tissue included elastic modulus, plasticity and adhesion force. Results showed that the micromechanical properties changed with increasing loading rate, deflection and dwell time. The micromechanical properties of oesophageal mucosa were related to time-dependent behaviours, such as contact stress, energy transformation, and strain gradient plasticity. Furthermore, the heterogeneity of oesophageal mucosa affected the micromechanical properties. The force mapping mode was a reliable and effective means to study the micromechanical properties of soft tissue. The results can provide a basis and technical support for the diagnosis of oesophageal diseases from a microscale as well as a material design perspective.

1 Introduction

With the increasing recognition of pathological conditions, the micromechanical properties of soft tissue have gradually become an important part of disease diagnosis [1]. Specifically, the micromechanical properties can be used as markers for the physiological state and function of the tissue. Deep understanding of the micromechanics of soft tissue, such as the oesophagus, is of great significance to the design of artificial oesophagi, endoscope materials and coatings for medical devices. Here, the micromechanical properties of oesophageal mucosa were studied under different loading rates, deflections, and dwell time by using atomic force microscopy. The micromechanical properties of soft tissue included elastic modulus, plasticity and adhesion force. Results showed that the micromechanical properties changed with increasing loading rate, deflection and dwell time. The micromechanical properties of oesophageal mucosa were related to time-dependent behaviours, such as contact stress, energy transformation, and strain gradient plasticity. Furthermore, the heterogeneity of oesophageal mucosa affected the micromechanical properties. The force mapping mode was a reliable and effective means to study the micromechanical properties of soft tissue. The results can provide a basis and technical support for the diagnosis of oesophageal diseases from a microscale as well as a material design perspective.

As a type of scanning probe microscopy, AFM can not only visualise the 3D surface topography but is also widely used in probing micromechanical properties. The curves combining the applying force and the displacement of the tip contain almost all the mechanical information, such as hardness and stiffness. In order to acquire the mechanical properties of the areas of interest, a force mapping mode is adopted: force curves are recorded while applying force and the displacement of the tip in an array is visualised. The curves combining the touching and deflection responses, uniaxial tensile behaviours and relaxation responses, are recorded. In this way, the mechanical properties of the areas of interest can be visualised.

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As mentioned above, it is a feasible method to study the micromechanical properties of soft tissues by AFM. In addition, there is little literature on the micromechanical properties of the oesophagus that are important for disease diagnosis, synthetic replacements for the oesophagus, endoscope materials and coatings on medical devices. As the counterpart of the endoscope, the mucosa of the oesophagus makes contact with the endoscope during endoscopy. Consequently, the goal of this study was to characterise the micromechanical properties of the oesophageal mucosa at different loading rates, deflections of the cantilever and dwell time, to determine the performance of elastic moduli, plasticities and adhesion forces, and ultimately provide a basis and technical support for oesophageal disease, and design of synthetic replacements.

2 Methodology

2.1 Tissue preparation and immobilisation

Porcine oesophagus in the thoracic region was selected as the test sample due to its similarity to the human oesophagus [16]. Three oesophagi (Chenghua pig) were obtained from the local slaughterhouse with the permission of the Agriculture and Veterinary Authority in China. The weight of each pig was about 65 kg and the age was about 25 weeks. The oesophagus was first preserved in an icebox with phosphate-buffered saline (PBS) solution and delivered to the laboratory within 2 h post-mortem; they were then tested within 4 h after extraction to preserve activity.

The microstructure of the oesophagus mainly contains mucosa, submucosa, muscle and adventitia inside and out. Along the boundary between the muscular layer and the mucosal layer, the mucosal layer can be acquired from the intact oesophagus with a scalpel. The thickness of the mucosal layer was close to 2 mm. The mucosal layer was then placed stuck in the middle of two stainless steel sheets tightened with ropes to keep the mucosa flat without extension, as shown in Fig. 1. The nether steel sheet has a bench that can extrude the sample over the upper surface of the top steel sheet. It can avoid the tip of an AFM striking the steel sheet. The area over the bench was ∼12 mm × 6 mm. The test area of the force mapping mode was 10 µm × 10 µm with a resolution of 10 × 10 pixels. Considering the total time of the force mapping mode when changing the dwell time, the test data were obtained using a single-point contact mode in different regions. All tests were performed under a controlled laboratory condition with a room temperature of 21–23°C and relative humidity of 50–60%.

2.2 Atomic force microscopy

A commercial AFM (MFP-3D, Asylum Research, Santa Barbara, CA, USA) with a Silicon Nitride sharp tip (MLCT, Bruker AFM probes), with a nominal force constant of 36 pN/nm was used to study the micromechanical properties of oesophageal mucosa. Before the test, the spring constant of the cantilever was determined using the thermal tune calibration method to obtain precise data. The PBS was injected into the surface of the sample with a syringe to simulate the human internal environment. Finally, a steel cover was used to enclose the setup to reduce noise. While the loading rates changed from 0.2 to 2.0 Hz, the deflection was 50 nm with a dwell time of 0 s. Similarly, when the deflections changed from 20 to 200 nm, the loading rate was 0.5 Hz with a dwell time of 0 s. When the dwell time changed from 0 to 12 s, the loading rate was 0.5 Hz and deflection was 80 nm.

2.3 AFM data acquisition and analysis

The mechanical properties of the samples were derived from the $F$ versus $z$ curves, where $F$ was obtained from the deflection $d$ and spring constant $k$ according to Hooke’s law $F = kd$, and $z$ was the vertical displacement of the tip. As shown in Fig. 2, $z_c$ means the tip-sample contact point and the red/blue lines correspond to the approach/withdrawal curves. When the tip probes an infinitely stiff surface, the displacement is equal to the deflection of the cantilever since the tip makes contact with the sample, and the slope of the $F$ versus $z$ curve represents the spring constant of the cantilever. In contrast, the $F$ versus $z$ curve displays a curved line of soft tissues, which represents the existence of indentation $\delta = z - z_c - d$.

2.3.1 Elastic modulus: The $F$ versus $\delta$ curves during unloading were fitted with the Oliver–Pharr model for the region of interest to yield the elastic modulus [21]. Briefly, the unloading curve can be expressed using a power-law equation: $P = m(h - h_f)^{\delta}$, where $P$ is the indenter force, $h$ is the vertical displacement of the tip and $h_f$ is the real displacement (Fig. 2b). The stiffness can be deduced by the derivative: $S_h = \partial P/\partial h$. There is a relationship between elastic modulus, stiffness and maximum penetration depth for tips [21]: $S_h(\text{max}) = 2\beta\sqrt{\pi}\sqrt{\text{A}_\text{max}\text{E}}$, where $\text{E}$ is the reduced modulus, $S_h(\text{max})$ is the stiffness at the maximum vertical displacement, $\text{A}_\text{max}$ is the projection of contact area at $h_\text{max}$ and $\beta$ is a parameter concerning the tip geometry. The fitting range is chosen from 10 to 90% of the ordinate for all curves. By combining the above relations with the unloading curve, the elastic modulus can be determined.

2.3.2 Plasticity: As shown in Fig. 2b, the plasticity is equal to the ratio of the area between the loading-unloading curves and horizontal axis (blue region) to the area between the loading curves and horizontal axis (blue region plus yellow region), i.e. $P = A/(A + B)$ [13, 15], where $A$ is plastic energy and $B$ corresponds to the recovery of the energy of materials. It was also a similar

![Fig. 1 Schematic diagram of sample fixation as well as indentation mode of AFM](image-url)
definition to fractional energy loss in the arterial wall [22]. It can be inferred that the plasticity is 1 when there is no energy recovery. In other words, it’s a pure plastic material. Accordingly, the plasticity is 0 when it’s a pure elastic material. In other cases, it’s a mixed state in which the material both contains elastic and plastic behaviours.

2.3.3 Adhesion force: Adhesion force in a loading-unloading loop is visualised using $F$ versus $d$ curves [23]. As shown in Fig. 2a, there are four schematic diagrams concerning the tip-sample interaction process. In the first stage, the tip moves toward the sample. Owing to the attractive effect of surface
molecules, there exists a negative force when the tip approaches the sample. The tip continues to advance and press the sample until the preset displacement. This makes up the second and third stages. After these stages, the tip backtracks to the initial point. During this process, there exists a bigger negative force relative to the first stage, namely, the adhesion force: the force between the minimum force on the unloading curve and the force on the tip when it is not in contact with the sample.

In order to observe the surface microstructure of mucosa more clearly, SEM (scanning electron microscope) observations were also conducted. The F-test (analysis of variance) was used to determine the significant difference; the level of statistical significance was set to $P<0.05$.

3 Results

3.1 Dependence of loading rates

The loading rate is the rate at which the tip moves toward or away from the sample. The error bars and the transverse lines of the rectangles from bottom to top in box graphs refer to the minimum, first quartile, median, third quartile and maximum, respectively. The asterisk in the box indicates the average value. There also exists some extrema outside the above range. According to Fig. 3a, with an increasing loading rate, the elastic modulus increases with a strong correlation ($P<0.05$). At higher loading rates, the value of the elastic modulus from 25 to 75% appears a magnitude of 1000 kPa. In contrast, the value of the elastic modulus is close to a magnitude of 10 kPa at lower loading rates. In other words, there is a difference between two orders of magnitude when comparing elastic moduli at different loading rates.

For the strong positive correlation of elastic modulus with loading rates, there is no clear tendency for plasticity with an increasing loading rate from 0.2 to 1.0 (Fig. 3b). The plasticity increases sharply from the loading rate of 1.5 ($P<0.05$). Moreover, the distribution of plasticity intensifies, implying that the tip-tissue contact is instable.

The tendency of adhesion forces is similar to that of the plasticity. According to Fig. 3c, the adhesion forces remain constant at the initial stages and significantly increase from the loading rate of 1.5 ($P<0.05$). The overall range of adhesion forces distributes similarly, except at a loading rate of 2.0.

3.2 Dependence of deflections of the cantilever

Firstly, with increasing deflection, the elastic modulus displayed a gradually downward trend in Fig. 4a ($P<0.05$). Furthermore, the elastic modulus varies by two orders of magnitude when the deflection increases from 20 to 200 mm, suggesting that the oesophageal mucosa is sensitive to the deflection.

There is a clear tendency of plasticity with an increasing deflection of the cantilever that can be observed not only from the position of the box but also from the average value of plasticity (Fig. 4b). While the deflections change from 20 to 80 mm, the plasticity of the sample decline accordingly and it remains stable with ever-increasing
deflections ($P < 0.05$). Furthermore, the distribution range of the plasticity tended to be stable with increasing deflection.

As can be seen from Fig. 4c, the tendency of the adhesion force descends with an increasing deflection except for the adhesion force at a deflection of 50 mm ($P < 0.05$). The distribution of the adhesion force at the smaller deflection is relatively unstable considering the loose surface of oesophageal mucosa. When the deflection continued to increase to 80 mm, i.e. the normal force increases, the distribution of the adhesion force started to stabilise and the trend became clear.

### 3.3 Dependence of dwell time

Compared with the routine dwell time of 300 s for soft tissues in macroscale experiments [21], there is a distinct difference in the elastic modulus with the dwell time of 3 s on the microscale (Fig. 5a). With an increasing dwell time, the elastic modulus presents an exponentially declining tendency ($P < 0.05$). The values of the elastic modulus also vary by two orders of magnitude. Moreover, the opposite trend is observed for plasticity. The dwell time is negatively correlated with the plasticity ($P < 0.05$) indicating that the deformation recovery ability of the sample weakens when the creep occurs at the microscale. The significance of the adhesion force is unique compared with other micromechanical properties (Fig. 5c). The adhesion forces with dwell time are an order of magnitude lower than that without dwell time and there is no obvious difference from 3 to 12 s ($P < 0.05$).

### 4 Discussion

As an indicator of the physiological state and function of soft tissues, the micromechanical properties play an important role clinically. As a result, the micromechanical properties of oesophageal mucosa, including elastic modulus, plasticity and adhesion force were studied at different loading rates, deflections of the cantilever and dwell time.

As can be seen from Figs. 3a–c, with increasing loading rate, elastic modulus, plasticity and adhesion force of the oesophageal mucosa increased to variable degrees. On the one hand, as a typical soft tissue, a process of energy transformation occurs when the tip comes into contact with the tissue, and the total work can be divided into two components: elastic work and plastic work [12]. At a smaller loading rate, the elastic deformation was the dominant contribution and the tissue had more time to form an internal equilibrium so that more energy could be recovered [24]. At a higher loading rate, the contact stress increased owing to stress relaxation which led to greater energy loss [10]. On the other hand, the viscous effect of tissue intensified with an increasing loading rate, resulting in a ‘hardening phenomenon’, implying that the elastic modulus increased correspondingly [4, 25]. This is also the reason the adhesion force increased. Furthermore, plasticity was related to the ability of the sample to respond freely; therefore, it could be influenced by the deformation rate. The proportion of elastic deformation declined when the tip made contact with samples at a higher loading rate, and there was more energy loss due to the viscous effects. In other words, tissue plasticity increased.

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**Fig. 5** Changes of the mechanical property of oesophagus at different dwell times

- **a** Elastic modulus
- **b** Plasticity
- **c** Adhesion force

The asterisk in the figures represents $P < 0.05$
According to Fig. 4, the elastic modulus, plasticity and adhesion force all declined with increasing deflection. In fact, this was the indentation size effect that had been observed in other materials at various deflections of the cantilever and the corresponding findings matched our results [15]. The fact that the elastic modulus evaluated at near-to-surface materials was higher than the bulk value could be explained by a physical mechanism described by strain gradient plasticity [26]. For the pyramid-shaped tip in our experiment, both experimental continuum-based methods and theoretical geometrical approaches suggested that the strain gradient decreased with increasing deflections [26]. For the plasticity, there existed two possible explanations. First, greater deflection imposed on the sample implied greater strain and more cooperative elements were required to accommodate the relevant strain at a molecular level [18, 19]. Compared with other elements, collagen fibre and elastin had better mechanical performances and the hysteresis energy was lower than that of other bearing elements (Fig. 4). Second, the activity of the tissue surface declined more quickly than that of the inner layers, owing to tissue exposure. It would be more difficult for a sample at the outer layer to deform. Furthermore, it’s difficult to find comparable data in the literature concerning the tissue adhesion forces at different deflections. As we know, there is a dynamic compound covering the surface of endothelial cells. It’s composed of glycoprotein and phosphatide, which participate in cell-to-cell or cell-to-extracellular matrix adhesion [27]. The increasing deflection could affect the structure and function of the compound, so the adhesion effect at near-to-surface layer was stronger.

As can be seen from Fig. 5a, there was a negative correction between the elastic modulus and the dwell time and the plasticity increased with increasing dwell time. These trends matched the results of Briscoe et al. [15] and Charitidis [14]. Similar to the results of loading rate, with an increasing dwell time, the reaction
time of the sample to external loading was prolonged and contact stress was reduced. In addition, it can be seen that the whole sample displayed a gradually hardening trend with increasing dwell time, and the ability of energy to transfer from kinetic energy into stored elastic energy declined. This plastic hardening behaviour responded to the increasing plasticity. Interestingly, there existed a difference in adhesion force with or without dwell time (Fig. 5c). It is more likely that the stored elastic energy and the surface energy were balanced with the dwell time [14] and there was no significant difference in the longer dwell time.

The elastic modulus spanned two orders of magnitude from 10,000 to 1,000,000 Pa at different loading rates, deflections and dwell time (Figs. 3a, 4a and 5a). Fig. 6 shows a schematic diagram of tip-sample interaction with potential bearing elements, including collagen fibres and elastin. The main bearing elements of tissue were fibres and elastin on a microscale. These results showed that the elastic modulus of a single collagen fibril can reach a magnitude of GPa [28], and the elastic modulus of the mucosal region was about 80,000–140,000 Pa [11]. The surface topography depended on the anatomic locations according to the SEM figures in Fig. 7. As a result, the micromechanism of oesophageal mucosa and the test area both affected the micromechanical properties. Combining the elastic modulus with the literature and tissue heterogeneity, it can be explained that the force mapping mode is a reliable and effective way to probe the micromechanical properties of soft tissues.

The above results will be of great significance for clinical diagnosis, surgical operation and artificial material development. As a trend of surgical development, a mechanical acquisition system in the equipment front end can be useful for in situ diagnosis and identifying the related diseases of the oesophagus. The micromechanical properties of oesophageal tissue can provide a reference to the clinical diagnosis of oesophageal diseases. Meanwhile, the morphocharacteristics of oesophageal tissues can provide mechanical parameters for the finite element modelling between medical instruments and oesophagus. Finally, the micromechanical properties of oesophageal tissue provide basic data for the development of artificial oesophagus.

5 Conclusions

The micromechanical properties of the oesophageal mucosa were studied at different loading rates, deflections and dwell time. Based on a series of experimental analyses, the conclusions can be summarised:

(i) The micromechanical properties, including elastic modulus, plasticity, and adhesion force, changed with increasing loading rate, deflection of the cantilever, and dwell time.
(ii) The micromechanical properties of mucosa were related to time-dependent behaviours, including contact stress, energy transformation, and strain gradient plasticity.
(iii) The heterogeneity also affected the performance of micromechanical properties.

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7 References

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