Experimental Study of Cortical Bone Microstructure and Its Toughening Mechanism

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Abstract: With the increasingly severe mechanical environment of the planetary detectors, fracture and failure are more likely to occur at their load-bearing structures. Therefore, it is urgent to improve the toughness of load-bearing structures. Because of the unique microstructures, goat tibia has remarkable toughness. In this investigation, firstly, the cortical bone of goat tibia was observed by SEM, and the characteristic microstructures were identified. Secondly, the cross section of cortical bone was loaded by long-term in-plane stress, then the toughness of cortical bone in different regions were obtained based on the orientation and distribution of cracks after the load. Finally, the toughening mechanisms of cortical bone were discussed. This study of the cortical bone toughening mechanism can be helpful for the biomimetic design of high-toughness materials.

1. Introduction

With the development of lunar exploration mission and Mars exploration project, detectors must have novel load-bearing structures that can maintain excellent mechanical properties under complex mechanical environment. When structures subjected to a load for a long time, even if the load is far less than the rated load, tiny cracks may still occur and continue to grow [1]. These tiny cracks can lead to structure fatigue, and reduce the strength of the structures. "Toughness" reflects the ability of the structure to resist the generation and propagation of tiny cracks [2]. Therefore, it is urgent to design and develop novel load-bearing structures with high toughness.

Bone is a composite composed mainly of protein and hydroxyapatite. As the main supporting and load-bearing structure in land-living animals, bone has excellent mechanical properties [3,4]. Goat tibia is tough enough to ensure that the large-scale structural deformation, fatigue or fracture would not happen under daily loads of a healthy adult goat. The outstanding toughness of bone is closely related to its microstructure [5,6]. Cortical bone and trabecular bone are two main types of bone. The cortical bone forms a dense, hard outer shell that mostly contributes to bone strength and toughness [3]. Therefore, this investigation focused on the cortical bone.

In recent years, a large number of researches on cortical bone have been carried out. Rho, J. Y. [3] observed and provided microscopic images of osteon and interstitial bone at different scales. Georgiadis M. et al. [7] made further observation on the lamella and its fibrous structure. S. Mohsin et al. [2] and Katsamenis, O. L. [8], at different scales, observed the propagation process of cracks in the middle region under one-direction load, and discussed the interaction between crack and osteon and cement line. Reznikov, N. et al. [9] made three-dimensional observation and component analysis of the outer region of the cortical bone, and studied its mechanical properties.
We find that in most of the experimental studies of cortical bone, the crack generation and propagation processes were conducted under the load of certain direction [2,8,9,10,11]. Moreover, most researchers focused on the middle region of the cortical bone, where the representative microstructure is osteon. However, Faingold A et al. found that for cracks with enough energy to grow across the osteon, the Haversian canal can promote the propagation [9]. This means the osteon in the middle region of the cortical bone can reduce the toughness under the particular circumstances.

In this investigation, we studied the relationship between the microstructure and toughness of the middle region as well as the outer region of the cortical bone from a healthy adult male goat's tibia. Firstly, the microstructures in different regions were observed by scanning electron microscopy (SEM). Then, a long-term inplane load was added to the specimens. The distribution and orientation of cracks in different regions were compared and analysed. Finally, the toughening mechanisms of cortical bone were discussed.

2. Materials and Methods

2.1. Specimen preparation and pre-treatment

The cortical bone sample for the experiment were taken from the tibia of a 36-week healthy black goat. Firstly, muscle and tissue were removed from the fresh tibia, shown in Figure 1 (a). Secondly, 1-2 cm bone segments were sectioned along the direction of bone growth and dehydrated; then in order to maintain the microstructure of cortical bone and facilitate the following operation, the segments were embedded with low-viscosity epoxy resin (by EXAKT520, Germany), shown in Figure 1 (b). Thirdly, about 1 mm thick slices were sectioned parallel to the cross section of the embedded cortical bone segment (by EXAKT300CP, Germany), such as Figure 1 (c). Finally, the thin slices were degummed and then sprayed, shown in Figure 1 (d), in order to obtain better observation results and eliminate the interference of embedding agent. And we got the well processed specimens reflecting the microstructure of cortical bone without loading.

2.2. Applying load

Based on the mechanism of polishing, we applied a long-term inplane load on the cross section of the embedded segments by a high precision two-axis polishing machine (LC-ZP820). Subsequently, we obtained the cortical bone specimens with tiny cracks under a long-term inplane load.

The loading process was conducted in two steps. Firstly, polish the cross section of the embedded cortical bone segments with polyurethane polishing pad and cerium oxide polishing powder of 3-micron and ensure the cross section of the segments reach 10-5-meter level smoothness. Secondly, the pre-polished cross section was precisely polished for 8 more hours with nylon cloth polishing pad and 50-nanometer silica polishing powder. After these two steps of polishing, segments after loading were obtained, as shown in Figure 1 (e).

The segment after loading was sectioned to 1-mm thick slice by tissue microtome. Because only the surface after polishing could reflect the cross section of cortical bone after omni-direction uniform surface load, it should be the only observation surface of the specimen after loading. Moreover, for the same reason as the treatment of the specimen without loading, the slice after loading was degummed and sprayed before observation, as shown in Figure 1 (f). Finally, we got the well-processed specimens of cortical bone after long-term inplane load.
2.3. Observation
Scanning electron microscopy (Pro-2017, Phenom-World, Eindhoven, Netherlands) was selected as the observation equipment. The resolution of SEM is beyond 1nm, which can clearly distinguish the micron-scale microstructures in different regions of the cortical bone. In this observation experiment, the SEM was set at 20kV with second electron mode.

3. Results
3.1. Middle region of the cortical bone
3.1.1. Typical microstructures in the middle region before loading
The SEM image of the middle region of unloaded cortical bone was shown in Figure 2 (a). It can be clearly seen that 5-7 layers of Haversian lamellae surround the Haversian canal in the form of concentric circles, forming "osteon" with diameters ranging from tens to hundreds of microns. The osteons are located in irregular “interstitial bone”, and separated from each other by the “cement line”. The interstitial bone is regarded as aging osteon [3], which is composed of closely arranged but randomly oriented interstitial lamellae arrays. The cement line is 1-2 micron thick containing little protein, and its strength is lower than other microstructures in cortical bone [3]. Figure 2 (b) shows a more specific image within an osteon, where the Haversian lamellae are arranged in parallel and tightly bonded.
3.1.2. The characteristics of the cracks after loading in the middle region

Figure 3 is the SEM image of the middle region after loading. We mainly focused on the cracks with the length over 10 microns and studied their distribution and orientation. Firstly, it can be found that after loading, the osteons were rarely destroyed, which means the integrity of the microstructure was basically maintained. Secondly, the characteristics of cracks distribution are summarized as follows: cracks seldom distributed within the osteon, but mostly distributed in the interstitial bone and at the junction of interstitial bone and osteon. Thirdly, the characteristics of cracks orientation are summarized as follows: under the long-term inplane random-direction load, the cracks were likely to grow in random directions within the interstitial bone at the beginning. When contacted with the cement line as well as the osteon, the cracks would be influenced by the microstructures and their growth direction would be changed.

The tiny cracks were likely to generate in the interstitial bone and grew with the energy provided by the load. The 3 white arrows in Figure 3, specifically indicate 3 different interactions between the growing cracks and the microstructures in the middle region. Case 1, The crack with relatively small energy would stop growing when contacting the cement line between the interstitial bone and osteon, which is defined as “cracks captured by the cement line”. Case 2: The crack with more energy would change their original growth direction and continue to grow along the cement line, which is defined as “cracks deflected by the osteon”. Case 3: With the further increase of contained energy, a crack would break through the cement line and penetrate several layers of Haversian lamellae in an osteon, which is defined as “cracks absorbed by the osteon”.

Figure 2. The SEM images of the middle region of unloaded cortical bone. (a) The white dotted circle shows an osteon, the red dotted line covers part of the cement line, and the irregular interstitial bone distributes between the osteon indicated by a white arrow; (b) The red dotted arrows show the orientation of three adjacent Haversian lamellae.

Figure 3. The SEM image of the middle region of cortical bone after loading. The white arrow 1 indicates the phenomenon “cracks captured by the cement line”, the white arrow 2 indicates the phenomenon “cracks deflected by the osteon”, and the white arrow 3 indicates the phenomenon “cracks absorbed by the osteon”.
As an aging osteon, the interstitial bone is stiffer and brittler than osteon [4]. This is a reason for most cracks distributed in the interstitial bone. However, the interstitial bone can also inhibit the growth and expansion of cracks with its internal microstructure. Figure 4 is the interstitial lamella on the separated sides of a crack, while a crack growing in the interstitial bone. It can be seen that the interstitial lamella is a splint structure formed by braiding arrangement of mineralized fibers. This internal microstructure of the lamella also deeply affects the growth of cracks. Case 1: a short mineralized fibre bundle was torn and raised, which was braided to one side of the lamella originally; this phenomenon is defined as “fibers pull-out”. Case 2: fibers still connected the separated interstitial lamellae, like a bridge; this phenomenon is defined as “fibers bridging”. Case 3: accompanied with Case 1 and the further development of Case 2, the fibers would break in the middle or at one end; this phenomenon is defined as “fibers breaking”.

Figure 4. Two interstitial lamellae separated from each other by a crack. The white arrow 1 indicates the phenomenon “fibers pull-out”, the white arrow 2 indicates the phenomenon “fibers bridging”, and the white arrow 3 indicates the phenomenon “fibers breaking”.

3.2. Outer region of the cortical bone

3.2.1. Typical microstructures in the outer region before loading
The SEM images of the outer region of cortical bone before loading were shown in Figure 5. There are no Haversian canals or other large canal tissues in the outer region. On both sides of the small blood vessels, “layered lamellae” are formed by several tightly bonded parallel straight lamellae. Unlike osteon in the middle region, there is no cement line boundary for the layered lamellae, hence the layered lamellae are adjacent to the interstitial bone directly. Figure 5 (b) shows a more specific image of the layered lamellae.

Figure 5. The SEM images of the outer region of cortical bone without loading. (a) The red dotted rectangles show parts of the layered lamellae, and the interstitial bone is between the red dotted rectangles; (b) The red dotted arrows show the orientation of three adjacent lamellae in the layered lamellae.
3.2.2. The characteristics of the cracks after loading in the outer region

FEM images of the outer region of cortical bone after loading were shown in Figure 6. Focusing on cracks larger than 10 microns in length, it can be found that the distribution of cracks in the outer region was similar to that in the middle region, but the orientation of cracks was entirely different. For the distribution: there are few cracks in the layered lamellae, while most cracks distributed in the interstitial bone and at the junction of interstitial bone and layered lamellae, as shown in Figure 6 (b).

The characteristics of cracks orientation are summarized as follow: Firstly, few cracks changed their direction of growth in large angles. However, when the tiny cracks pierced through the micropores in the interstitial bone, they could be deflected at small angles, as shown in the white dotted rectangle in Figure 6 (b). Secondly, the cracks parallel to the layered lamellae was less than those perpendicular to the layered lamellae, but the average length of parallel ones was much longer than that of perpendicular ones. Finally, two typical types of “large cracks” with the length of more than 100 microns were observed, both of which grew parallel to the lamina, indicated by the white arrows in Figure 6 (a). One type generated and grow at the interface between the layered lamellae and the interstitial bone; and the other one were from the expansion and connection of the lacunas in the center of the layered lamellae and the micro-pores in the interstitial bone.

![Figure 6](image)

Figure 6. The SEM image of the outer region of cortical bone after loading. (a) The white arrow 1 indicates the large crack at the interface between the layered lamellae and the interstitial bone; the white arrow 2 indicates large crack from the expansion and connection of the lacunas in the center of the layered lamellae; (b) in the white dotted rectangle, two tiny cracks deflected at small angles after pierced through a micro-pore in the interstitial bone.

4. Discussion

In this study, a long-term inplane load was applied to the cross-section of a cortical bone. Subsequently, the SEM images of different regions of the cortical bone before and after loading were observed. By analyzing the interaction between the cracks and the microstructures, the toughening mechanism of the cortical bone can be understood. The toughening mechanism of typical microstructures in cortical bone such as interstitial bone, osteon, cement lines, layered lamellae and micro-pore is discussed here.

4.1 Interstitial bone

Interstitial bone, form by closely arranged but randomly oriented interstitial lamellae arrays, is the main distribution area of cracks in both middle and outer regions. However, according to Figure 3 and Figure 4, the interstitial bone also has unique toughening mechanisms, which would be discussed from two perspectives.
4.1.1 Change of crack growth direction
Firstly, when a crack changing the growing direction, as shown in Figure 3, the current stress field at the crack tip would change. The process of stress field changing would reduce the stress intensity factor and increase the structure resistance to crack growth.

Secondly, when the crack deflected, the phenomenon of "bone plate fracture" could occur, as shown in Figure 3 at the left end of the crack. A segment of the tightly arranged lamellae array break with the deflection of the crack, which not only has to overcome the cohesive forces between the interstitial lamellae, but also has to structurally destroy layers of the interstitial lamellae. Thus the energy consumption is a lot more compared with a straight crack.

4.1.2 Internal microstructure of the interstitial lamellae
The interstitial lamella is a splint structure formed by braiding arrangement of mineralized fibers. When a crack grew in the interstitial lamellae array, the phenomena of “fibers pull-out”, “fibers bridging”, and “fibers breaking” would happen, as shown in Figure 6. The fibers that make up the interstitial lamellae are tightly bonded. All these phenomena would consume the energy of crack and effectively inhibit the expansion and growth of the crack.

4.2 Osteon and cement line
Osteons, always surrounded by the cement line, are the representative microstructures of the middle region of cortical bone. The toughness of the cement line is less than that of the interstitial bone and osteon. Therefore, when a crack growing and contacting the cement line, it would have a tendency to grow along the cement line, in which direction the stress intensity factor is minimum. Since the cement line is rarely straight, the crack would keep changing the growth direction, leading to a greatly increased the energy consumption. Thus, although the cement line is less tough than the interstitial bone and the osteon, it can still improve the overall toughness of cortical bone.

When a crack was absorbed by an osteon, it passed through the interface between the cement line and the osteon, and pierced across several layers of Haversian lamellae. Accompanied with the structurally broken of these Haversian lamellae, the crack would consume a lot of energy in a very short growth distance within the osteon. This is an omni-direction toughening mechanism of the osteon.

4.3 Layered lamellae
Layered lamellae is the representative microstructure in the outer region of cortical bone, which is arranged parallel with each other and distributed in the interstitial bone. The layered lamellae has major influence on the anisotropic toughness of the outer region. Therefore, the toughening mechanisms of the layered lamellae are analyzed in different directions, respectively.

4.3.1 Toughening mechanism in the direction parallel to load
When subjected to loads parallel to the layered lamellae, cracks perpendicular to the layered lamellae would generate and grow. these cracks would pierce through several layers of the closely arranged lamellae. The structurally broken of every single lamella is accompanied with high energy consumption. Therefore, the layered lamellae has remarkable toughing effect in the direction parallel to load.

4.3.2 Toughening mechanism in the direction perpendicular to load
Under the load perpendicular to the layered lamellae, cracks parallel to the layered lamellae would generate and grow, always at the interface between layered lamellae and interstitial bone. The main toughening mechanisms at the interface is crack deflection. However, the arc of the interface is usually small. Therefore, the cracks have no trend of deflecting continually or deflecting to a large angle. In addition, "fibers pull out", "fibers bridging" and "fibers breaking" phenomena will also happen at the interface, which can help with the structure toughening to some extent. It can be seen that the toughening effect of layered lamellae perpendicular to load is far less than that parallel to load.
4.4 The micro-pore

The micro-pore, with a size of about 10 microns, is widely distributed in cortical bone, functioning mainly in biological aspects, such as providing channels for blood vessels and accommodating osteocytes. In this study, we found the micro-pores could also influence the toughness.

The toughness of the interface between the micro-pore and its surrounding is much lower than that of other microstructures in cortical bone. When a crack is approaching the micro-pore, it would tend to enter the micro-pore, and redistribute energy at the micro-pore inner interface. Therefore, with high energy micro cracks could develop into large cracks by continuously penetrating and connecting several micro-pores in the same direction, as indicated by the white arrow 2 in Figure 6 (a). From this point, the micro-pore reduces the toughness of the cortical bone.

However, because of the irregular shape of the micro-pore and the changing of ambient microstructures, the toughness along the interface varies. Thus, as shown in the white dotted line frame in Figure 6 (b), the crack may change its growth direction after penetrating the micro-pore, which will consume additional energy for the crack propagation. In addition, if the energy is not enough for the micro crack to destroy the micro-pore boundary after the redistribution, the micro crack would be captured by the micro-pore, as indicated by the white arrow 2. Under these circumstances, the micro-pores exert a positive influence on the toughness.

In a word, the micro-pore will exert complex influence on the toughness of the cortical bone, which is closely related to the size and distribution of the micro-pore, as well as the loading on the structure.

5. Conclusions

In this study, the cross section of cortical bone from a goat tibia was subjected to a long-term inplane load, applied by a polishing machine. The microstructures in different regions before loading, and the properties of cracks in different regions after loading were observed with SEM. Furthermore, the main toughening mechanisms in cortical bone were determined by studying the interaction between cracks and the typical microstructures.

The authors conclude that in the middle region of the cortical bone, the cortical bone is effectively toughened in all directions, mainly by the microstructure of onston and cement line. While in the outer region of the cortical bone, the toughness in the direction parallel to the layered lamellae far excels that perpendicular to the layered lamellae, which mainly due to the toughening mechanism of the layered lamellae.

Thus, the toughness of the cortical bone is connected with some typical micro-structural characteristics, which provides a reference for the design of bionic materials or structures with outstanding toughness.

References

[1] Bažant, Z.P.; Chen, E. Scaling of Structural Failure. Advances in Mechanics 1999, 50, 593-627.
[2] Mohsin, S.; O'Brien, F.J.; Lee, T.C. Osteonal crack barriers in ovine compact bone. J ANAT 2010, 208, 81-89.
[3] Rho, J.Y.; Kuhnspearing, L.; Zioupos, P. Mechanical properties and the hierarchical structure of bone. MED ENG PHYS 1998, 20, 92.
[4] Meyers, M.A.; Chen, P.Y. Structural biological materials: critical mechanics-materials connections. SCIENCE 2013, 339, 773-779.
[5] O Brien, F.J.; Taylor, D.; Lee, T.C. The effect of bone microstructure on the initiation and growth of microcracks ☆. J ORTHOP RES 2005, 23, 475-480.
[6] Sabet, F.A.; Raeisi, N.A.; Hamed, E.; Jasiuk, I. Modelling of bone fracture and strength at different length scales: a review. INTERFACE FOCUS 2016, 6, 20150055.
[7] Georgiadis, M.; Müller, R.; Schneider, P. Techniques to assess bone ultrastructure organization: orientation and arrangement of mineralized collagen fibrils. J R SOC INTERFACE 2016, 13, 20160088.
[8] Katsamenis, O.L.; Jenkins, T.; Thurner, P.J. Toughness and damage susceptibility in human cortical bone is proportional to mechanical inhomogeneity at the osteonal-level. BONE 2015, 76, 158-168.

[9] Faingold, A.; Cohen, S.R.; Reznikov, N.; Wagner, H.D. Osteonal lamellae elementary units: lamellar microstructure, curvature and mechanical properties. ACTA BIOMATER 2013, 9, 5956-5962.

[10] Zhang, X.; Liu, X.; Yan, Z.; Cai, J.; Kang, F.; Shan, S.; Wang, P.; Zhai, M.; Guo, X.E.; Luo, E. Spatiotemporal characterization of microdamage accumulation in rat ulnae in response to uniaxial compressive fatigue loading. BONE 2018, 108, 156-164.

[11] Jimenezpalomar, I.; Shipov, A.; Shahar, R.; Barber, A. Mechanical behavior of osteoporotic bone at sub-lamellar length scales. Frontiers in Materials 2015, 2, 9.