Experimental Study on Boundary Lubrication Mechanism at Superficial Layer of Articular Cartilage

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（関節軟骨表面層における境界潤滑メカニズムの実験的研究）

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論 文 内 容 の 要 旨

Healthy mammalian synovial joints feature an excellent biotribological system characterized by remarkably low friction, a significant load-bearing capacity, and extended durability. The extracellular matrix components in articular cartilage govern the joint's load-bearing capacity, while interstitial fluid, synovial fluid (SF), and surface adsorbed molecules influence lubrication. Identifying crucial boundary lubricants is essential for understanding the normal physiology and pathology of cartilage diseases, such as osteoarthritis In this study, the lubricating function of the superficial area of articular cartilage and SF constituents were examined. Cartilage specimens were processed with four different treatments: gentle and severe washing with detergent, incubation in NaCl solution, and trypsin digestion to selectively remove certain constituents from the cartilage surface. The angularly reciprocating tests with a spherical glass probe and articular cartilage specimens were performed over a range of sliding speed and extremely low contact loads in the mN range lubricated with lubricants containing representative SF constituents, such as phospholipids, proteins and hyaluronic acid (HA) by Nano Tribometer. Meanwhile, the morphological examination and the assessment of protein adsorption onto the cartilage surface were performed using the fluorescence microscope and water-immersion objectives to identify the underlying cause of the difference in friction.

In chapter 1, the background and objective of this thesis were described. First, articular cartilage and osteoarthritis was explained. Then, the composition and structure of articular cartilage, composition and function of synovial fluid and lubrication of synovial joints were described. Finally, the motivation and objective of this study were identified.

Chapter 2 primarily introduces the experimental materials, instruments, methods, and the production of the lubricant used in the study.

In Chapter 3, the friction characteristics of untreated cartilage specimens were evaluated in PBS and SF. As a result, it was confirmed that the coefficient of friction (COF) of the prepared cartilage specimens was varied from 0.05 to over 0.3 in PBS. However, a certain group of cartilage specimens exhibited a low COF of less than 0.1 with limited variation.

Chapter 4 aim to employ four methods for removing specific lubricating elements from the

adsorption layer on the cartilage surface, conduct an in-depth study on the effects of these methods on cartilage friction properties in PBS and SF. The friction characteristics of four treated cartilage specimens were evaluated in PBS and SF. Results showed that for the low COF group of specimens, all four treatments increased the COF in PBS to different extents, and fluorescence microscopy revealed that the integrity of the cartilage surface was deteriorated by treatments. This means that the intact cartilage surface had lubricating constituents to maintain low friction, and the removal of such constituents resulted in the loss of the intrinsic boundary lubricity of the cartilage surface. The variation in the COF of the cartilage specimens was suppressed in SF because it had a clear boundary lubrication effect on the cartilage surface. The lubricating effect of SF could be confirmed even after degenerative treatment.

To understand the effect of synovial fluid constituents on boundary lubrication of superficial area of articular cartilage, the friction characteristics of untreated and treated cartilage specimens were evaluated in PBS and lubricants containing representative SF constituents, such as phospholipids, proteins and HA in Chapter 5. Results showed that the lubricating effect of fresh SF could be confirmed even after degenerative treatment. The experimental results indicated HA and phospholipids had no lubrication effect. The addition of albumin or γ-globulin to a saline solution exhibited distinct frictional properties in the samples, depending on the surface condition. The lubricant containing albumin and γ-globulin maintained a consistently low COF even after degenerative treatment. The significance of the interaction between albumin and γ-globulin should be emphasized.

In Chapter 6, the boundary lubrication mechanism on articular cartilage surface was discussed. These results led us to suggest the structure and composition of the superficial layer on the cartilage surface. The uppermost superficial zone of cartilage is covered with an adsorbed film forming a biomembrane structure primarily composed of a phospholipid bilayer and proteins. Beneath this, there exists an underlying proteoglycan-derived gel layer. Additionally, a model of absorbed protein boundary layer composed of albumin and γ-globulin was proposed. In this model, albumin could maintain low shear, while γ-globulin may act as a substrate for albumin adsorption. Establishing the proper layer structure of albumin and γ-globulin is crucial for achieving low friction for cartilage.

In Chapter 7, the results obtained from this study were summarized, and conclusions were listed.