The effect of synovial fluid constituents on boundary lubrication of superficial area of articular cartilage

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1. INTRODUCTION

Healthy synovial joint is an extremely efficient tribological system with the coefficients of friction (COF) in the range of 0.001 to 0.01 [1]. Synovial joints are covered with compliant and hydrated articular cartilage and lubricated with synovial fluid (SF). Extensive research has been conducted on the synergistic effects of SF constituents on boundary lubrication of superficial area of articular cartilage. However, the lubrication mechanism under boundary lubrication conditions remains unclarified.

Two different degenerative treatments were applied to remove specific lubricating elements from the cartilage surface. The friction behavior of treated and untreated cartilage specimens was examined under different lubricants to elucidate the synergistic effect of the lubricating elements existing in the superficial area of the articular cartilage and SF constituents.

2. MATERIALS AND METHODS

2.1 Bovine cartilage preparation and specimen treatment

Square cartilage samples (side length 8 mm) were harvested from the proximal end of the bovine middle phalanx within 24h of sacrifice. Two different degenerative treatments samples were tested. A solution of 10%(v/v) detergent (Triton-X-100) was used to remove adsorbed lipids and protein molecules from the cartilage surface. The first group of cartilage specimens was gently washed with the detergent solution by using a tube mixer, called the gentle wash group. The second group was immersed to the 1.5M NaCl solution for 20 min to remove lubricin from the cartilage surface, termed the “NaCl group”.

The component of the lubricants was hyaluronic acid (FCH-150, Kikkoman), dipalmitoylphosphatidylcholine (DPPC, P0763, Sigma-Aldrich) bovine serum albumin (A7030, Sigma-Aldrich), and γ-globulins (G5009, Sigma-Aldrich). Fresh SF was extracted from bovine metacarpal-phalangeal joints. To minimize variability between samples, SF obtained from several joints was mixed.

2.2 Friction tests

A sliding pair of prepared cartilage specimens, mated against a smooth glass probe with a 2 mm diameter, were tested in rotational reciprocating sliding experiments with an angular amplitude of 30° using a Nano Tribometer (Anton Paar NTR^3). The glass probe was connected to the cantilevers for force measurement through the stainless steel stem and mounted on the piezo actuator integrated in the tribometer. The dual quad-beam cantilever with two separate high-resolution capacitive sensors were used to measure normal and friction forces. Simultaneously, the piezo actuator reads the normal force measured by the sensor in real-time and adjusts the vertical position of the glass probe instantly to maintain a constantly normal force during the sliding test.

To evaluate the surface friction of each of the cartilage specimen, the glass probe was pressed against the cartilage surface at constant load, and the cartilage specimen in the liquid bath was rotationally reciprocated by a servo motor at constant speed. Initially, the contact load and the sliding speed was set at 5 mN and 0.05 mm/s, respectively. The sliding speed was subsequently increased to 0.1, 0.2, 0.5, 1.0, 2.0 mm/s in a stepwise manner with the COF recorded for 5 cycles at each of the sliding speed. After finishing the friction measurement at 5 mN, the same procedure was repeated with increased contact load of 20 mN and 100 mN.

The process of friction measurements is summarized schematically in Fig. 2.

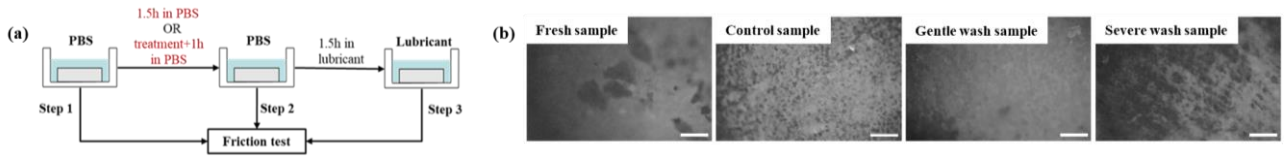

Fig.1(a) Schematic representation of the overall experimental design. (b) Fluorescent images of articular cartilage surface for 4 types cartilage specimens in PBS (Scale bars, 250 μm).

3. RESULT AND DISCUSSION

Fig. 1(b) shows the fluorescence images of the cartilage specimen surfaces by using a fluorescent microscope and water-immersion objectives. The surface of the fresh specimen was consistently and uniformly smooth. This represents the ideal appearance of the intact uppermost superficial layer of the articular cartilage. However, a slightly uneven surface was observed after immersion in PBS for 1.5 h. The gently washed cartilage maintained a relatively smooth surface; however, randomly distributed dark spots were observed. These dark spots might be defects caused in the superficial area of the cartilage surface by gentle washing in detergent solution. The surface of the NaCl group appeared to be slightly modified and had some similarities to that of the gentle-wash group. The fluorescent images of albumin absorbed on the cartilage surface are shown in Fig. 2. In gentle

and NaCl sample, the area of the adsorption film on the cartilage surface increased significantly when albumin and γ-globulin were mixed, compared to the area observed in the albumin solution alone. Conversely, in control sample, there was no significant increase in the adsorption film area. These results indicate that albumin and γ-globulin synergistically contribute to the formation of adsorption film on the cartilage surface in gentle and NaCl sample, whereas this effect is less pronounced in control sample.

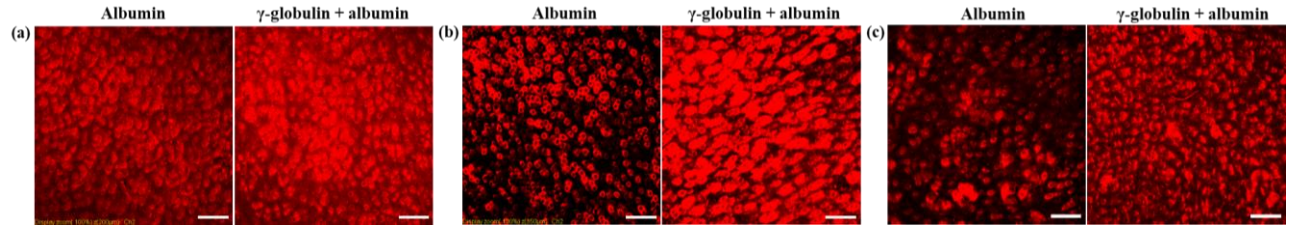

Fig. 2 Fluorescent images of the articular cartilage surfaces in albumin and albumin+γ-globulin solution. (a) Control specimen; (b) gentle wash group; (c) NaCl group. (Scale bars: 100 μm)

Fig. 3 showed the COF obtained from the control (a), gentle wash (b) and NaCl (c) cartilage specimens in Steps 1, 2, and 3 lubricating with PBS, PBS and single SF constituent respectively at 5 mN, 0.05 mm/s. In Step 1 for all samples, similar fluctuating behavior was observed in the data points of the COF, with a range of variation between 0.049 and 0.31. This could be attributed to slight surface damage in some samples' initial state. In Step 2, the minimum COF increased, and the COF range expended to 0.077-0.503. Compared with the control samples, the gentle wash sample and the NaCl sample had larger minimum COF values, both exceeding 0.1, while the control sample was only 0.077. These differences are likely due to the impact of degenerative treatment on the boundary lubrication function of the cartilage surface. Upon comparing PBS in Step 2 with fresh SF in Step 3, it was observed that fresh SF could reduce COF even in the presence of some damage to the cartilage surface. However, HA and DPPC did not exhibit lubricating effect. γ-globulin displayed similar friction behavior to PBS from Step 2 in the control samples, resulting in ineffective lubrication. However, the gentle wash and NaCl samples exhibited the lowest friction behavior. It is worth noting that among the single SF component lubricants, only albumin showed the smallest COF in the control sample, even smaller than that of fresh SF, and with very minimal error bars.

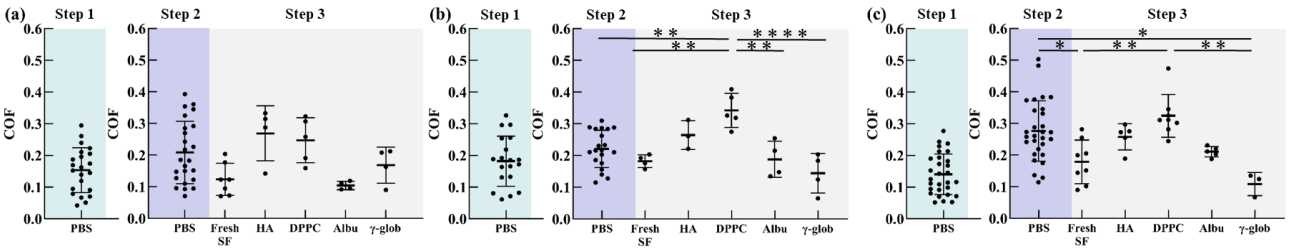

Fig. 3 Comparison of the COFs for (a) control, (b) gentle wash, and (c) NaCl specimens lubricated with PBS, PBS, single synovial fluid constituent in Step 1, Step 2 and Step 3 at the 0.05 mm/s, 5 mN.

Fig. 4 show the COF obtained from the control (a), gentle wash (b) and NaCl (c) cartilage specimens in Steps 1, 2, and 3 lubricating with PBS, PBS and lubricants containing albumin respectively at 5 mN, 0.05 mm/s. Compared with the albumin solution, the coexistence of albumin and DPPC did not effectively reduce friction, but it was still lower than that of the PBS solution in Step 2, except for the gentle wash samples. However, the addition of γ-globulin to the albumin solution showed a lower COF than albumin alone, even when compared to fresh SF, and this trend was consistent across all samples. When HA and DPPC were added to the albumin and γ-globulin mixture solutions, it is found that this combination exhibited similar friction behavior to the mixture of albumin and γ-globulin, with both showing the smallest COF and very minimal error bars. These reduction in friction is believed to be due to the restoration of the lubricating absorbing film on the cartilage surface. These findings confirmed the synergy between these two proteins and emphasize their importance as components constituting the adsorption film on the surface of cartilage.

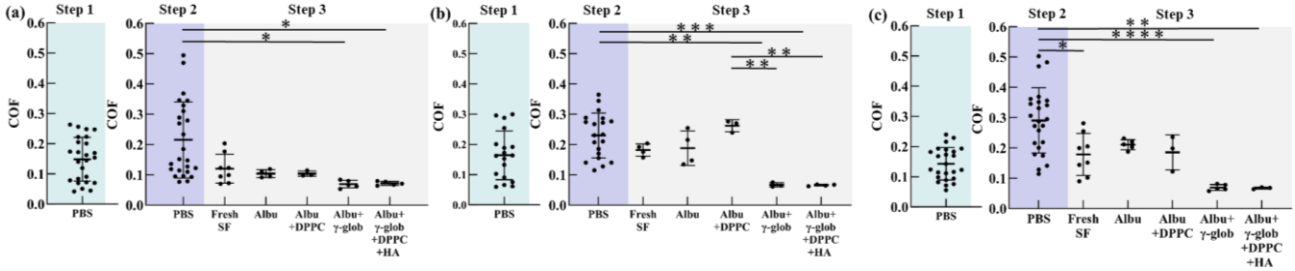

Fig. 4 Comparison of the COFs for (a) control, (b) gentle wash, and (c) NaCl specimens lubricated with PBS, PBS, lubricant containing albumin in Step 1, Step 2 and Step 3 at the 0.05 mm/s, 5 mN.

REFERENCES

1) T. Murakami, K. Nakashima, S. Yarimitsu, Y. Sawae et al. Effectiveness of adsorbed film and gel layer in hydration lubrication as adaptive multimode lubrication mechanism for articular cartilage. *Proc Inst Mech Eng J: J Eng Tribol* **225** (2011) 12.