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ORIGINAL PAPER

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Highly viscous sodium hyaluronate and joint lubrication

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Abstract We studied the natural lubrication mechanism of synovial joints. We determined the effect of sodium hyaluronate (HA) on lubricating joints without the normal lubrication mechanism. The coefficient of friction (CF) of fresh pig hip joints was measured with the cartilage intact, washed, scoured with gauze and finally with sandpaper, to model cartilage degradation. Three formulas of HA (8×10^5 daltons 1%, 20×10^5 daltons 1%, 20×10^5 daltons 1.5%) and physiologic saline were used as lubricants. We observed the cartilage using light microscopy (LM) and scanning electron microscopy (SEM). The latter showed that the most superficial layer observed in the washed joint was disrupted after gauze scouring. Compared with intact cartilage the CF did not increase with washing. CF increased more after scouring with sandpaper than with gauze. Each formula of HA decreased the CF of joints scoured with gauze, but only the two more viscous HA formulas decreased the CF of sandpaper-scoured joints. A negative correlation was found between the CF of the sandpaper-scoured joints and the logHA viscosity (r=-0.733, P=0.0001), suggesting that HA with higher viscosity was more effective in lubricating the joints.

Résumé Nous avons étudié la lubrification naturelle des articulations synoviales. Nous avons déterminé l'effet de l'hyaluronate du sodium (HA) lorsqu'il n'y a pas le mécanisme normal de lubrification. Le coefficient de frottement (CF) de l'articulation fraiche de la hanche de porc a été mesuré avec le cartilage intact, lavé, frotté avec une gaze et finalement altéré avec du papier de verre pour modeliser la déchéance du cartilage. Trois formules de HA (8×10^5 daltons 1%, 20×10^5 daltons 1%, 20×10^5 daltons 1.5%) et du sérum physiologique ont été utilisés comme lubrifiants. Nous avons observé le cartilage en microscopie optique et microscopie électronique. Cette dernière a montré que la couche la plus superficielle observée dans l'articulation lavée a été interrompue après nettoyage à la gaze. Comparé avec cartilage intact, le CF n'a pas augmenté avec le lavage. Le CF a augmenté plus après avoir frotté avec le papier de verre qu'avec la gaze. Chaque formule de HA a diminué le CF des articulations frottées avec la gaze, mais seulement les deux formules HA plus visqueuses ont diminué le CF des articulations altérées avec du papier de verre. Une corrélation négative a été trouvée entre le CF des articulations altérées avec du papier de verre et la viscosité du logHA (r=–0.733, p=0.0001), suggérant que le HA avec viscosité élevée était plus efficace pour lubrifier les articulations.

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Introduction

In normal synovial joints friction is extremely low and the cartilage does not abrade, even over several decades. This suggests that fluid film lubrication is formed by the cartilage and synovial fluid. In osteoarthritis (OA) on the other hand, the disease progresses with the attrition of the articular cartilage, resulting in contact between the friction surfaces of the cartilage during articular movement. This is believed to be caused by cartilage degeneration, subsequent cartilage surface irregularity, and the height of the irregularities becoming larger than the thickness of the fluid film. In addition, it is known that, in comparison to normal synovial fluid, OA synovial fluid has a reduced viscosity due to the decline in both concentration and molecular weight of its sodium hyaluronate (HA) [1, 26]. The reduction in viscosity of OA synovial fluid is also thought to be a cause of the thinning of the fluid film.

The intraarticular injection of HA has been established as a conservative treatment for OA, and numerous reports have been published concerning its pharmacological effects and clinical results [8]. However, there are no uniform conclusions on either the testing methods or results of the lubrication improvement effect of HA. In addition, if the thickening of the fluid is the lubrication improvement effect of HA, then one would expect that the higher the viscosity of the HA the greater the lubrication improvement effect.

Our objective was to investigate the role of articular cartilage and synovial fluid in the lubrication mechanism of natural synovial joints and to investigate the improvement of lubrication of highly viscous HA on joints in which the normal lubrication mechanism has failed.

Materials and methods

Test materials

OA synovial fluid was harvested from 20 knees of 20 outpatients diagnosed with OA of the knee with articular edema. This study was limited to those patients who had no history of receiving intraarticular injections of HA or steroids in the 3 months prior to the study. The mean age of the patients was 72.3 (61–82) years.

The three formulations of HA used were a 1% sodium HA solution with a mean molecular weight of 800,000 daltons (HA80–1), a 1% sodium HA solution with a mean molecular weight of 2 million daltons (HA200-1), and a 1.5% sodium HA solution in a gel state with a mean molecular weight of 2 million daltons (HA200-1.5), all of which were manufactured by a fermentation process using *Streptococcus epui*. In the friction test 12 hip joints from 12 pigs raised for human consumption, which had been placed in cold storage within 48 h of slaughter, were used. With the exception of the articular capsule, all soft tissue was removed and the joint was left at room temperature for 15 min prior to the commencement of the test.

Viscosity measurement of OA synovial fluid, physiologic saline, and three formulations of HA

The viscosity of the OA synovial fluid, saline, and the three formulations of HA were measured at 20°C using a cone plate viscosimeter (DV-1, Brookfield, Middlebro, Mass., USA).

Articular coefficient of friction (CF) measurement by a pendulum-type friction tester

The friction test was carried out using a pendulum-type friction tester at 20°C. Firstly, the CF of the intact joints was measured immediately after removal of the soft tissue. Next, saline was used to wash the synovial fluid from the cartilage surface (CS1), whose CF was measured with saline as a lubricant. After that, the cartilage surface was scoured with gauze (CS2), and the CF of the joint was measured using saline and the three formulations of HA as lubricants. Following this, we scoured the same joints with sandpaper (CS3) and repeated the measurements (Figs. 1a,b). In this way, a single joint was washed with physiologic saline, then scoured with gauze, and finally scoured with sandpaper.

Statistical method

Statistical analysis was performed using SPSS computer software (SPSS Inc., Chicago, Ill., USA). Statistical significance was calculated by Tukey method with a risk ratio of under 5%.

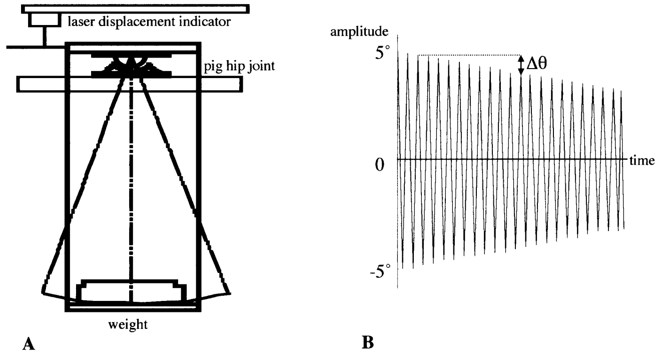

Fig. 1 The friction test was carried out using a pendulum-type friction tester. The pendulum load was 60 kg and the initial swing was 5°, which was commenced immediately after the load was set. The amplitude of the pendulum was detected by a laser displacement indicator (**a**). The coefficient of friction (CF) was calculated using the following equation: CF=l/4r×Δθ m, l; pendulum length, *r*; radius of the femoral head, Δθ; the amplitude deference between the second single swing and the 12th swing on a decreasing curve, Δθ m; Δθ/(12–2) Δθ (**b**)

Microscopic observations of cartilage

Light microscopy (LM) and scanning electron microscopy (SEM) observations were made of the CS1, CS2, and CS3 of the femoral head.

Results

Viscosity of OA synovial fluid, saline, and three HA formulations

The viscosity of the OA synovial fluid was negligible compared to that of each HA formulation, which, in sum, demonstrate that viscosity increases with both HA concentration and molecular weight. The viscosity of the saline was nearly identical with that of water (Table 1).

Changes in CF due to differing treatments of the cartilage and lubricants

The following results are shown as mean±standard deviation (SD). The CF of the intact joints was 0.015±0.003. The CS1 joints using saline as a lubricant had a CF of 0.015±0.009, which was not a significant increase from the intact joints and was used as a control. In the CS2 joints in which saline was used as lubricant, CF was 0.0307±0.0033. This value was significantly higher than the control value ($P<0.001$). On the other hand, in using the three formulations of HA as a lubricant, that is, HA80-1, HA200-1 and HA200-1.5, there was a significant decrease to 0.0265±0.0033 ($P<0.01$), 0.0265±0.0023 ($P<0.01$), and 0.0267±0.0029 ($P<0.01$). However, there was no significant difference between the three formulations of HA, nor was there a decrease to the level of the control value (Fig. 2). Using saline as a lubricant the CF of the CS3 joints increased significantly

Table 1 Viscosity (mPa·s) of osteoarthritis (*OA*) synovial fluid, physiological saline, and three formulations of sodium hyaluronate (*HA*)

Shear rate (1/s)	OA synovial fluid	Physiological saline	HA80-1	HA200-1	HA200-1.5
15.0	36.8	1.06	>1000	>1000	>1000
4.5	–	–	3620	10380	36250

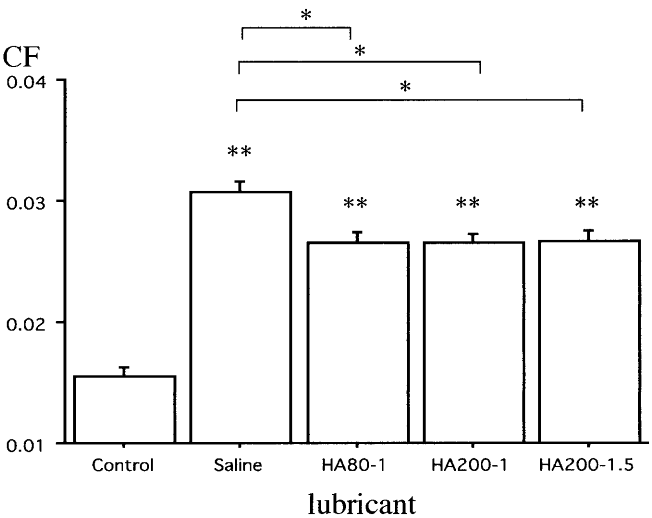

Fig. 2 Coefficient of friction (*CF*) of the C2 joints (* *P*<0.01, ** *P*<0.001)

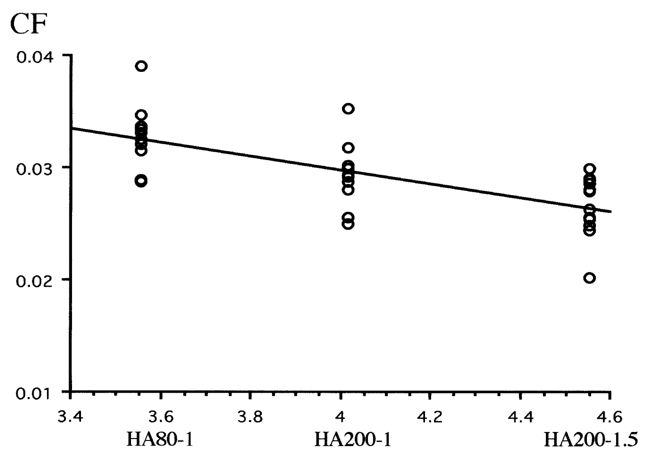

Fig. 4 A negative correlation was found between the coefficient of friction (*CF*) of the C3 joint and the logHA viscosity. (r=–0.733, P=0.0001)

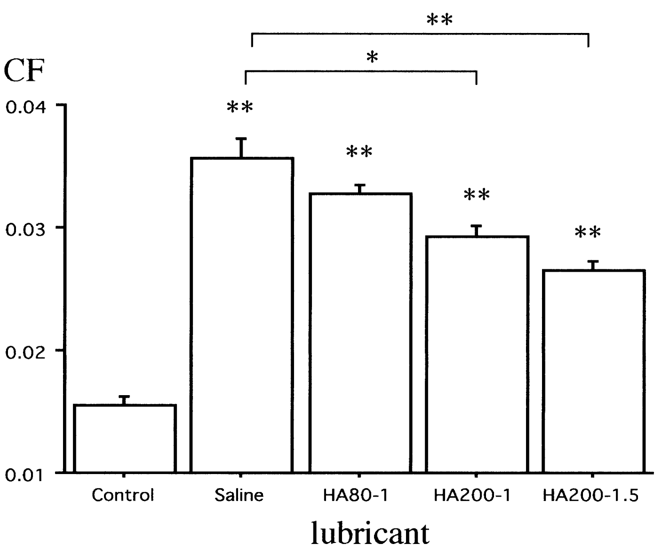

Fig. 3 Coefficient of friction (*CF*) of the C3 joints (* *P*<0.01, ** *P* <0.001)

to 0.0356±0.0054 (P<0.01) in comparison to the CS2 joints. Even using HA80-1 as a lubricant the CF of the joints was 0.0327±0.0027, indicating no significant decrease from the CF with saline as a lubricant. The CF of the joints using HA200-1 and HA200-1.5 were 0.0293±0.0029 (P<0.01) and 0.0268±0.0029 (P<0.001) respectively, which was a significant decrease from the CF with saline (Fig. 3). In addition, a negative correlation was found between the CF of the CS3 joints and the logHA viscosity (r=–0.733, P=0.0001) (Fig. 4).

Morphological differences in the cartilage by treatment type

Under LM, both the CS1 and CS2 presented normal cartilage images. In the CS3 the superficial layer of the cartilage was disrupted, and the intermediate layer was exposed as an irregular surface (Fig. 5). SEM findings showed a slightly high-intensity amorphous layer of approximately 5 μm in thickness on the surface of the CS1. In the CS2, however, this layer was disrupted. In the CS3, as in the LM findings, the intermediate layer was exposed as an irregular surface (Fig. 6).

Discussion

Several reports have demonstrated that the normal joint has fluid film lubrication [2, 19, 24]. Others have demonstrated that the CF of natural synovial joints is extremely small (0.001–0.042) [3, 13, 28]. In our study the CF of the intact joints was also exceedingly small, averaging 0.015, a condition in which one would expect fluid film lubrication to be established.

The components forming the normal lubrication mechanism of synovial joints are cartilage and synovial fluid. The mechanical action of the cartilage is reported

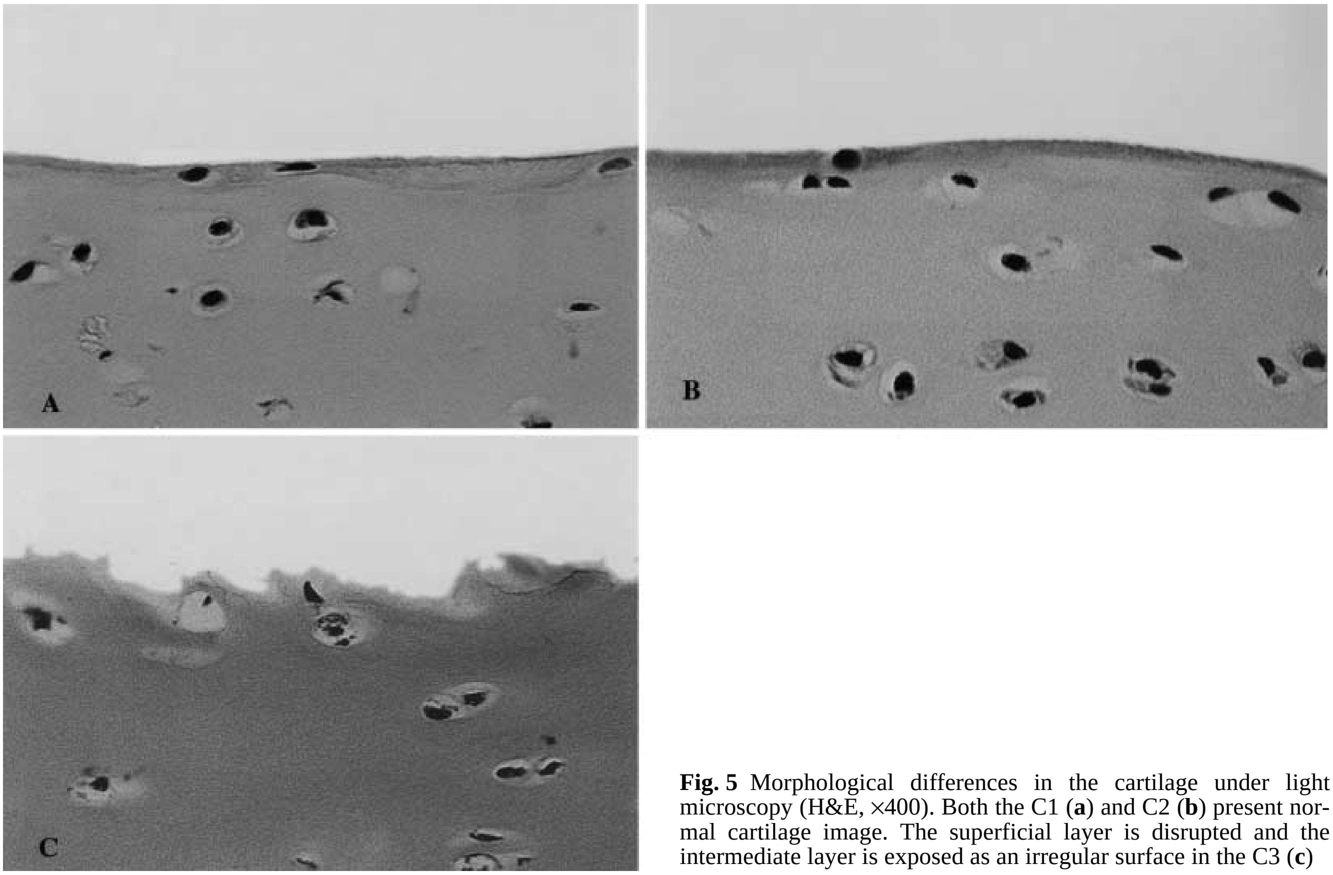

Fig. 5 Morphological differences in the cartilage under light microscopy (H&E, ×400). Both the C1 (**a**) and C2 (**b**) present normal cartilage image. The superficial layer is disrupted and the intermediate layer is exposed as an irregular surface in the C3 (**c**)

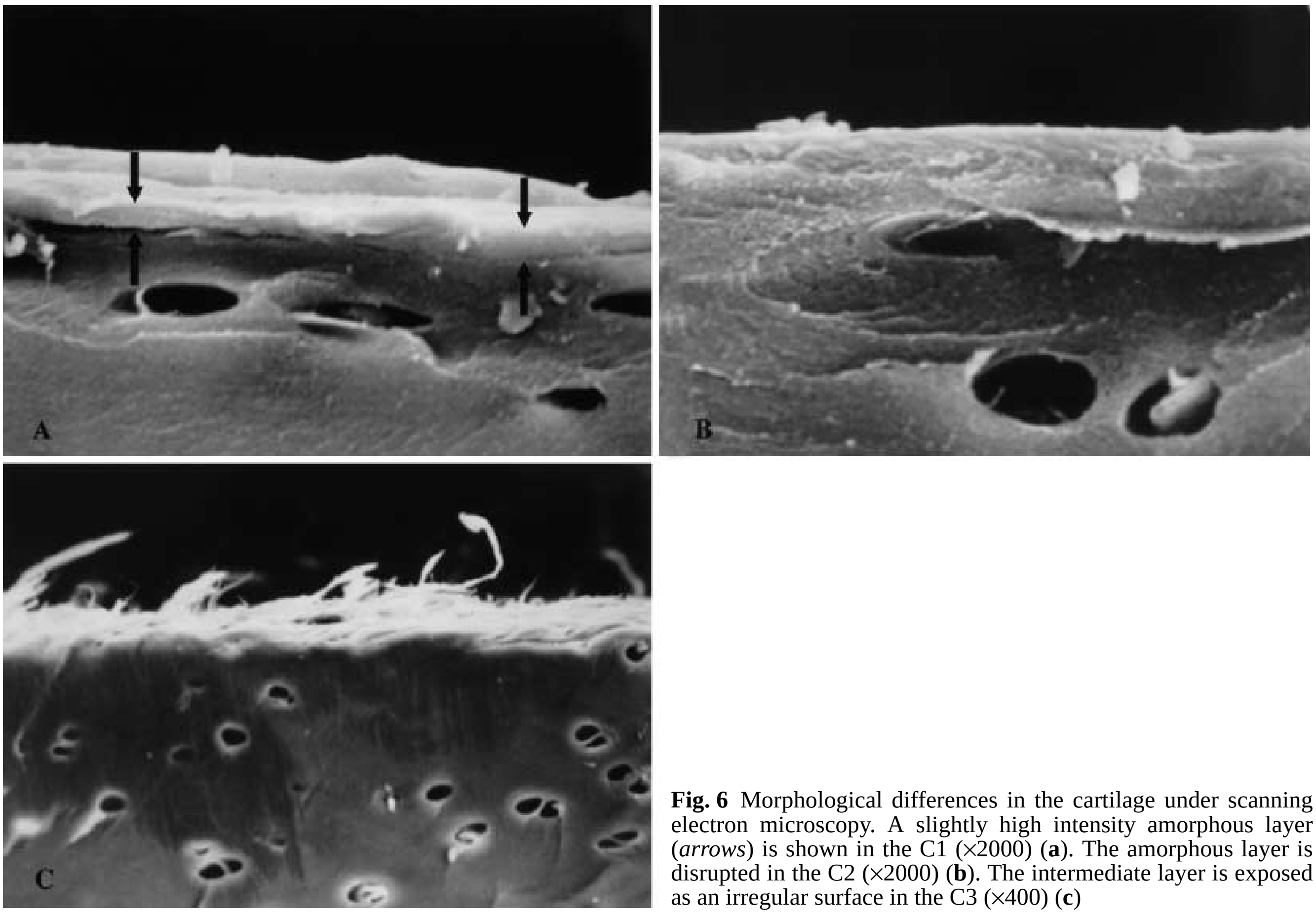

Fig. 6 Morphological differences in the cartilage under scanning electron microscopy. A slightly high intensity amorphous layer (*arrows*) is shown in the C1 (×2000) (**a**). The amorphous layer is disrupted in the C2 (×2000) (**b**). The intermediate layer is exposed as an irregular surface in the C3 (×400) (**c**)

to be due to elastic distortion and movement of the enchondral fluid [2, 4, 17]. There have been numerous reports on the lubrication layer of the cartilage surface, the so-called most superficial layer. MacConail was the first to report on a morphological investigation of the most superficial layer, calling it lamina splendens [15]. Subsequently, using SEM [9, 12, 25], transmission electron microscopy (TEM) [6, 18, 21, 23], and atomic force microscopy (AFM) [11], this has been described as an amorphous layer ranging from several nanometers to several micrometers. Kobayashi reported that the CF of the joint increased when the most superficial layer was stripped by mechanical manipulation [12].

It has been reported that the lubrication effect of synovial fluid apart from the synovial joint is unsatisfactory [27]. Our friction test results confirmed that, even when low viscosity physiologic saline was employed as a lubricant, the CF of the washed joints was unchanged when compared to the CF of the intact joints. That is, the lubrication effect of the synovial fluid in normal cartilage was nearly equivalent to that of the saline. In the washed joints the amorphous layer thought to be the most superficial layer could be clearly defined on the cartilage surface under SEM, while this layer was disrupted after gauze scouring – at which point the CF of these joints also began to increase. From this result it was thought that, irrespective of the viscosity of the lubricant, the most superficial layer plays an important part in satisfactory lubrication and that satisfactory lubrication cannot be established by the physical action of the cartilage alone.

SEM observations have indicated this most superficial layer was the first to disappear in an experimental OA model [7, 10]. This suggests that scouring with gauze reproduces a state similar to the start of OA. Furthermore, the rough cartilage surface after sandpaper scouring resembled the cartilage of advanced OA and had the largest CF [16]. The establishment of a fluid film in such cases would be difficult, and one would expect contact between cartilage to occur over a wide area.

Mabuchi et al. reported that the lubrication improvement effect of HA for OA is due to a reduction in contact between cartilage, which in turn was brought about by increasing the already lowered viscosity of OA synovial fluid and thickening the fluid film [14]. Our friction test results indicate a negative correlation between the CF of the sandpaper-scoured joints and the logHA viscosity. When applied to a Stribeck linear diagram, this would be equivalent to a mixed lubrication [28]. That is, our experimental results indicate the above-mentioned effect. On the other hand, although the CF of the gauze-scoured joints decreased significantly with the use of HA80-1, there was no correlation between the CF and logHA viscosity. Not only is the most superficial layer of the cartilage in a gel state and the viscosity extremely high, it has been reported that it is histologically united with the cartilage surface [11, 25]. In our tests the most superficial layer was not disrupted by just the action of washing with physiologic saline solution. In contrast, the HA200-1.5, although being in a gel state, was only applied to the cartilage surface during the pendulum-type test and therefore would not be expected to have completely filled the cartilage friction surface. That is to say, the lubrication improvement effect of HA will rise to a certain extent with an increase in HA viscosity, but is still limited.

Although we have not taken into consideration the in vivo metabolism of HA after being intraarticularly injected [5, 20], this study suggests that highly viscous HA – in which both concentration and molecular weight are high – would be effective on OA joints for lubrication improvement.

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