Effects Of Corticosteroids And Hyaluronic Acid On Torn Rotator Cuff Tendon

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Disclosures:
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Introduction: Intra-articular injection of corticosteroids and hyaluronic acid are commonly used for the treatment of rotator cuff tears. However, little has been clarified with regards to their effects on the torn rotator cuff tendons. The purpose of this study was to determine the effects of corticosteroid (CS) and hyaluronic Acid (HA) on torn rotator cuff tendon, using fibroblasts from human torn tendons (in vitro) and cuff tear model in rat (in vivo).
Methods: Cell Culture: Tendon specimens were harvested from patients with cuff tears during surgery (N=19). Tendon fragments were transferred into 75cm^2 flasks and DMEM containing 10%FBS, and incubated at standard condition (37℃, 95% air / 5% CO2-humidified atmosphere) until reached 80% confluence. Then, the cells were subcultured and used for the experiments after the first passage. Corticosteroid (CS) and hyaluronic acid (HA): CS was purchased from MSD Co. (DECADRON) and HA from KAKEN Co. (ARTZ). MTT assay; The cells were exposed for 24h in the presence of CS and HA at various concentrations (0.01, 0.1, 0.5 and 1.0 mg/ml). Then, the relative number of viable cells was determined by using cell count reagent WST-8(Nacalai tesque). Wst-8 solution was added to each well, including 8 wells containing medium alone to be used for background substraction. The absorbance at a wave length of 450nm was measured by microplate reader. Live/Dead staining and viable cell counts with fluorescence microscopy; Fibroblasts were plated at 1×10^{5}cells/chamber on slides and exposed for 24h in the presence of CS and HA at 1.0mg/ml. The adherent cells were stained by Live-Dead Cell Staining Kit (BioVision). Five random fields from each chamber were photographed and counted with using Image J.FACS assays for quantification of live/dead cells; The cells were exposed for 24h in the presence of CS and HA at 1.0mg/ml. Floating cells and trypsinised adherent cells were combined and diluted to 1×106 cells/ml using the 1×PBS. C12-Resazurin and SYTOX GREEN were added to stain live and dead cells. After incubation four 15min at 37℃, the live and dead cell counts were obtained using FACS machine(BD Bioscience).Rotator cuff tear model; Adult Sprague-Dawley rats were used (N=10). Complete tears were made in mid portion of the supraspinatus tendon in bilateral shoulders. (Image 1) After laceration, 0.4mg/ml of CS and HA was applied to the right shoulder (N=5 in each) and PBS to the left shoulder as control (N=10). The animals were sacrificed at 2 weeks after the surgery and subjected to the experiments: 8 specimens for biomechanical testing and 2 specimens for histological analysis. Biomechanical testing; All specimens were immediately tested after sacrifice. The tendon was loaded with a preload 0.1 N, followed by 5 cycles of loading and unloading with 0mm-0.5N at cross head speed 5mm/min, and then loaded until failure at 1mm/min. The ultimate load to failure was calculated from the resulting load elongation curve. Histological analysis; The supraspinatus tendon-humerus complex was fixed in 10% buffered formalin with decalcification. The specimens were subjected to HE staining. Outcomes included immunohistochemical analysis to determine the number of PCNA positive cells as an index of cell proliferation. (Image 2) Data analysis; Results were expressed as mean ± standard error (SE) and analyzed be using variance (ANOVA) with Tukey's post-hoc analysis. Differences of $P<0.05$ were considered significant.

Results: Cell proliferation: Compared with control, CS significantly decreased cell proliferation in a dose-dependent manner ($p<0.01$), in contrast to no inhibitory effect of HA (Figure 1). Significant differences were also noted between CS and HA, except 0.01mg/ml (Figure 2). For control, the value (optical density) was 100% (0%). For CS, the value was 97.6% (1.6%) in 0.01mg/ml, 79.0% (3.5%) in 0.1mg/ml, 43% (3.8%) in 0.5mg/ml, and28.2% (2.9%) in 1.0mg/ml. For HA, the value was 99.1% (1.1%) in 0.01mg/ml, 98.5% (1.4%) in 0.1mg/ml, 99.1(2.3%) in 0.5mg/ml, and 96.2% (1.3%) in 1.0mg/ml.Live/Dead cell counts with fluorescence microscopy: Viable cell counts with fluorescence microscopy was examined at 1.0mg/ml. CS resulted in significantly less live-cells (46.7±9.2), compared with HA (138±13.3) and control (141±10.0) ($p<0.01$, respectively) (Figure 3). Live/Dead cell counts with FACS assays: Consistent with the data from fluorescence microscopic analysis, the FACS quantification also showed significantly decreased cell-viability in the CS-treated cells (% Live cells: 51.3±9.9) than in the HA-treated cells (%Live cells: 84.8±2.5) and controls (%Live cells: 86.0±3.4) ($p<0.01$, respectively) (Figure 4~5). Analysis by rotator cuff tear model in rats. Biomechanical testing: All specimen tested failed at the tendon-bone interface. The ultimate failure load was significantly decreased in CS group (11.32±2.0N, $P<0.05$) than in HA (20.8±2.01N) and Control groups (19.9±1.42N) (Figure 6). Histological evaluation In the control group, the injured site was repaired with abundant fibro-vascular tissue at the extra-articular side (Image 3). This healing pattern was similar in the steroid group; however, the fibro-vascular tissue appeared to be less with fewer PCNA positive cells (Image 4).

Discussion: Injections of CS and HA are used in the treatment of rotator cuff tears. It is well known that both drugs have an anti-inflammatory effect and can give pain relief, although the effects of these agents on tendon fibroblasts in the torn rotator cuff tendon has not been sufficiently clarified. Therefore, the present study compared the effect of CS and HA on the tendon fibroblasts in cuff tear in vitro and in vivo. As a result, CS significantly inhibited cell proliferation and decreased cell viability, compared with HA and controls. Concurred with the data from the in vitro analysis, CS inhibited the coverage by the fibro-vascular tissue at the repaired site in rotator cuff tear model, consequently weakening the biomechanical strength at the site. On the other hand, HA did not show inhibitory effects in vitro and in vivo. Our previous studies have demonstrated that HA possesses anti-inflammatory effects on subacromial-synovial fibroblasts and anti-adhesive effects on both tendon and glenohumeral-synovial fibroblasts in rotator cuff tears.[1] Although the direct comparisons of anti-inflammatory/adhesive effects between CS and HA were not examined in this study, we conclude that clinician should recognize the different characteristics of these agents when treating rotator cuff tears.

Significance: Compared with HA, CS significantly decreases cell viability and weakens the biomechanical strength at the repaired site in rotator cuff tear model. The different characteristics of these agents should be recognized for the treatment of rotator cuff tears.

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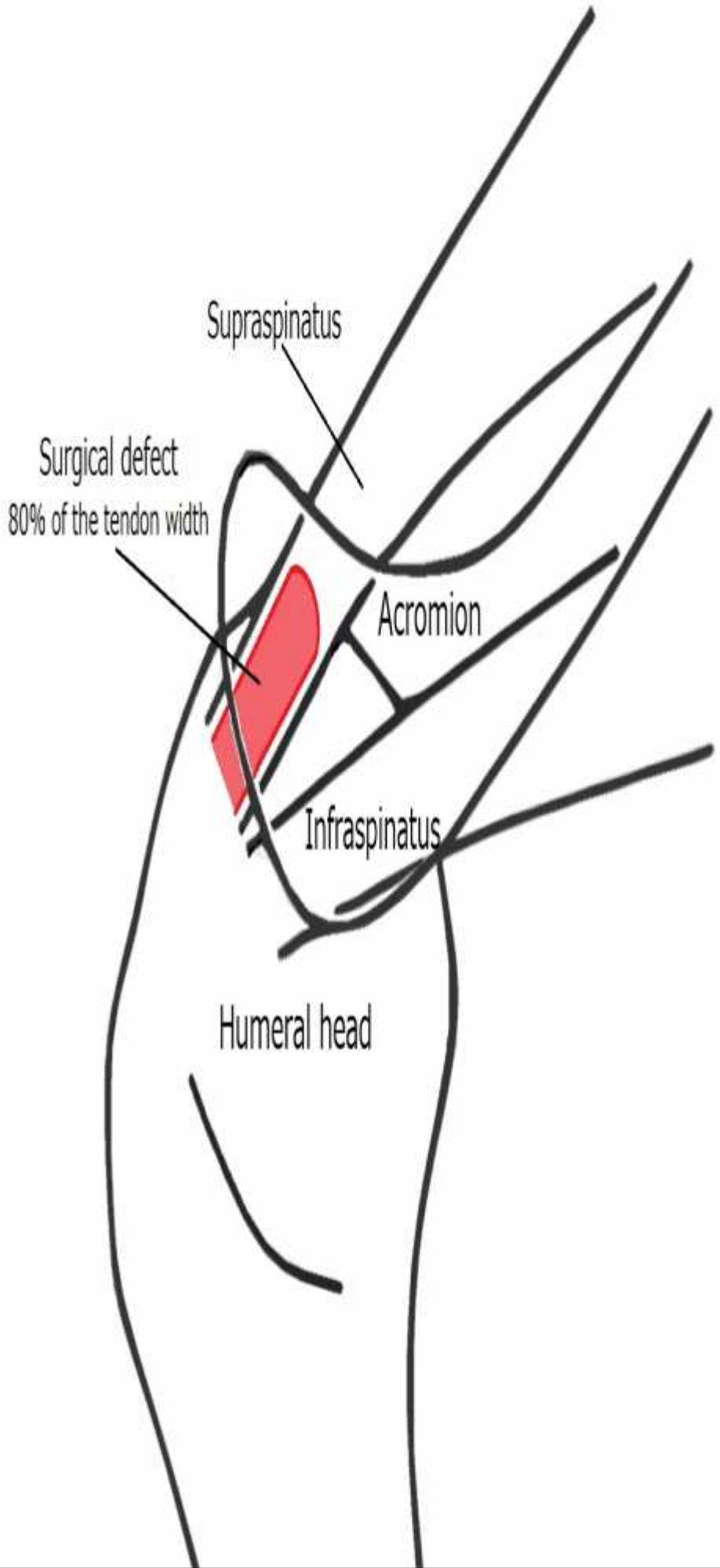
References: 1.Mitsui Y, Gotoh M, Nagata K. Hyaluronic acid inhibits mRNA expression of proinflammatory cytokines and cyclooxygenase-2/prostaglandin E(2) production via CD44 in interleukin-1-stimulated subacromial synovial fibroblasts from patients with rotator cuff disease.J Orthop Res. 2008 Jul;26(7):1032-7.


Supraspinatus
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80% of the tendon width
Acromion
Infraspinatus
Humeral head

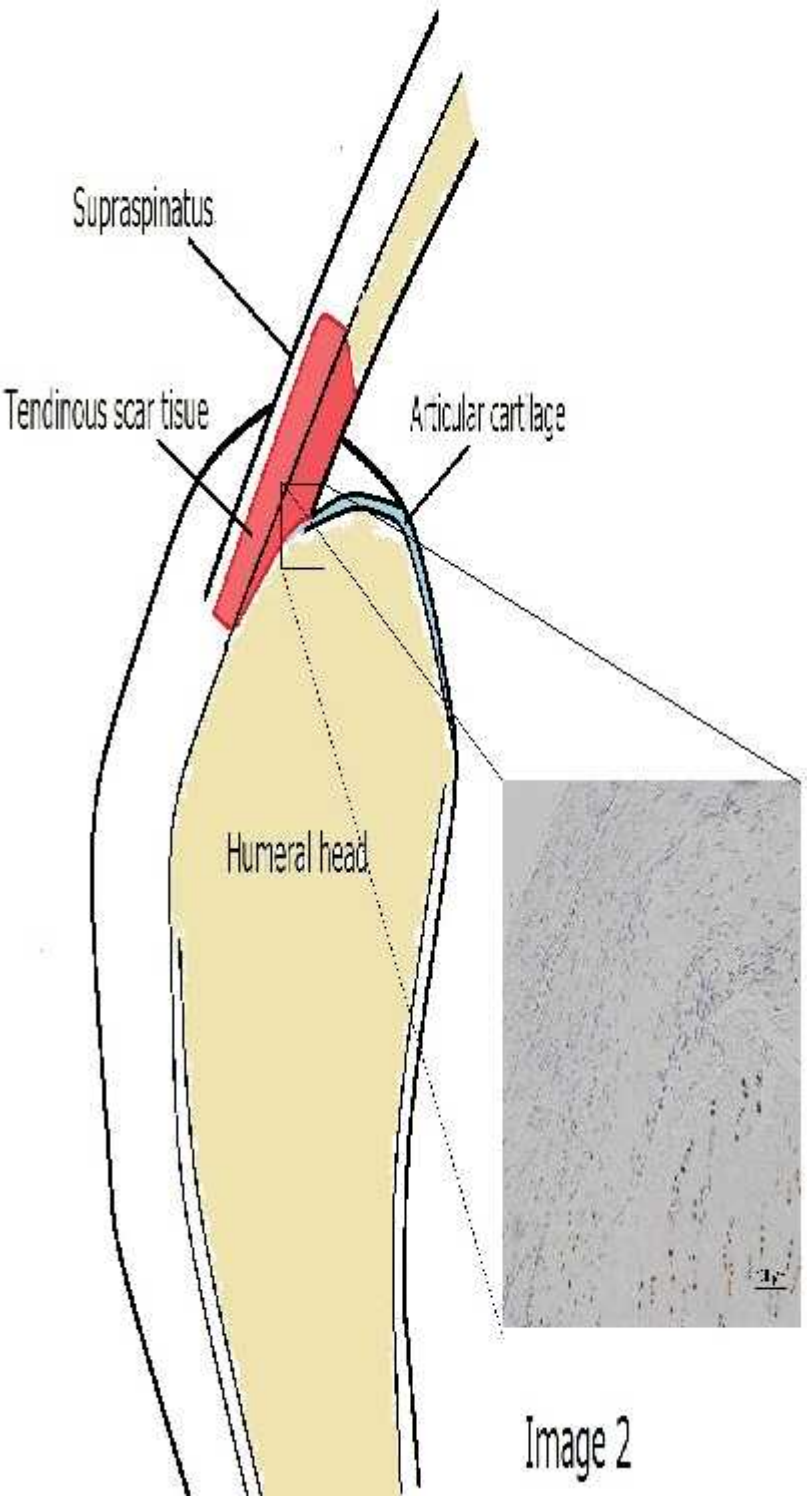

Supraspinatus
Tendinous scar tisue
Articular cartilage
Humeral head

Image 2

Fig.1 MTT assay

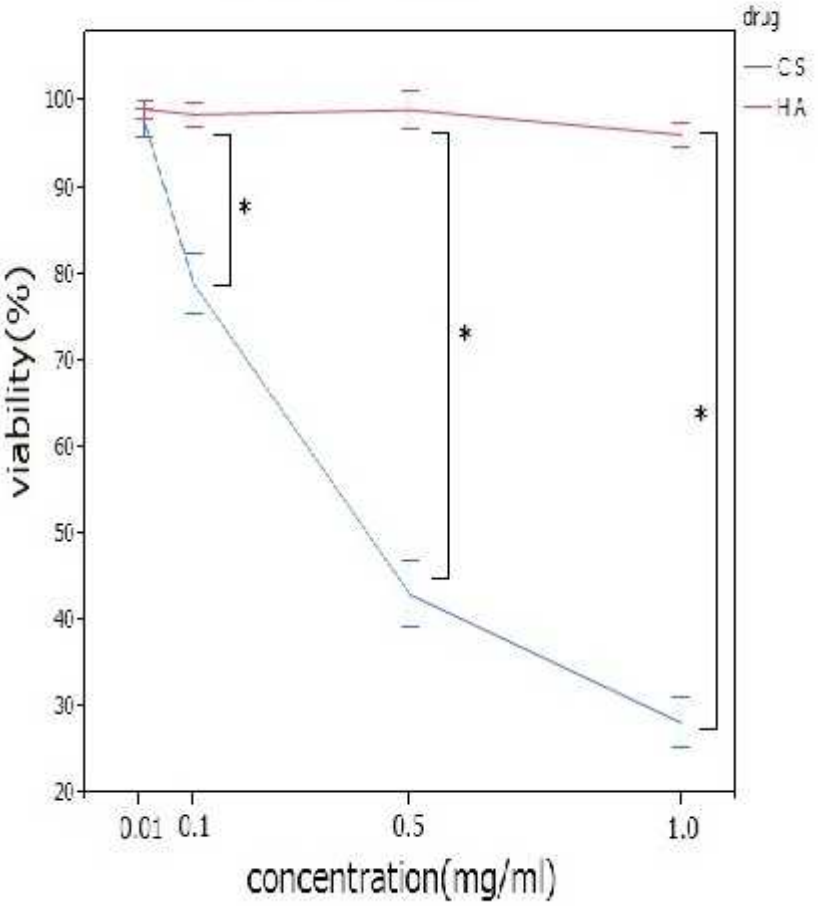


Fig.2 MTT assay

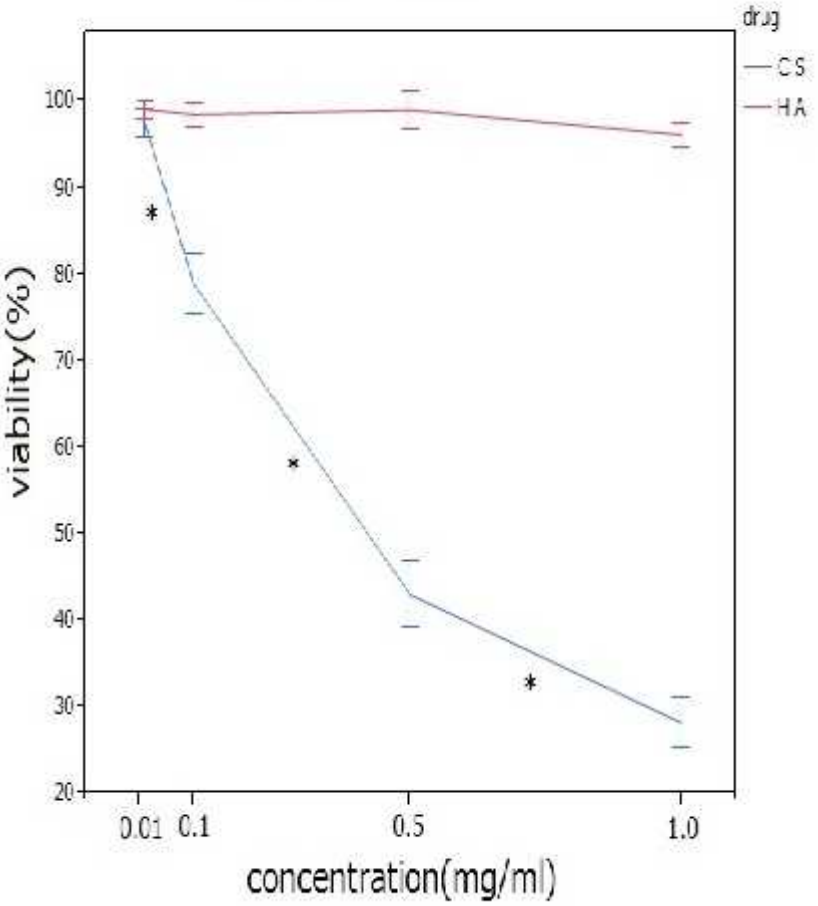


Fig.3 Viable cell counts with fluoroscence microscopy

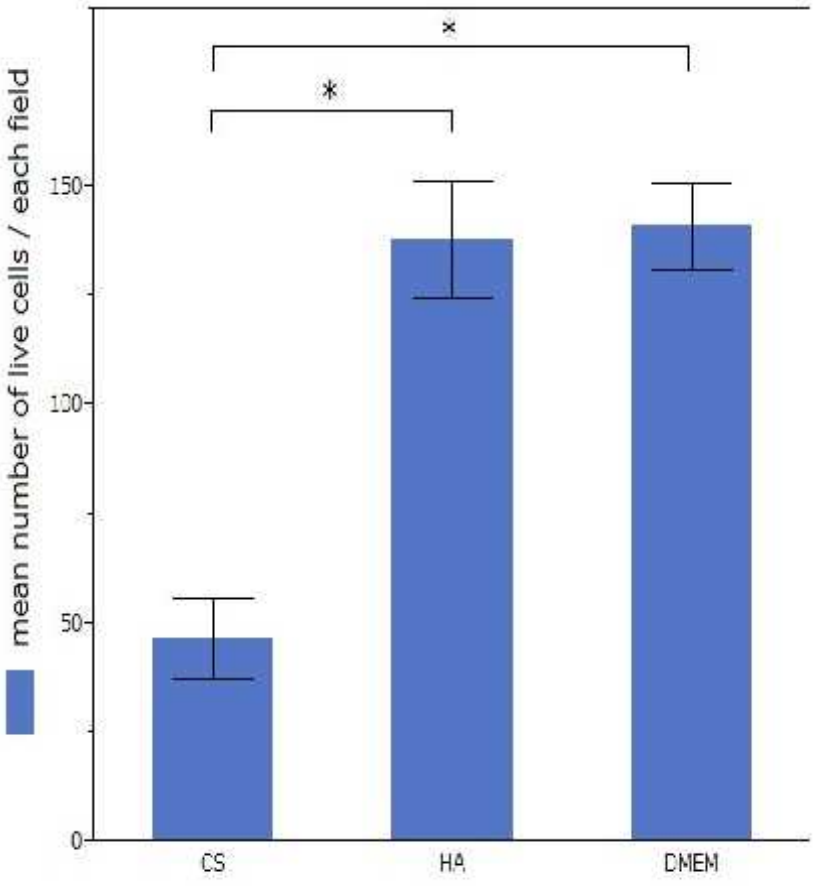


Fig.4 FACS

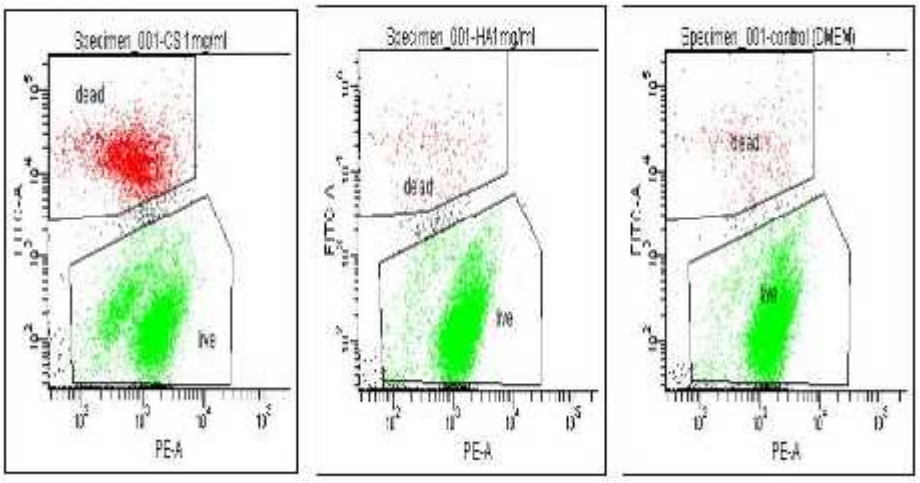


Fig.5 FACS

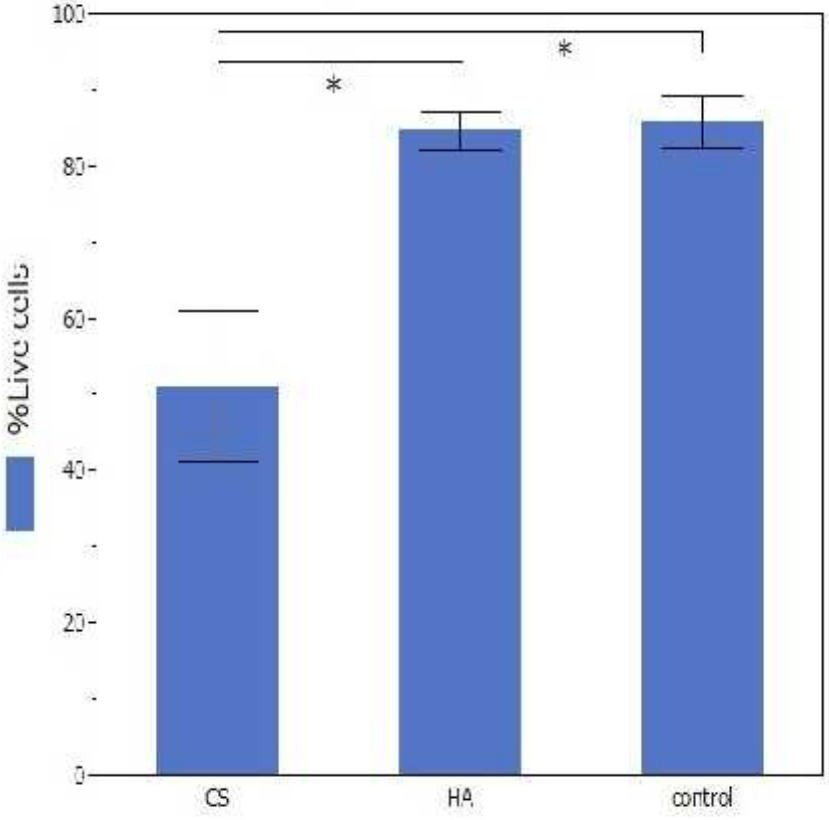
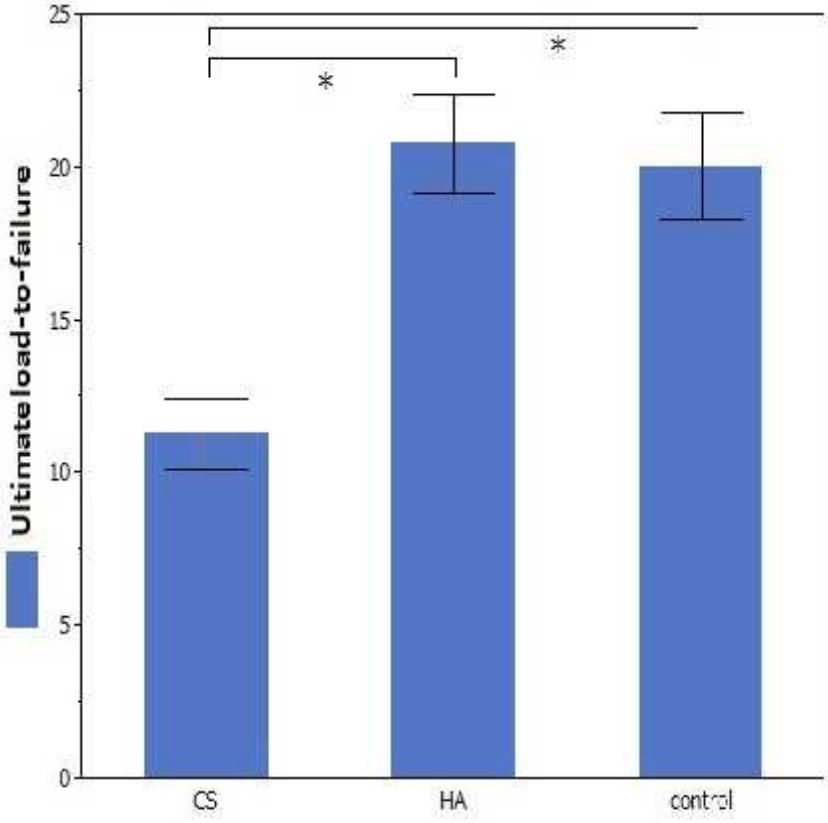
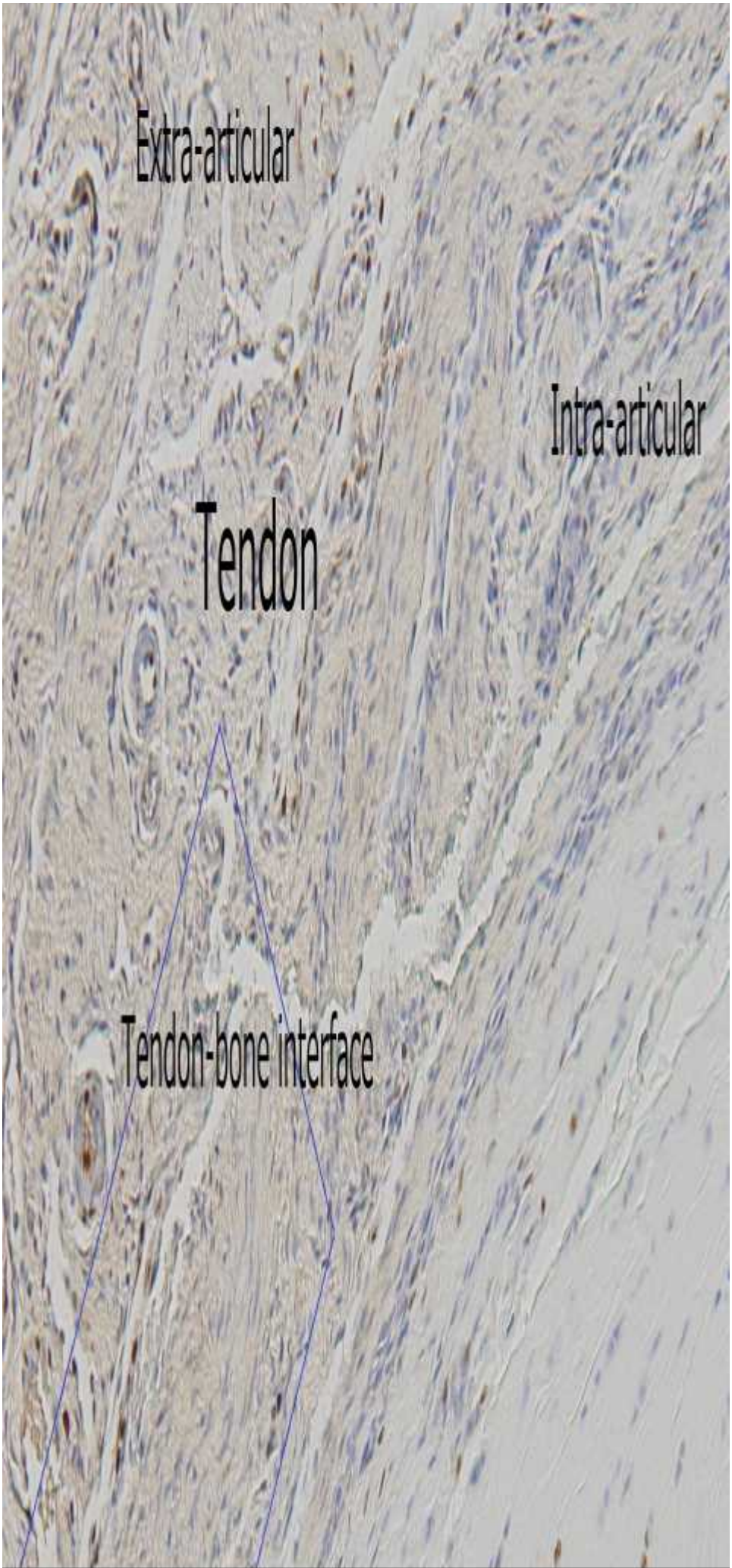
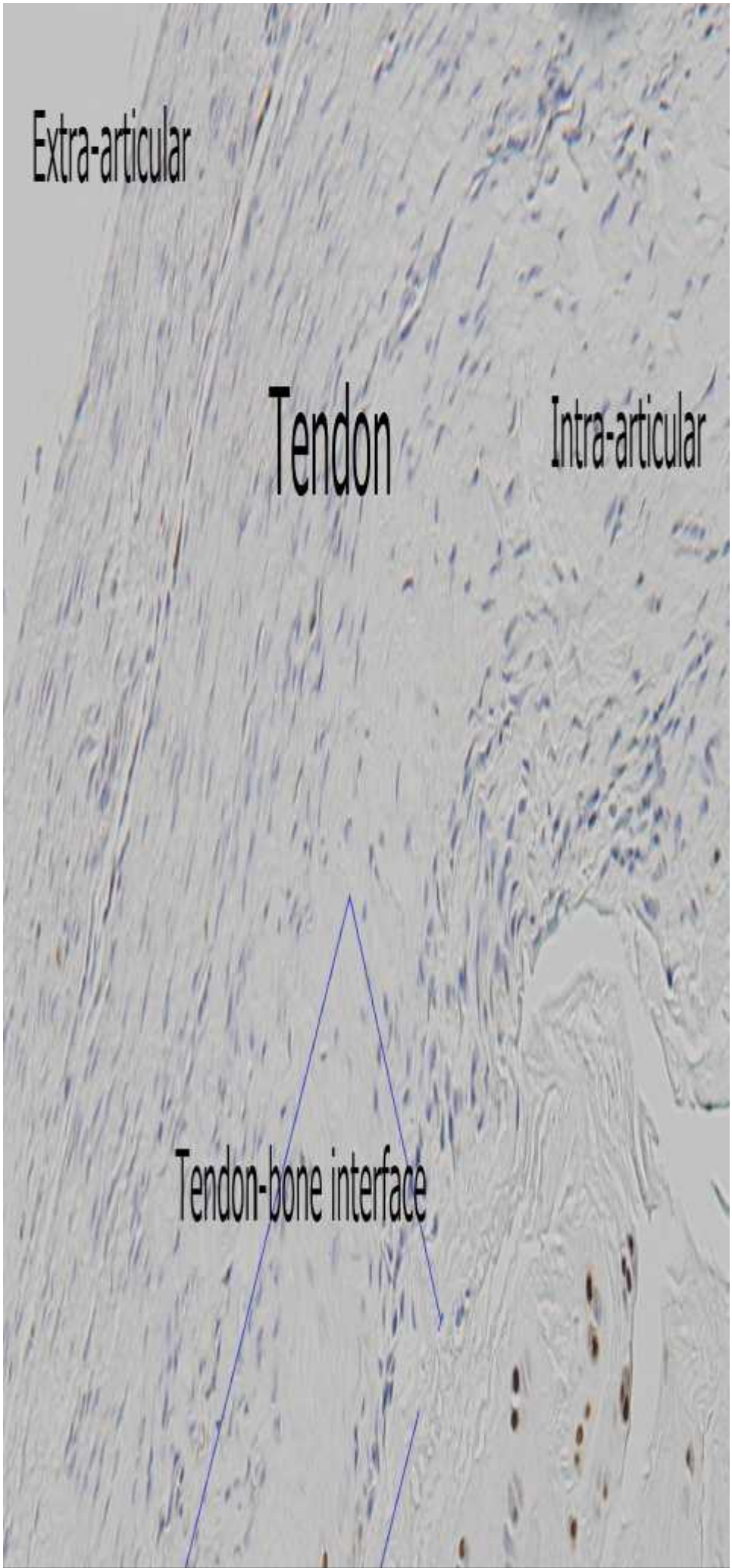


Fig.6 Biomechanical testing




Extra-articular
Intra-articular
Tendon
Tendon-bone interface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