INTRODUCTION:
Anterior cruciate ligament (ACL) reconstruction with a graft of semitendinosus or/or gracilis tendon is common and well-established treatment for ligament rupture. However, the weak link during the early healing process in the attachment site between the tendon and bone and the rate of healing and strength of this attachment could be one of the critical factors for successful ACL reconstruction. To improve this limitation, recent studies have shown that various growth factors have potential for tendon-bone integration1,2,3. In this study, we focused on enamel matrix derivative (EMD) for tendon-bone integration. This EMD has a potential for stimulation of mesenchymal stem cells following up-regulation of Sharpey’s fiber formation and has already been applied to dental field for treatment of tooth root. Inspired these backgrounds, we hypothesized that EMD applied to tendon-bone junction during ACL reconstruction could accelerate tendon-bone healing with up-regulation of Sharpey’s fiber formation. The purpose of this study is to evaluate the histological and mechanical effect of EMD on tendon-bone interface in ACL reconstruction. Then, long term result of the mechanical properties and immunohistochemical examination of the presence of enamel matrix protein in the attachment of ACL are added to the last ORS meeting presentation and discussed.

METHODS:
The institutional animal care and use committees of the Hiroshima University approved all animal procedure.

Surgical Procedure
Female Sprague-Dawley rats (12 weeks old, 250-280g) were used in this study. The both knees of each animal were underwent ACL reconstruction using the flexor digitorum tendon. Each animal was anesthetized with an intraperitoneal injection of pentobarbital sodium. Using 0.2mm of the dental drill, bone tunnels were made in the proximal tibia and the distal femur. Then the graft was passed through the bone tunnels. After fixation of proximal side on femur, the distal side of graft was secured on the proximal tibia under pretension 100g (Fig. 1). Rats were allowed to bear full weight with no limitation of range of motion.

Material preparation
We used commercially available EMD (EMDOGAIN®; Seikagaku Corporation, Osaka, Japan). In left knee joint, around the tendon-bone interface on tibia side was filled in 40μl of EMD with propylene glycol alginate (PGA) as a carrier of proteins (EMD). In right knee joint around the tendon-bone interface on tibia side was eutetted and filled in only PGA (control). All animals were evaluated at 4, 8, 12 and 16 weeks with histological and biomechanical examination (n = 6 in each time point).

Histological Evaluation
At 4, 8 and 12 weeks, the knee joints were harvested and fixed in 10% buffered formalin. After decalcification, samples were embedded in paraffin and cut into 5-μm thick longitudinal sections to the bony tunnels in the femur and tibia. Haematoxylin- eosin (H-E) and Azan staining were carried out using these slides.

Mechanical Evaluation
The mechanical properties of the graft were measured using the both knee specimens of each rat in a conventional tensile tester (1840NT:500, AIKOH Engineering, Osaka, Japan). After removal of all extraneous soft tissues from the femur-graft-tibia units, the femur and tibia were set and measured the ultimate load to failure at a cross-head speed of 5mm/min in line with the long axis of the ACL.

Statistical analysis
Results are depicted as mean ± SE. Means were compared by Student t test. Comparisons of groups at different time points and between control groups were performed with by 1-way analysis of variance (ANOVA) and were considered to significantly different if p < 0.05.

RESULTS:
Histological analysis
We examined six samples at 4, 8, and 12 weeks after treatment in both groups. In the EMD group, significant increase of collagen fibers and progressive maturation in the interface was observed (Fig.1a, b) although tendon-bone interface was composed of cellular and vascular fibrous tissues in the control group (Fig.1c, d).

DISCUSSION:
In this study, it was demonstrated that EMD has a potential for improvement of tendon-bone infiltration after ACL reconstruction histologically and mechanically especially in early period. To our knowledge, this is the first report that showed efficacy of EMD to the acceleration of the tendon-bone interface maturation in ACL reconstruction in vivo. But, there was no report focusing the presence of enamel matrix protein in the attachment of ACL. Therefore, immunohistochemical examination would be required for detection of the enamel matrix proteins in the attachment of ACL. In result, the localization was not admitted in the attachment of ACL. In the clinical setting, ACL reconstruction procedures require healing of tendon grafts in the surgically created bone tunnel. Firm attachment of the tendon graft to the bone is a critical factor allowing earlier and more aggressive rehabilitation and earlier return to sports and work. However, more precise investigations would be required for applying to clinical situations, this clinically available material could be an attractive option supporting successful ACL reconstruction in the near future.

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REFERENCES:
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Figure 1. Reconstructed anterior cruciate ligament (ACL) with the flexor digitorum tendon in the left knee.

Figure 2. Histological evaluation of tendon-bone interface of a specimen from each group at 8weeks after surgery (left panels; H-E staining, right panels; Azan staining, T: tendon, B: bone, original magnification; x100). In the tendon-bone interface, the abundant perpendicutar collagen fibers connecting the bone. Sharpey’s fibers, were observed in the EDM group at 8weeks after surgery (a, b), whereas the fibers were not observed in the control group (c, d).

Figure 3. The average load to failure in the specimen of each group at 8weeks after surgery (left panels; H-E staining, right panels; Azan staining, T: tendon, B: bone, original magnification; x100). On the tendon-bone interface, the abundant perpendicutar collagen fibers connecting the bone. Sharpey’s fibers, were observed in the EDM group at 8weeks after surgery (a, b), whereas the fibers were not observed in the control group (c, d).

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