Generation of Bone-Tendon-Bone graft using rhBMP and application to the ACL Reconstruction
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ABSTRACT INTRODUCTION:
Graft healing in the bone tunnel is a one of the important factor for the success in anterior cruciate ligament (ACL) reconstruction. The healing process of tendon to bone (ex, hamstring graft) is slowly and type of an indirect insertion [1]. While, that of bone-tendon-bone graft is type of a bony fusion and an original bone-tendon junction “enthesis” has been maintained in the graft. Thus, bone-tendon-bone graft may be suitable for the ACL reconstruction histologically and physiologically. However, use of bone-tendon-bone graft is controversial due to the associated donor-site morbidity [2]. In previous study, we produced the regenerated enthesis structure using rhBMP [3]. These findings suggest that it is possible to successfully regenerate a direct tendon-to-bone enthesis.
In this study, we succeeded the generated bone –tendon-bone structure injecting rhBMP to the tendon and could perform the ACL reconstruction using the newly bone tendon bone graft.

METHODS:
Preparation of RhBMP-Induced Bone-tendon-bone graft in the Semitendinosus Tendon
Healthy, adult female New Zealand White rabbits were utilized in the study. RhBMP-2 was dissolved into 0.01 N HCl at a concentration of 5 µg/µL. The semitendinosus tendon of the animals in the experimental group (n=5) was exposed. To prevent from spreading rhBMP-2 solution, the tendon was tied with 3-0 nylon at four points, which the both ends were to become bone after the injection of rhBMP. A 30 µg portion of rhBMP-2 in 12 µL of BMP solution was injected with a micro-syringe and 28G needle (Ito Corp, Shizuoka, Japan) into the intervals between first and second knot, and third and forth knot. In the control group (n=5), the left hind limb was treated in the same fashion but only HCl solution (12µL) was injected into the tendon. After 6 weeks, the semitendinosus tendons were harvested (fig1,a) and processed for radiological and histological examination.

Anterior Cruciate Ligament Reconstruction with induced Bone-tendon-bone graft
6 weeks after the injection with rhBMP-2 to the semitendinosus tendons of 6 healthy adult rabbits, ACL reconstruction with induced bone-tendon-bone graft. Original ACL was excised from tendon-bone junction, and bone tunnels (2.8 mm in diameter) were created by drilling at ACL original footprint of the femur and the tibia, respectively. The semitendinosus tendon including the ectopic bone was exposed and cut at approximately 5 mm peripheral to the ectopic bone. The harvested tendon was sutured at the end of tendon by stainless wire and the graft was fixed to the bone tunnels by pins. And temporary fixation by a pin was performed in the Tibia side. (BMP+)
In the control group (BMP-), another 6 healthy adult rabbits were injected with HCl/ICG solution alone in the semitendinosus tendon. At 4 and 8 weeks after surgery, three rabbits from each group were sacrificed, examined CT scan and processed for histological examination.

RESULTS SECTION:
In radiography, bone-tendon-bone structure was seen at the semitendinosus tendon 6 weeks after the injection of rhBMP (fig1, b). No calcification was seen in the control group. In histology, as the same of the previous study, enthesis-like tissue had been successfully reproduced between the ectopic bone and the tendon by 6 weeks after injection of rhBMP-2(fig1, c). This data showed newly bone-tendon-bone structure were generated from semitendinosus tendon using rhBMP. On gross inspection and 3D-CT imaging in ACL reconstruction, induced bone-tendon-bone graft were recognized through the bone tunnels and consistently incorporated in the bone tunnel in the experimental group at 4 and 8 weeks, though no significant bone formation was observed in the controls (fig2,a,b).

In histology of the ACL reconstruction, the transferred induced bone-tendon-bone graft has integrated with the bone tunnels and the structure of the enthesis at the generated bone-tendon junction has been maintained at 4 and 8 weeks, while no significant enthesis structure was observed in the controls but Sharpey fibers were appeared the interface between tendon and bone at 4 and 8 weeks.

DISCUSSION:
This study is the first report that generated bone-tendon-bone is reproduced at semitendinosus tendons using rhBMP. And the ACL reconstruction using generated bone-tendon-bone was successfully performed, which bony fusion was recognized between the graft and the bone tunnel and the structure of the enthesis at the generated bone-tendon junction has been maintained.

In previous study we demonstrated that it is possible to induce ectopic bone formation in tendon consistently and then successfully generate a tendon-bone junction similar to normal enthesis at the tendon-bone interface. Further, the regenerated enthesis produced by transfer of the tendon containing the rhBMP-2-induced bone mass into host bone was shown to be functionally competent by mechanical load testing [4]. So, this technique generated bone-tendon-bone may have provided a strong physiological fixation, a less donor-site morbidity, and resulting in biomechanically better ACL reconstructions.
A limitation of this study is that two surgeries are required, one to ossify the graft tendon, and another to harvest and reconstruct the knee, perhaps making clinicians reluctant to use this technique.

However, hopefully our approach will avoid postoperative widening of the bone tunnels, which probably occurs due to incomplete bonding of the transferred tendon to the bone tunnel walls [5], and it might yield improved long-term outcomes for knees with ACL reconstruction.

Fig1 generated bone-tendon-bone graft

a) gross inspection
b) X-P
c) H-E stain

Fig2 gross inspection of ACL reconstruction

BMP- BMP+

ectopic bone

REFERENCES: