

# Biological fixation between calcium phosphate-hybridized tendon graft and tibial bone tunnel in anatomic single-bundle ACL reconstruction - A case report

Hirotaka Mutsuzaki<sup>a,\*</sup>, Tomonori Kinugasa<sup>b</sup>, Masataka Sakane<sup>c</sup>

<sup>a</sup> Department of Orthopaedic Surgery, Ibaraki Prefectural University of Health Sciences, 4669-2 Ami Ami-machi, Inashiki-gun, Ibaraki, 300-0394, Japan

<sup>b</sup> Department of Orthopaedic Surgery, Ichihara Hospital, 3681 Oozone, Tsukuba, Ibaraki, 300-3295, Japan

<sup>c</sup> Department of Orthopaedic Surgery, Tsukuba Gakuen Hospital, 2573-1 Kamiyokoba, Tsukuba, Ibaraki, 305-0854, Japan

## ARTICLE INFO

### Keywords:

Calcium phosphate hybridization  
Biological fixation  
Tendon-to-bone healing  
Anatomic single-bundle anterior cruciate ligament reconstruction

## ABSTRACT

The purpose of this study was to histologically observe a specimen of a calcium phosphate (CaP)-hybridized tendon graft-bone interface at the posterior side of tibial bone tunnel obtained during the revision anterior cruciate ligament (ACL) reconstruction.

We present the case of a 15-year-old female who was returning to sports 7 months and 12 days after primary anatomic single-bundle ACL reconstruction with no instability. Re-rupture was diagnosed 9 months and 12 days after the surgery.

At the joint aperture site, a firm biological fixation via direct bonding area, cartilaginous tissue, and dense collagen fiber were observed without the presence of a nonbonding gap area.

## 1. Background

Following anterior cruciate ligament (ACL) reconstruction using a soft tissue graft, only fibrous tissue was noted at the interface between the grafted tendon and bone tunnel.<sup>1,2</sup> To improve tendon-to-bone healing, a calcium phosphate (CaP)-hybridized tendon graft using an alternate soaking process was developed.<sup>3</sup> Direct bonding area and normal ACL insertion-like cartilage layer at the joint aperture sites between the CaP-hybridized tendon graft and bone tunnel was observed at 1 year after ACL reconstruction in goats.<sup>2</sup> In a clinical trial of anatomic single-bundle ACL reconstruction using CaP-hybridized tendon graft,<sup>4</sup> a specimen of the grafted tendon-bone tunnel interface was obtained from the posterior side of the tibial bone tunnels during re-creation of the tibial bone tunnel during one case of a revision ACL reconstruction. The purpose of the present study was to histologically observe the specimen of the CaP-hybridized tendon graft-bone tunnel interface.

## 2. Case report

This is the case of a 15-year-old female, 159 cm in height and 63 kg weight participating in competitive level football.

A semitendinous tendon alone was used as the tendon graft for

primary ACL reconstruction. The length of the multi-stranded tendon graft was 60 mm, and graft diameter was 8 mm in the femoral portion, 8 mm in the intraarticular portion, and 9 mm in the tibial portion. The surgical procedure of the anatomic single-bundle ACL reconstruction using the outside-in tunnel technique is described in a previous study.<sup>4</sup> The graft was suspensory fixed with an initial tension of 10 N using a tension meter at 20° of knee flexion.

The intraoperative CaP hybridization method was as described in a previous study.<sup>4</sup> After covering the intraarticular portion of the tendon graft, the tendon graft was soaked in a calcium solution for 30 s, and in a NaHPO<sub>4</sub> solution for 30 s. Before each soaking, the grafts were washed in a saline solution. This cycle was repeated 10 times.<sup>4</sup>

Postoperative rehabilitation was performed in accordance with a previous study.<sup>4</sup> Full weight-bearing walking was allowed at 3 weeks postoperatively. Running was allowed at 3 months postoperatively, and the return to sports was allowed after 6–12 months.

There was no instability in 7 months and 12 days after the primary ACL reconstruction. Just prior to resuming sports, a re-rupture was diagnosed by the second-look arthroscopy 9 months and 12 days after the primary ACL reconstruction. Revision ACL reconstruction was performed 10 months after the primary ACL reconstruction. During the revision surgeries, the specimen was obtained.

The specimens were fixed in 10% neutral buffered formalin,

\* Corresponding author.

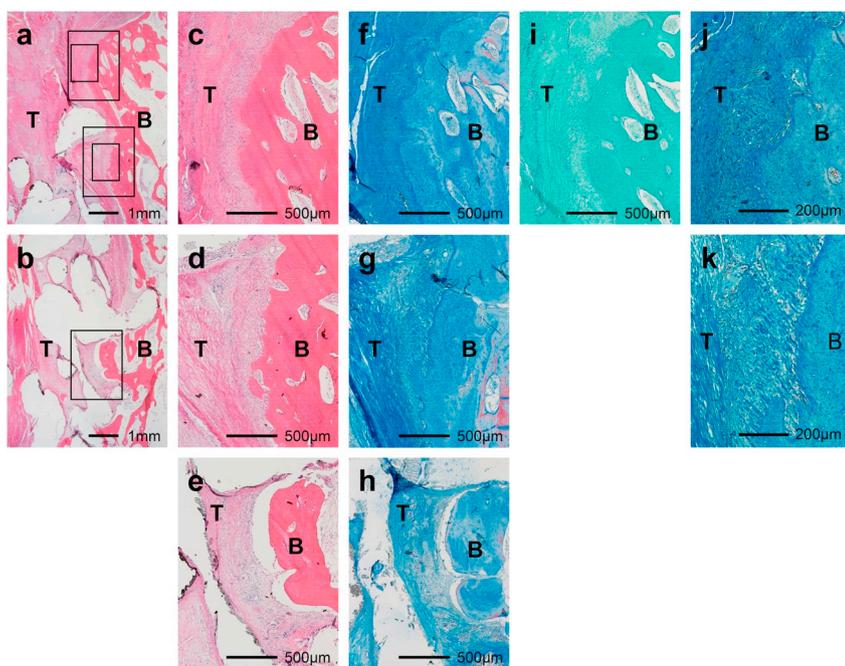
E-mail addresses: [mutsuzaki@ipu.ac.jp](mailto:mutsuzaki@ipu.ac.jp) (H. Mutsuzaki), [yan-k@da2.so-net.ne.jp](mailto:yan-k@da2.so-net.ne.jp) (T. Kinugasa), [sakane-m@tsukuba-seikei.jp](mailto:sakane-m@tsukuba-seikei.jp) (M. Sakane).

<https://doi.org/10.1016/j.jor.2019.04.014>

Received 8 February 2019; Accepted 15 April 2019

Available online 02 May 2019

0972-978X/ © 2019 Professor P K Surendran Memorial Education Foundation. Published by Elsevier B.V. All rights reserved.



**Fig. 1.** Histologic sections of the grafted tendon-bone tunnel interface at posterior side of tibial bone tunnels in Case 1. a, low magnified image ( $12.5\times$ ) by hematoxylin and eosin (H&E) staining at proximal site; b, low magnified image ( $12.5\times$ ) by H&E staining at distal site; c, large square area of upper boxed image in Fig. 1a ( $40\times$ ) by H&E staining; d, large square area of lower boxed image in Fig. 1a ( $40\times$ ) by H&E staining; e, large square area of the boxed image in Fig. 1b ( $40\times$ ) by H&E staining; f, large square area of upper boxed image in Fig. 1a ( $40\times$ ) by MT staining; g, large square area of lower boxed image in Fig. 1a ( $40\times$ ) by MT staining; h, large square area of boxed image in Fig. 1b ( $40\times$ ) by MT staining; i, large square area of upper boxed image in Fig. 1a ( $40\times$ ) by Safranin-O staining; j, small square area of upper boxed image in Fig. 1a ( $40\times$ ) by MT staining; k, small square area of lower boxed image in Fig. 1a ( $40\times$ ) by MT staining. T, tendon graft; B, bone.

decalcified, and embedded in paraffin. Thereafter, they were sliced sagittally. Hematoxylin and eosin (H&E) staining, safranin-O staining for identification of glycosaminoglycans in the cartilage layer, and Masson's trichrome (MT) staining for identification of collagenous fiber tissue were then performed. The grafted tendon-bone tunnel interface was examined by light microscopy.

The institutional ethics committee reviewed and approved the study (approval number: 1101). Informed consent was obtained from the patient.

### 2.1. Findings

The grafted tendon, approximately 15 mm in length, was observed in the tibial bone tunnel. In the grafted tendon-bone tunnel interface, at the joint aperture site (proximal 5 mm), the direct bonding area, cartilaginous tissue with chondrocytes via round cell, and dense collagen fibers were observed without a nonbonding gap area (Fig. 1a, c, f, i, and j). Area of red Safranin-O-stained glycosaminoglycans in the cartilage layer was not observed (Fig. 1i). At the mid portion of the tibial bone tunnel (central 5 mm), an interfacial layer with Sharpey's fibers-like fibers was observed (Fig. 1a, d, g, and k). At the distal portion of the tibial bone tunnel (distal 5 mm), thin fibrous tissue and loose scar tissue were observed (Fig. 1b, e and h).

### 3. Discussion

In this case, a direct bonding area without the nonbonding gap between the grafted tendon-bone tunnel interface was observed at the joint aperture site after re-rupture of anatomic single-bundle ACL reconstruction using a CaP-hybridized tendon graft. The osteogenic effect of the CaP-hybridized tendon graft was enhanced by bone formation on the surface of the tendon graft in the tibial bone tunnel. In the previous study, firm biological fixation with direct bonding and cartilage layer at the joint aperture site in the CaP group were observed compared with the conventional method after ACL reconstruction in goats.<sup>2</sup> Moreover, the biomechanical knee stability in the CaP group was superior to that in the conventional method after the ACL reconstruction.<sup>2</sup> The firm biological fixation using the CaP-hybridized tendon grafts may contribute to knee stability after ACL reconstruction.

The firm biological fixation was observed at the joint aperture site only. Mechanical unloading degenerated the insertion structure as observed histologically in rabbits.<sup>5</sup> The mid and distal portion of the tibial bone tunnel showed loose fibrous tissue, the interface can be mechanical unloaded. The area of red safranin-O-stained glycosaminoglycans in the cartilage layer was not observed. The period from re-rupture to revision surgery can be influence the structural changes.

In this case, firm biological fixation between the CaP-hybridized tendon grafts and posterior tibial bone tunnels was seen at the joint aperture site after re-rupture of the anatomic single-bundle ACL reconstruction. CaP-hybridized tendon grafts can provide firm biological fixation between the grafted tendon and bone.

### Conflicts of interest

The authors declare that they have no conflicts of interest.

### Ethics approval and consent to participate

The institutional ethics committee reviewed and approved the study (approval number: 1101). Informed consent was obtained from the patient.

### Consent for publication

We have obtained written consent from the patient for publication.

### Acknowledgments

We would like to thank Editage ([www.editage.jp](http://www.editage.jp)) for English language editing. This work was supported by the Japan Sports Medicine Foundation, 2015.

### References

1. Nebelung W, Becker R, Urbach D, Röpke M, Roessner A. Histological findings of tendon-bone healing following anterior cruciate ligament reconstruction with hamstring grafts. *Arch Orthop Trauma Surg.* 2003;123:158–163.
2. Mutsuzaki H, Sakane M, Fujie H, Hattori S, Kobayashi H, Ochiai N. Effect of calcium

- phosphate-hybridized tendon graft on biomechanical behavior in anterior cruciate ligament reconstruction in a goat model: novel technique for improving tendon-bone healing. *Am J Sports Med.* 2011;39:1059–1066.
3. Taguchi T, Kishida A, Akashi M. Hydroxyapatite formation on/in hydrogels using a novel alternate soaking process. *Chem Lett.* 1998;8:711–712.
  4. Mutsuzaki H, Kinugasa T, Ikeda K, Sakane M. Anatomic single-bundle anterior cruciate ligament reconstruction using a calcium phosphate-hybridized tendon graft: a randomized controlled trial with 2 years of follow-up. *J Orthop Surg Res.* 2018;13:327.
  5. Mutsuzaki H, Nakajima H, Wadano Y, Takahashi H, Sakane M. Influence of mechanical unloading on histological changes of the patellar tendon insertion in rabbits. *Knee.* 2015;22:469–474.