Augmentation of the Tendon-Bone Interfacial Healing by Calcium Phosphate Cement

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Introduction
In many instances of joint stability reconstructive surgery, an autografted tendon is passed through the bone tunnel. Therefore, the healing on the interfacial gap between bone and tendon will greatly influence the post-operative rehabilitative programs and ultimately the clinical results. The bone ingrowth had been found to be the most important factor that influenced the mechanical strength of interface healing. (1,2,3) Therefore, we hypothesize that filling the gap between grafted tendon and bone tunnel with an osteoconductive material will augment the interface healing by inducing more bone ingrowth. And, the present rabbit model study was done to evaluate if filling the bone-tendon gap with calcium phosphate cement (CPC) will encourage the interface healing or not.

Materials and Methods
New Zealand White rabbits were used for this in-vivo study. Bilateral knees were approached anteromedially and the anterior cruciate ligament (ACL) was excised first. The semitendinosus tendon was dissected out and passed through the tibial bone tunnel and femoral bone tunnel to reconstruct the ACL. (4) Randomly, on one side of the knee, the interface between the grafted tendon and bone tunnel was filled with calcium phosphate cement. One ml of CPC was injected into the interface by a 5ml syringe. Total 22 rabbits were used for this study. For mechanical testing, 6 rabbits were sacrificed respectively at the end of first and second post-operative week For serial histological observation of the interface healing, 2 rabbits were sacrificed sequentially on the end of 1st, 3rd, 6th, 12th & 24th post-operative week.

The injectable calcium phosphate cement (CS-1537, Biomaterial Lab. National Chenchung University, Taiwan) in this study was fabricated by mixing equal molar tetra calcium phosphate Ca4(P04)2 (TTCP) and dicalcium phosphate anhydrous CaH04. 2H2o(DCPA) pretreatment powder in a diluted phosphate-containing solution (25mM) at a powder/liquid rate of 4.0 (8mg/ml).

The Instron material testing machine model #1322 with a 50kg load cell was used to detect the maximal tensile strength of the healing tissue. The test samples were dissected to be a bone-tendon-bone model, the bone of which was mounted on each end. The tensile strength was detected at a rate of displacement of 5.0 minimeters per second, until the tendon was pulled out of the femoral bone tunnel. The maximal tensile strength was collected and the data was statically analyzed by pair-t test.

For histological study, the dissected knee joints were fixed in the neutralized Formalin for 72 hours first, then decalcified with mixed solution containing 20% sodium citrate and 50% formic acid for 1 week. After decalcification, the samples were embedded in paraffin for slice cut perpendicular to the bone tunnels axis. Hematoxylin and eosin stains were done for light microscopic study.

Results
Gross Findings: In CPC group, the gross pictures of transverse sections made perpendicular to the long axis of the bone tunnel revealed most part of the tendon-bone interface was filled out by calcium phosphate cement in one-week specimen. (Fig.1-A). Progressive absorptions of CPC and bone ingrowth were observed in the three-week (Fig.1-B), six-week (Fig.1-C), twelve-week (Fig.1-D) and twenty-four-week specimens. The gap obliterated progressively by the new growing bone. In non-CPC group, the gross pictures of transverse sections revealed the most part of the tendon-bone interface was filled by CPC in one-week specimen. (Fig. 2-A). Although the amount and maturation of collagen fibers increased in three-week (Fig. 2-B) and six-week specimens (Fig.4-C), new continuity to the bone tunnel was only occasional observed. No new bone formation observed within the gap and a layer of new lamellar bone formation was observed on the rim of native bone tunnel in twelve-week (Fig. 4D) and twenty-four-week specimen.

Histology Findings: In CPC group, Histological pictures of these transverse sections revealed the most part of the tendon-bone interface was filled by CPC in one-week specimen (Fig. 3-A). New growing bone islands observed progressively within the CPC in three-week(Fig 3-B) and six-week specimens (Fig. 3-C). Most portions of the interface were filled out by newly formed bone and adjacent to the surface of grafted tendon the continuity between collagen fibers of the grafted tendon and surrounding bone could be clearly observed in the twelve-week (Fig. 3-D) and twenty-four-week specimens.

Mechanical Test: One-week specimen: Six specimens in the CPC group and non-CPC group respectively were tested. All the specimens failed by pullout of the tendon from the femoral bone tunnel. The mean maximal tensile strength of CPC group was 0.664 ± 0.174 kg and the mean maximal tensile strength of non-CPC group was 0.209 ± 0.097kg. While tested by pair-t test, the CPC group was significantly stronger than the non-CPC group.(T=5.603, P<0.01) Two-week specimen: Six specimens in the CPC group and non-CPC group respectively were tested. All the specimens also failed by pullout of the grafted tendon from the femoral bone tunnel. The mean maximal tensile strength of CPC group was 1.173 ± 0.292kg and the maximal tensile strength of non-CPC group was 0.556 ± 0.404kg (Table II). While tested by pair-t test, the CPC group was significantly stronger than the non-CPC group (T=3.023 P<0.05).

Discussion
In the histological observation, the calcium phosphate cement was found with obvious augmenting effects on the biological healing response of tendon graft by conducting massive bone ingrowth, and in the mechanical study, the calcium phosphate cement also was found with significant reinforcement effects on the maximal tensile strength. One of the biggest disadvantages of using the hamstring tendons used to be the fixation and healing. From the results of this study, we believe that CPC is a potential and promising material to reinforce the fixation and to augment the tendon healing to bone in the clinical practice.

Reference