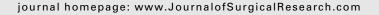


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Anterior cruciate ligament reconstruction in a rabbit model using canine small intestinal submucosa and autologous platelet-rich plasma

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ABSTRACT

Background: The bone—ligament interface is the main point of failure after anterior cruciate ligament reconstruction. Synthetic ligament materials have problems such as a greater failure rate of the bone—ligament insertion than autografts. Small intestinal submucosa (SIS) is a biologic scaffold that has been used to repair musculoskeletal tissue and has been shown to promote cell migration and enhance collagen fiber regeneration. Autologous platelet-rich plasma (PRP) has also been investigated as a potential promoter of tendon healing. We investigated SIS and PRP as biomaterials that might strengthen the bone—tunnel interface and improve tendon structure formation.

Methods: Anterior cruciate ligament grafts were formed of braid-twist canine SIS. These canine SIS ligament grafts were used for anterior cruciate ligament reconstruction in 20 New Zealand white rabbits. The rabbits were divided into 2 treatment groups. In 1 group (SIS group; n=10), we only implanted the canine SIS grafts. In the second group (PRP group; n=10), we applied autologous PRP to the surgical area after implantation of canine SIS grafts. We determined the cytokine level of the autologous PRP using a transforming growth factor- $\beta 1$ enzyme-linked immunosorbent assay kit. At 1 and 4 wk after surgery, magnetic resonance imaging was performed to evaluate the grafts. The femur-graft—tibia complex was assessed histologically and biomechanically at 8 wk after surgery.

Results: At 1 wk after surgery, the magnetic resonance imaging scans of the PRP group showed high signal-intensity lesions. In biomechanical tests, the SIS group had a significantly greater maximum load, maximum stress, and ultimate load and strain than the PRP group. The histologic findings of the PRP group revealed a greater cellular response, fibrotic tissue regeneration around the graft, broad chondrocyte cell infiltration, and collagen fibers that were loosely attached to the bone.

Conclusions: The PRP group had significantly lower tension load values than the SIS group, and there was greater cellular response in a broad area around the grafts of the rabbits in

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the PRP group compared with those in the SIS group. The early inflammatory responses around the canine SIS grafts in the PRP group and the altered cytokine or growth factor concentration in the intra-articular capsule of the rabbits in PRP group might explain their relatively low tensile strength results.

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1. Introduction

The anterior cruciate ligament (ACL) is the major intraarticular ligament of the knee and is critical to normal kinematics and stability. The ACL is composed of dense, highly organized, combinations of type I, III, and V collagen, elastin, proteoglycans, water, and cells [1]. In ACL reconstruction, autografts (patellar, semitendinosus/gracilis, and quadriceps tendons) are limited in availability and require additional surgery for tissue harvest that can cause donor site morbidity. After surgery, knee pain will likely persist in patients who undergo this type of reconstruction owing to insufficient strength of the knee stabilizing muscles [2]. Using interference screw fixation of tendon grafts can cause problems such as divergent screw placement, laceration of sutures or grafts by the screw threads, or increased difficulty of revision surgery by the presence of screws [3]. To overcome these problems, various synthetic, allograft, and xenograft materials have been used in ACL reconstruction surgery [4,5].

To be successful, a replacement ligament should be designed such that the tissue ingrowth and cell populations are well organized in a manner analogous to the original ligament. In addition, a replacement ligament must be able to withstand the physiologic stresses placed on it. Small intestinal submucosa (SIS) is a biologic scaffold that has been used to repair musculoskeletal tissue both experimentally and clinically [6]. Musahl et al. [7] applied a single layer of SIS to an injured medial collateral ligament in a rabbit model. Within 26 wk of surgery, the SIS was organized into a neoligament formation, increasing the mechanical strength of the ligament and the diameter of the collagen fibrils [6]. When used to treat canines with surgically created defects of the Achilles tendon, SIS increased the ultimate load at failure [8].

Platelet-rich plasma (PRP) has been shown to clinically accelerate healing of both soft and hard tissues, including in orthopedic, dental, dermatologic, plastic, and maxillofacial surgery [9]. Many studies have focused on the growth factors related to PRP and are known to have mitogenic effects in musculoskeletal tissue. Fibroblast growth factor, transforming growth factor (TGF), platelet-derived insulin-like growth factor, epidermal growth factor, insulin-like growth factor, and growth and differentiation factor have all shown the capacity to improve cell proliferation or matrix elaboration in ligament engineering constructs [10] and bone reconstruction [11].

We adapted a braid-twist method of ligament graft construction to create grafts from canine SIS [12]. Braiding is a technique that has been used to create products designed to bear axial loads, supply reinforcement, or serve as protective covers [12,13]. These structures are shear resistant and conformable and can transfer large loads and provide extension [12,14]. The aim of the present study was to compare the outcomes of replacement of the ACL with canine SIS grafts

and replacement of the ACL with SIS grafts plus application of autologous PRP.

2. Materials and methods

2.1. Experimental design

Twenty male New Zealand white rabbits (Samtako, Korea) weighing 1.8–2.0 kg were used in this experiment. The Institutional Animal Care and Use Committee of Konkuk University approved the present study. We randomly divided the rabbits into 2 groups. We implanted 1 group of 10 rabbits with braid-twist canine SIS ligament grafts in place of the ACL (SIS group). We implanted the second group with the same type of grafts but also injected autologous PRP into the bone tunnels and intra-articular capsule of the surgical area (PRP group). All procedures were performed in the left knee of the rabbits and the right knee was left intact as a control. After 8 wk, the rabbits were sacrificed, and their excised knees were submitted for biomechanical tests and histologic evaluation.

2.2. Preparation of braid-twist canine SIS ligament grafts

Canine SIS fabrics were isolated from German Shepherd dogs provided to the animal body donation program at the Veterinary Medical Teaching Hospital, University of Konkuk because of "at risk of euthanasia" and other causes. Dogs with infectious diseases or tumors were excluded. Segments of jejunum were obtained from the dogs, and no macroscopic evidence was found of specific pathologic features in any of the segments. Canine SIS was prepared as previously described under sterile conditions [15]. We prepared three 12 cm \times 3 cm strips of canine SIS for each graft. The end of each strip was grasped with forceps and twisted 10 times in 1 direction while pulling the strip taut. Three twisted strands of canine SIS were braided to create a ligament graft (Fig. 1). We placed 30-35 knots (stitches) in each ligament graft. All procedures were done manually under strictly sterile conditions. The biomechanical strength of these grafts was measured by performing the tensile load test with the model 3366 tensiometer (Instron, Canton, MA). The crosshead speed was set to 20 mm/min, and the test was performed until the graft failed.

2.3. Autologous PRP preparation and application method

Autologous rabbit PRP was prepared as described by Wu et al. [16]. In brief, while under anesthesia, 10 mL of whole blood was drawn from the marginal auricular vein immediately before surgery using an 18-gauge catheter. The blood was placed into a sterile conical tube containing 1.5 mL sodium

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