Enhancement of Polyethylene Terephthalate Artificial Ligament Graft Osseointegration using a Periosteum Patch in a Goat Model

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Key words
- polyethylene terephthalate
- periosteum
- osseointegration
- artificial ligament
- ACL

Abstract
The purpose of this study is to investigate whether a periosteum patch could enhance polyethylene terephthalate (PET) artificial ligament graft osseointegration in a bone tunnel. 12 female goats underwent ACL reconstruction with a PET artificial ligament graft in the right knees. Right knees in 6 goats were reconstructed with periosteum patch-enveloped PET grafts (Periosteum group) in the tibia bone tunnel, whereas the other 6 goats had no periosteum patch and served as the Control group. All the goats were sacrificed at 12 months after surgery. 3 tibial-graft complex samples in each group were harvested consecutively for microcomputed tomography (micro-CT) scan, magnetic resonance imaging (MRI) scan and histological evaluation. The other 3 tibial-graft complex samples in each group were harvested for biomechanical testing. The mean pull-out load of the Periosteum group (208±25N) at 12 months was significantly higher than that of the Control group (107±13N) (p=0.0044). According to the micro-CT scan, more new bone formation was observed at the graft-bone interface in the Periosteum group compared with the Control group. Furthermore, MRI showed that the Periosteum group appeared to have a better graft osseointegration within the bone tunnel compared with the Control group. Histologically, application of a periosteum patch induced more new bone and Sharpey’s fiber formation between the graft and bone tunnel compared with the controls. The study has shown that periosteum enveloping of the PET artificial ligament has a positive effect in the induction of artificial ligament osseointegration within the bone tunnel.

Introduction
In the treatment of the anterior cruciate ligament (ACL) injury, autograft or allograft tendons were mostly chosen to reconstruct ACL. However, autograft or allograft tendons have some associated problems, such as donor site-related problems, disease transfusion and immunological rejection. In order to avoid these problems, ligament advanced reinforcement system (LARS; Surgical Implants and Devices, France) artificial ligament has been used clinically in the reconstruction of ACL in many countries [14,21]. The LARS ligament has several clinical benefits in ACL reconstruction surgery, such as rapid recovery and a quicker return to sports activity in the early post-operative period [7,15]. However, the LARS artificial ligament made of polyethylene terephthalate (PET) material is hydrophobic. It is reported to have poor osseointegration within the surrounding bone tunnel after implantation [15,18].

Previously, Gao et al. [7] observed that an interposed layer of fibrous scar tissue appeared at the interface between the graft and the bone tunnel after ACL reconstruction in some failure cases using the LARS artificial ligament graft. Unlike the fibrocartilage transitional zone found in the native ACL insertion site, this type of fibrous scar tissue affords a poor anchorage of the graft in the bone tunnel, and long-term motion could possibly lead to graft laxity and failure [8]. Therefore, this raises questions as to how to decrease the fibrous scar tissue and improve the artificial ligament graft osseointegration within the bone tunnel.

To date, several kinds of materials have been used to modify the surface of PET grafts in order to improve their surface bioactivity, including polymers and bioceramics [5,19–21,24,27]. These materials have been demonstrated to be effective in the acceleration of graft osseointegration by decreasing fibrous scar tissue and increasing new bone or fibrocartilage formation at the graft-
bone interface. However, these approaches have not been applied clinically yet. Clinically, periosteum has been reported to be applied to enhance graft osseointegration in the human body [1–3]. In 2004, Chen et al. [3] applied periosteum into ACL reconstruction for enhancing tendon graft-bone tunnel healing and indicated the periosteum-enveloping hamstring tendon graft achieved a satisfactory effect in ACL reconstruction. In a later study with a mean follow-up of 4.6 years (range, 2–7 years), Chen et al. [1] investigated 312 patients who received a periosteum-enveloping hamstring tendon graft in ACL reconstruction. It showed that minimal tunnel widening can be achieved with the periosteum-enveloping hamstring tendon graft in single-bundle ACL reconstruction. Thus, it can be seen that enveloping the tendon graft with the periosteum is an effective and favourable way to enhance graft osseointegration in the bone tunnel. To our knowledge, there is no report investigating the effect of periosteum on the PET artificial ligament graft. Therefore, the aim of this present study was to investigate if periosteum could be applied to improve the PET artificial ligament graft osseointegration after ACL reconstruction. It was hypothesized that the periosteum could prevent scar tissue formation and facilitate graft osseointegration after ACL reconstruction.

Materials and Methods

PET artificial ligament graft

PET sheets (Taken from LARS artificial ligament, Surgical Implants and Devices, Arc-sur-Tille, France) were prepared and immersed in 75% alcohol solution to remove dirt. They were washed with pure water and dried under vacuum at 37 °C for 24 h. The sheet was rolled into a cylinder graft 10 cm in length and 4.5 mm in diameter.

Animal study design

All animal experiments were approved by the Animal Care and Use Committee of Shanghai jiaotong University Animal Department (201401050001). We have read and understood IJSM’s ethical standards document [9]. ACL reconstruction experiments were performed on 12 healthy skeletally mature female goats. The right knees in 6 goats were reconstructed with periosteum-enveloping hamstring tendon graft in single-bundle ACL reconstruction. The right knees in 6 goats were reconstructed with periosteum patch-enveloped PET grafts (Periosteum group) in the tibia bone tunnel (Fig. 1), whereas the other 6 goats with no periosteum patch served as the Control group. All the goats were sacrificed 12 months after surgery. 3 tibial-graft complex samples in each group were harvested for microcomputed tomography (micro-CT) scan, magnetic resonance imaging (MRI) scan and histological evaluation consecutively. In order to acquire detailed information of graft-bone interface, the bone tunnel was divided into 3 parts (proximal site, middle site and distal site) (Fig. 1). The region of interest will focus on the graft-bone interface of these 3 sites respectively according to micro-CT, MRI and histological images. The other 3 tibial-graft complex samples in each group were harvested for biomechanical testing.

ACL reconstruction

The animals were sedated with 5 mg/kg ketamine hydrochloride and 0.5 mg/kg Diazepam. Anesthesia was induced by 25 mg/kg sodium pentobarbital. Each goat was then positioned supine on the experimental table, and the right knee was prepared for aseptic surgery. After lateral arthrotomy was made, the patella was retracted medially. The native ACL was exposed and removed from the insertion site by sharp dissection. For the Periosteum group, a 30 × 20 mm section of periosteum was taken from the proximal tibia, wrapped around the tibial tunnel part of the graft. The cambium layer was placed outside to face the tunnel wall. For the Control group, no periosteum was used. Then, a 4.5 mm-diameter tunnel was drilled in the femoral and tibial insertion sites of the ACL. The graft was pulled manually into the bone tunnel. The graft ends outside the bone tunnel were sutured with adjacent soft tissue using No. 5 Ethibond sutures. The wound was closed in layers. The postoperative animals were returned to the animal care facilities. All the goats were sacrificed at 12 months respectively after surgery.

Micro-CT analysis

After sacrifice, the graft-tibia samples (n = 3) were fixed in 10% formalin for 48 h. All the bone tunnel model images were acquired via Siemens Inveon Micro PET/CT scanner (Siemens Medical Solutions, Germany). The acquisition parameters were as follows: voltage = 80 kV, current = 500 uA, exposure time = 800 ms, binning = 2, magnification=med (the transaxial and axial FOV are 61.05 mm and 40.70 mm respectively). Mineralized tissue was distinguished from non-mineralized tissue using a global thresholding procedure with a value approximating 1.20 g · cm⁻³ (25% lower than 1.6 g · cm⁻³).

Fig. 1 The schematic illustration for ACL reconstruction with periosteum patch enveloping artificial ligament. ps, proximal site; ms, middle site; ds, distal site.
MRI scan
On the same day after CT scan, a 3.0-T MRI scanner (Magentom Verio, Siemens, Germany) was used to perform imaging on these graft-tibia complex samples. Sagittal and axial images were obtained with oblique proton density-fat saturation (PD-FS): repetition time, 5000 ms; echo time, 25 ms; matrix, 320*272; field of view, 100*100 mm; and slice thickness, 3 mm. The images were imported into Siemens Software Packages (NUMARIS/4, SyngoMR B17, Siemens, Germany) to analyze the graft-bone interface.

Histological examinations
After MRI and CT scan, these samples were decalcified in 10% EDTA, changed twice weekly, for 4-6 weeks, after which they were embedded in paraffin. The samples were sectioned with a thickness of 5μm perpendicular to the longitudinal axis of the graft. These sections were treated with hematoxylin-eosin (H&E) and masson staining for histological evaluation. All images were visualized with inverted light microscopy (IX71SBF-2, Olympus Co., Japan). Digital images were taken using a DP Manager (Olympus Optical Co., Japan).

Biomechanical testing
Immediately after sacrifice, the graft-tibia samples (n=3) were harvested from each knee and prepared for mechanical testing. Furthermore, the other healthy knees were also harvested to undergo ACL reconstruction, and these time zero samples were used to analyze the graft pull-out strength at time zero. All pull-out load of the graft in the bone tunnel was tested using an Instron materials testing system machine (8874, Instron Co. USA). The graft protruding from the articular tunnel entrance was sutured by a No. 5 Ethibond suture for traction. The bone part was fixed firmly in a clamp. Care was taken to keep the bone tunnel oriented parallel to the testing axis. Before the tensile test was conducted, the specimen was preloaded with a preload of 1N for 5 min. Immediately after preconditioning, the ultimate pull-out load was performed with an elongation rate of 2mm/ min. For each specimen, testing was completed when the graft ruptured or was pulled out of the bone tunnel. The maximal pull-out load (N) was recorded.

Statistical analysis
The mean and standard deviation were used to describe the data, and the data analysis was performed using Stata10.0 software (Stata Corp, USA). At first, post hoc power analysis was performed. If a minimal difference of 40N was found in the pull-out load between groups, it was considered a clinically significant difference of graft pull-out load. Given the SD of graft pull-out load in the data, the sample size of the group (n = 3) has a power of 80% when level of significance was set at 0.05. A statistical analysis of the biomechanical results was carried out with the paired Student’s t-Test. The statistical significance level was set at 0.05.

Results
Three-dimensional (3-D) CT images of the bone tunnels are shown in Fig. 2. According to CT scan, both groups showed an enlarged bone tunnel shape at the proximal site. However, the Periosteum group clearly showed better bone regeneration ability than the Control group at the middle site. The bone tunnel area of the Periosteum group appeared much smaller than that of the Control group at the middle site. The bone tunnels implanted with the Periosteum-PET grafts were filled with much mineralized tissue, while the bone tunnels implanted with pure PET grafts revealed an enlarged bone tunnel at the middle site of the bone tunnel.

MRI images further revealed the healing quality of the graft-bone region for 2 groups after 12 months of surgery (Fig. 3). Both groups showed a high-intensity signal band around the graft at the proximal site. For the Control group, a swelling and diffused hyper-intense area was present at the graft-bone interface in the middle site. For the Periosteum group, there was a low-intensity signal band across the bone tunnel at the tendon-bone interface in the middle site and the graft showed a better osseointegration within the bone tunnel compared with that of the Control group.

Histological results were shown in Fig. 4, 5. At the proximal site, no significant difference of the graft-bone interface was found between the Control group and the Periosteum group. At the middle site, some new bone tissue formation was observed at the graft-bone interface of the Periosteum group while the Control group had much fibrous tissue at the graft-bone interface. Particularly on the Periosteum group, some protruding new fibrocartilage tissue formation was found at the interface between host bone and graft. Interestingly, Sharpey’s collagen tissue was observed integrating and mixing together with graft and bone tunnel at the interface. However, the PET group still had disorderly fibrous scar tissue at the graft-bone interface, and it was difficult to find graft osseointegration at 12 months postoperatively. At the distal site, the Control group had a thick fibrous scar tissue band formed at the graft-to-bone interface. In the Periosteum group, newly formed bone grew from host bone to the graft. The interface width appeared much narrower, and there was less scar tissue formation.

In biomechanical testing, all the specimens failed by pull-out the from bone tunnel and no graft rupture occurred. At time zero, the mean pull-out load of the Periosteum group was 31 ± 13N and the mean pull-out load of the Control group was 12 ± 6N. The mean pull-out load of the Periosteum group (208 ± 25 N) was significantly higher than that of the Control group (107 ± 13 N) at 12 months postoperatively (p = 0.044) (Fig. 6).

Discussion
In this study, periosteum was successfully applied to envelop the PET artificial ligaments in order to enhance graft osseointegration. Periosteum can be easily harvested at the proximal tibia from a routine incision clinically. Actually, we do not need to harvest a large size periosteum. In clinically, we only need to harvest a periosteum with size of 3 cm * 3 cm given that the bone tunnel is 8 cm in diameter. Considering the graft strength in the tibia bone tunnel is weaker than that in the femoral bone tunnel, we only wrap the periosteum onto the graft of the tibia part. This present study displayed that the periosteum-enveloped PET grafts possessed better osseointegration than the pure PET grafts, and the biomechanical strength of the Periosteum group was also significantly higher than that of the Control group. Our results indeed suggest that periosteum is an efficient and safe method to enhance PET artificial ligament osseointegration after ACL reconstruction.
In this study, CT scan revealed that more new bone formation was observed at the graft-bone interface in the Periosteum group. Furthermore, MRI showed that the Periosteum group appeared to have a better graft osseointegration within the bone tunnel compared with the Control group. As reported, periosteum has the capacity to form all varieties of connective tissue, to initiate endochondral bone formation by inducing mesenchymal cells, to differentiate into chondroblasts and then into osteoblasts, to augment bone ingrowth into collagenous tissue, and to induce ossification and bone formation [4, 13]. With osteoblasts and osteoclasts infiltration, periosteum induces the formation of bone or fibrocartilage at the graft-bone interface.

Interestingly, it was noted that application of the periosteum patch induced Sharpey’s fiber between graft and bone when histologically compared with the controls. Sharpey’s fibres, indirect insertion, were usually observed in the tendon graft reconstruction [11, 12, 23]. After implantation, the reconstructed graft in the bone tunnel will undergo a series of biologic healing processes, namely the osseointegration process [17, 26]. Immediately after graft implantation, hematoma formation occurs at the graft-bone interface. Subsequently, inflammatory response is present with neutrophils and macrophages producing scar tissue-related cytokines, such as transforming growth factor-beta [6]. For artificial ligament, severe inflammatory responses with many foreign body giant cells were observed in the pure PET grafts [16]. It was presumed that the periosteum could support a micro-environment-like autograft tendon, alleviate inflammation and deter bone resorption. Moreover, the advantages of a periosteum patch include the fact that this tissue meets the 3 primary requirements for tissue engineering: a source of progenitor cells, a scaffold for recruiting cells and growth factors, as well as being a source of local growth factors [22]. These growth factors contribute to the formation of Sharpey’s fibres at the graft-bone interface.

Actually, the final purpose of satisfactory graft-bone healing is to enhance the graft biomechanical property in the bone tunnel, for example, by improving the load-to-failure property. In the present study, the mean pull-out load of the Periosteum group at 12 months were significantly higher than those of the Control group, indicating that the newly-formed bone and Sharpey’s fiber had a positive effect upon improving graft anchorage in the bone tunnel. Previously, Chen et al. [4] transplanted the long
digitorum extensor tendon into a bone tunnel of the proximal tibia using a periosteum in a rabbit model, and a significant increase was found in the interface strength between the Periosteum and Control group at 8 weeks (72.32 ± 4.41 vs. 57.66 ± 5.85) and 12 weeks. Similarly, transplanting the long digitorum extensor tendon into a bone tunnel of the proximal tibia using a periosteum in a rabbit model, Karouglu et al. [10] compared 3 groups (Periosteum group, Bone marrow group, Control group). They demonstrated that periosteum had a positive effect when compared to bone marrow and control groups on the tendon-to-bone healing at an early time point (6 weeks), and bone marrow was also effective at the 12-week time point compared to the control group. Their findings indicated periosteum had a positive effect at an early time point (6 weeks). Based on these positive results, our present study further demonstrates that periosteum is a very effective enhancement of graft-bone healing in a goat model at 12 months after implantation.

Previously, in an ovine reconstruction experiment, Viateau et al. [25] found that the pull-out load of the polystyrene sodium sulfonate-coated group was found to be a little higher than the non-coated ligaments (322 ± 170 vs. 260 ± 126 N at 12 months), but there was no significant difference detected between the 2 groups. This difference might be because the interference titanium screws were inside the graft-bone tunnel and such a biomechanical examination tested both the fixation and graft-bone anchorage property. In our study, no interference screws were used and only the graft-bone anchorage property was used.

Finally, we admit there are several limitations to our study. First, only 12 goats were investigated in this study, which is a small sample size. Further studies are necessary with a larger sample size. Second, no fixation screws were used in the bone tunnel of the present study. Instead, the graft ends outside the bone tunnel were sutured with adjacent soft tissue using No. 5 Ethibond sutures, and the sutured knot at the tunnel aperture can be made as a suspensory fixation to prevent graft slippage. This fixation method could not only facilitate the histological analysis of graft-bone healing capability but also avoid the artifact of interference screw on the MRI image. Particularly, the future direction of artificial ligament fixation will be changed into suspensory fixation (like Endobutton CL fixation). Another limitation is how to make periosteum firmly wrap the graft and facilitate the implantation of the periosteum-wrapped graft. Actually, the periosteum is not entirely flat and uniform, and it is not easy to enter into the bone tunnel together with the artificial ligament.
ligament graft. Before implantation, we needed to suture the periosteum to the graft firmly. Finally, the present study is only an animal experiment, and it remains uncertain whether the periosteum patch can effectively enhance graft osseointegration clinically in human body, which suggests that clinical application of the periosteum-enveloping method in artificial ligament is required to ascertain the clinical effect of ACL reconstruction.

Conclusions

In summary, the findings of the present study indicate that periosteum can promote and enhance PET ligament graft osseointegration in bone tunnel in a goat model. We found that this method could improve the biomechanical property of the graft after implantation in the bone tunnel. Importantly, periosteum
is safe and easy to harvest from the proximal tibial. In clinical practice, this technique may be applied to ACL reconstruction to enhance PET artificial ligament graft healing within the bone tunnel. This approach may open a new door for orthopaedists to accelerate artificial graft osseointegration after implantation in bone tunnel, thereby promoting rapid recovery and a quicker return to sports activity, especially early after operation.

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References


Fig. 6 Biomechanical results of 3 grafts at each time point after surgery. The maximal pull-out load of the Periosteum group was significantly higher than that of the Control group at 12 months (indicated by #).