The Knee xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

The Knee

In vivo heterotopic culturing of prefabricated tendon grafts with mechanical stimulation in a sheep model

M. Petri [a,*], P. Lu [a], M. Omar [a], M. Ettinger [b], C. Krettek [a], C. Neunaber [a,1], M. Jagodzinski [c,1]

[a] Trauma Department, Hannover Medical School (MHH), D-30625 Hannover, Germany
[b] Department of Orthopedic Surgery, Hannover Medical School (MHH), Hannover, Germany
[c] Department of Orthopedic Trauma, Agaplesion Ev. Hospital Bethel, Bückeburg, Germany

ARTICLE INFO

Article history:
Received 11 November 2017
Received in revised form 21 January 2018
Accepted 19 February 2018
Available online xxxx

Keywords:
Heterotopic culturing
Prefabricated tendon grafts
Anterior cruciate ligament reconstruction
Mechanical stimulation
Tendon-chamber construct

ABSTRACT

Background: The goal of this study is to investigate the biomechanical and histological properties of in vivo heterotopically prefabricated cruciate ligament replacement grafts with and without mechanical stimulation. The clinical goal is to heterotopically prefabricate a bone–tendon–bone graft for anterior cruciate ligament reconstruction, which allows rapid ingrowth and early full weight bearing.
Methods: In a sheep model, eight quadriceps tendon grafts were harvested and introduced into culture chambers at their proximal and distal ends. In group S, four tendon-chamber constructs were mechanically stimulated by direct attachment to the quadriceps tendon and patella. In group NS, the same constructs were cultured without proximal attachment. All sheep were sacrificed six weeks postoperatively and the constructs were examined biomechanically and histologically. The healthy contralateral ACL and quadriceps tendon were used as controls.
Results: Macroscopically, no obvious ossification could be observed at the ends of the tendon-chamber constructs six weeks postoperatively. Histologically, the tendon tissue from the mechanically stimulated constructs revealed higher counts of cells and capillaries. However, there was less regular cell distribution and collagen fiber orientation compared to the control group. In addition, osteoblasts and osteogenesis were observed in the prefabricated constructs both with and without mechanical stimulation. Biomechanically, there were no significant differences in stiffness, elongation and ultimate failure load between the groups.
Conclusion: In vivo heterotopic culture of prefabricated tendon grafts may have the potential to stimulate osteoblasts and induce osteogenesis. Future studies with longer follow-up and modifications of the surgical technique and culture conditions are desirable.

1. Introduction

The anterior cruciate ligament (ACL) plays an important role in maintaining knee joint stability and is the most commonly injured ligament in the knee, particularly in association with sports-related injuries [1]. Episodic pain and instability of knee, chondral and meniscal impairment, and early osteoarthritis have been reported in patients who were subjected to chronic ACL

* Corresponding author.
E-mail address: petri.maximilian@mh-hannover.de (M. Petri).
[1] C. Neunaber & M. Jagodzinski contributed equally to this study.

https://doi.org/10.1016/j.knee.2018.02.006
0968-0160/© 2018 Published by Elsevier B.V.

insufficiency [2]. Anterior cruciate ligament reconstruction is a common procedure performed by orthopedic surgeons concerning the poor self-healing capacity of ACL. In the United States, the incidence of ACL reconstruction increased from 86,687 in 1994 to 129,836 in 2006 [3]. Current approaches to ACL reconstruction include replacement of the torn ACL by biological grafts, such as autograft and allograft. Due to the possible immunogenicity, delayed graft incorporation and risk of disease transmission, allografts are less commonly used than autografts which have been traditionally used as the gold standard for ACL reconstruction [4]. Clinically, bone–patellar tendon–bone (BPTB) and hamstring tendon are the most commonly used autografts [5].

In ACL reconstruction, tendon grafts fixed into the bone tunnels at both the femoral and tibial sides require sufficient strength and stiffness. The incorporation of tendon grafts into the bone tunnel is critical to achieve a satisfactory clinical result after ACL reconstruction. Aglietti et al. [6] compared the patellar tendon (bone–patellar tendon–bone) and the hamstring tendon for anterior cruciate ligament reconstruction. The study showed similar functional results, however, after a minimum of five-year follow-up, better stability with diminished extension deficits was obtained in the BPTB group. Petersen and Laprell [7] examined the structure of the insertion of autologous hamstring tendon graft and bone–patellar tendon–bone graft in ACL reconstruction. The results showed that the insertion of patellar tendon grafts incorporated with bone plug resembles the chondral enthesis of the normal anterior cruciate ligament, revealing a more biological connection to the bone than hamstring grafts. In a rabbit model, Park et al. [8] compared the healing of tendon to bone and the healing of bone to bone. They found that the healing of the tendon to bone was more difficult to achieve and required 12 weeks, while healing of bone to bone required eight weeks. Thus, the bone–patellar tendon–bone graft has been considered as a more competitive graft choice for its high initial quality of incorporation into bony tunnel and more extensive bony ingrowth. However, donor site morbidity and anterior knee pain after harvest of bone–patellar tendon–bone graft have been reported [9,10]. As a further drawback, the length of the harvested BPTB graft in some cases is too long and needs to be shortened. Furthermore, tendon graft resources may not be sufficient in patients, especially with multiligamentary knee joint injuries.

Not only applying for ACL reconstruction, the issue of bony ingrowth of tendons and grafts is also crucial e.g. in rotator cuff tears and Achilles tendon ruptures. Bony healing gets impeded and slowed down by the tendon-to-bone interface causing tunnel enlargement, such as in hamstring tendon grafts. Partial weight bearing has been identified to be beneficial for tendon to bone healing [11].

A potential solution to these issues might be heterotopic in vivo cultivation of grafts with biphasic structure and mechanical stimulation. For example, heterotopic graft cultivation of the triceps tendon at the elbow might be a promising option in an effort not to additionally weaken the injured knee joint.

In this study, we chose cultivation of quadriceps tendon grafts incorporated into the extensor mechanism in a sheep model for that purpose. The goal of this study is to investigate the biomechanical and histological properties of in vivo heterotopically prefabricated quadriceps tendon grafts with mechanical stimulation. The clinical goal is to heterotopically prefabricate a bone–tendon–bone graft as a viable option for ACL reconstruction. The hypothesis was that heterotopically cultivated quadriceps tendon grafts would show signs of a bone–tendon–bone structure.

2. Materials & methods

2.1. Study design and operative procedure

This study was carried out in accordance with the German Animal Welfare Legislation and was approved by the local institutional animal care and research advisory committee, as well as it was permitted by the local government of Lower Saxony, Germany (Approval number: 33.12-42502-04-14/1560). Twelve Schwarzkopf (SKF) sheep with an average age of 9.8 months and average weight of 51.1 $\pm$ 4.5 kg were included in this study. The sheep were randomly assigned to either the mechanical stimulation group (group S) or the non-mechanical stimulation group (group NS). The sheep were anesthetized by the intravenous administration of 0.2 mg/kg midazolam and four milligrams per kilogram carprofen. After endotracheal intubation, anesthesia was maintained by inhalation of O_2 and two to five percent end-tidal isoflurane. The sheep was placed in a lateral decubitus position with its right hind leg on top and was prepared in the standard sterile manner (Figure 1A). As first step an autologous cancellous bone graft (14 $\times$ 2 mm) from the ipsilateral anterior iliac crest was harvested with a Jamshidi needle (Figure 1B, C). Afterwards, in each sheep, a 50 $\times$ 5 mm quadriceps tendon graft was harvested from the right hind leg (Figure 1D–F). The proximal third of the quadriceps tendon graft was fixed into a culturing chamber sutured together with the additional autologous cancellous bone graft (14 $\times$ 2 mm) from the ipsilateral anterior iliac crest (Figure 2A, B). The distal third was fixed into a plain culturing chamber without bone graft (Figure 2B, C). Before fixation of the construct, multiple microfractures were created at the superior border of the patella. In the mechanical stimulation group (S), the proximal and distal ends of the construct were sutured to the quadriceps tendon and fixed to the superior border of the patella, respectively. Thus, the contraction of the quadriceps muscle would exert mechanical forces along the longitudinal axis of the construct (Figure 2D, E). In the non-mechanical stimulation group (NS), the constructs were only embedded into the quadriceps muscle in line with its longitudinal axis without fixation of its two ends. The healthy contralateral anterior cruciate ligament and quadriceps tendon (QT) were harvested as controls. The sheep were allowed to move freely and were weight bearing as tolerated under analgesia with carprofen (two milligrams per kilogram of body weight per day) for three days. Nursing support was provided with a quiet, clean resting place, wound maintenance and a progressive recovery to normal feeding. Daily surgical wound monitoring for pain, swelling, exudate and dehiscence was conducted. Particular attentions have been paid to surgical related complications such as organ failure, thrombosis and ischemia. Six weeks after the surgery, the sheep were euthanized with pentobarbital and then the hind limbs were removed. The constructs and the healthy ACL and QT were obtained for biomechanical and histological analysis.

Please cite this article as: Petri M, et al, In vivo heterotopic culturing of prefabricated tendon grafts with mechanical stimulation in a sheep model, Knee (2018), https://doi.org/10.1016/j.knee.2018.02.006