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Original article

Allograft integration in a rabbit transgenic model for anterior cruciate ligament reconstruction

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ABSTRACT

Background: Tissue engineering strategies include both cell-based and cell homing therapies. Ligamentous tissues are highly specialized and constitute vital components of the musculoskeletal system. Their damage causes significant morbidity and loss in function.
Hypothesis: The aim of this study is to analyze tendinous graft integration, cell repopulation and ligamentization by using GFP+/– allografts in GFP+/– transgenic New Zealand white (NZW) rabbits.
Material and methods: Graft implantation was designed to closely mimic anterior cruciate ligament (ACL) repair surgery. Allografts were implanted in 8 NZW rabbits and assessed at 5 days, 3 weeks and 6 weeks through: (1) arthroCT imaging, (2) morphological analysis of the transplanted allograft, (3) histological analysis, (4) collagen type I immunochemistry, and (5) GFP cell tracking. Collagen remodeling was appreciated at 3 and 6 weeks.
Results: Graft repopulation with host cells, chondrocyte-like cells at the tendon-bone interface and graft corticalization in the bone tunnels were noticed at 3 weeks. By contrast we noticed a central necrosis aspect in the allografts intra-articularly at 6 weeks with a cell migration towards the graft edge near the synovium.
Discussion: Our study has served to gain a better understanding of tendinous allograft bone integration, ligamentization and allograft repopulation. We believe that both cell-based therapies and cell homing therapies are beneficial in ligament tissue engineering. Future studies may elucidate whether cell repopulation occurs with pre-differentiated or progenitor cells. We believe that both cell-based therapies and cell homing therapies are beneficial in ligament tissue engineering.
Level of evidence: Level V (animal study).

1. Introduction

Tissue engineering solutions aim to regenerate failed or defective organs and tissues. Cell-based approaches used to help heal defects and replace native cells have constituted the mainstay of research for many years [1]. Recent advances in tissue engineering have evaluated the emerging theme of cell homing whereby using the host's endogenous cells, including stem or progenitor cells, to repopulate tissue engineering scaffolds for use in regeneration therapies [1].

Injury to the anterior cruciate ligament (ACL) affects more than 200,000 people each year in the United States [2]. The repair of ACL defect often requires the harvest of a replacement graft from the patient causing significant pain and morbidity [2]. In addition to autografts, much research has looked at allograft replacements for ruptured and defective tendons [3,4]. Incorporation of the tendon occurs through tendon graft ligamentization and integration into the bone tunnel [5,6]. Due to this reason, it becomes increasingly important to study, understand, and optimize cell growth and healing in the bone tunnel after ACL repair [7]. Despite some attempts to improve the biomechanical strength of the bone-tendon interface by instilling substances such as insulin-growth factor 1 and

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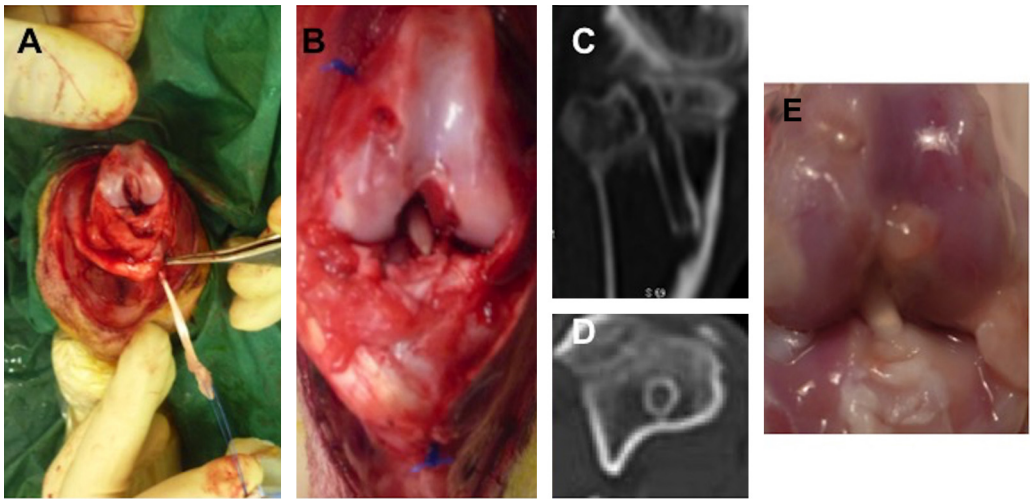

Fig. 1. A. Photography of the surgical procedure showing tendon graft passage in tibial bone tunnel. B. Macroscopic final aspect of implantation of tendon graft as native ACL. C. Sagittal tibial slide. D. Axial tibial slide of arthroCT revealing corticalization signal all the length of bone tunnels. E. Allograft macroscopic aspect 3 weeks after the surgery.

matrix-metalloprotease inhibition [8,9], cell growth and tendinous graft integration in the host's bone tissue still remains a challenge [10,11].

Considerable research has been focused on graft osteointegration in bone tunnels and intra-articular graft ligamentization after anterior cruciate ligament (ACL) reconstruction [12–18]. The development of an easy and reliable method that would allow cell fate monitoring in transplant recipients is a pressing concern for the field of ligament allografts. The transduction of detectable genetic markers, such as those encoding gene fluorescent protein (GFP), allow stable and reliable long-term labeling of transplanted cells and provide an important vehicle for the study of cell fate in the implanted graft.

We describe here a process used to better understand cell survival and healing in a bone tunnel following surgical graft reconstruction of ruptured ACL in a GFP transgenic rabbit model [19]. The GFP allows specific tracking of cells derived from the host organism and the cells pertaining to the ACL allograft. In addition, it allows the possibility of clearly following the fate of host and graft cells in order to determine cell survival and graft incorporation after allograft implantation. It further allows the opportunity to study the relative contributions of graft versus host cells is tendon tissue allografts. We hypothesized that allografts in GFP transgenic model for ACL reconstruction could give a better understanding of graft integration, cell repopulation and ligamentization.

2. Materials and methods

2.1. GFP animals

Transgenic GFP animals were obtained by the technique of pronuclear microinjection of DNA into naturally ovulated and fertilized rabbit eggs previously described by Houdebine [19] (INRA, Jouy-en-Josas, France). Eight skeletally immature New-Zealand white rabbits (average age = 10 weeks old, age distribution = 6 to 14 weeks old, average weight = 2.35 ± 0.6 kg) were used. Of these, four were GFP-expressing animal models (GFP+) and 4 did not express GFP (GFP–).

2.2. Surgical technique

The technique of ACL reconstruction was performed under general anesthesia. All procedures were approved by the Animal Experiment Ethics Committee of Lariboisiere-Villemin Number 09 (CEEA-LV/2010-01-01) and were previously described [20]. In order to study graft integration and allow cell tracking in the fresh allografts, one GFP– (control specimen) and one GFP+ rabbit were operated simultaneously by the same surgeon. The allograft taken from a GFP+ rabbit was implanted in a GFP– host and the allograft taken from a GFP– rabbit was implanted in a GFP+ host.

Each right extensor digitorum longus (EDL) tendon was harvested and prepared for implantation. After excision of the ACL, tibial and femoral bone tunnels were created to implant the allograft in the ACL isometric native position (Fig. 1A). The graft was fixed with non-absorbable 3.0 polypropylene sutures (Fig. 1B). All operated animals were allowed to move freely after the surgical technique.

2.3. Specimen collection

One GFP+ and one GFP–rabbit were sacrificed 5 days after ACL reconstruction. Two GFP+ and two GFP-rabbit were sacrificed 3 weeks after ACL reconstruction procedure. One GFP+ and one GFP–rabbit were sacrificed 6 weeks after ACL reconstruction procedure.

2.4. Imaging technique

Arthro CT (GE; lightspeed 64 slice) was used to image the position of the allograft in the knee joint and the integration of the graft in the bone tunnel.

2.5. Histology and immunochemistry

At the end of each predetermined time, the rabbits were sacrificed and the femur-graft-tibia complexes were removed and processed for immunohistochemistry. Frozen sections (14 μm thick) were collected on slides and processed for GFP and type I collagen immunohistochemistry using standard procedures with mouse monoclonal antibodies (anti-GFP Clontech ref: 632381 and anti-collagen-I Sigma ref: C2456). In order to check the specificity of the immunostaining, alternative sections were treated similarly but incubated without the primary antibody. Moreover, no GFP-immunostaining was observed in non-GFP rabbits. Morphologic analysis was performed on serial sections with hematoxylin/safranin-O/fast green staining.