INTRODUCTION:
Considerable research has been focused on graft osteointegration in bone tunnels and intra-articular graft ligamentization after anterior cruciate ligament (ACL) reconstruction. However, the question of cell survival after implantation, and the sequence of graft necrosis and cellular repopulation remain unresolved. 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 orthopaedic allograft surgery. Among the different techniques that have been proposed to follow the fate of implanted grafts in vivo, only the transduction of detectable genetic markers, such as those encoding for Green Fluorescent Protein gene (GFP) allow stable and reliable long-term labeling of transplanted cells. This study was conducted using transgenic GFP rabbits to define tendinous graft incorporation and cell survival in ACL allograft reconstruction. Surgical procedure was evaluated by imagery. The allografts were assessed macroscopically, histologically and by immunohistochemistry at 3, 6 and 12 weeks after surgery. Biomechanical properties of the graft were evaluated at each time point.

METHODS:

Animals: Transgenic animals were produced by pronuclear DNA microinjection of the GFP gene into naturally ovulated and fertilized rabbit eggs. Twenty five skeletally immature transgenic New-Zealand White rabbits (10 weeks old, average weight: 2.35±0.6 kg) were used. For surgery and post-operative maintenance of the animals, an established protocol was followed in accordance with the guidelines published by the European Committee for Care and Use of the Laboratory Animals.

Surgery: ACL reconstruction was performed under general anesthesia and strictly aseptic conditions. GFP rabbits were used either as tendon graft donors or recipients in a fresh allograft animal model. Two matched sibling animals (brother and sister) were operated simultaneously to reduce the risk of allograft rejection. The extensor digitorum longi was harvested and prepared for implantation. The native ACL was excised, tibia and femoral bone tunnels were drilled independently with a 2.0mm diameter drill-bit, and graft fixation was achieved with a non-absorbable 3.0 polypropylene suture. Accurate tunnel placement was confirmed under fluoroscopy. All rabbits were allowed to move freely after surgery.

Collection of Specimens: At each time point, 3, 6 and 12 weeks after surgery, grafts were processed for CT-scan, histology, immunohistochemistry (using a monoclonal antibody to Type 1 collagen and a monoclonal antibody to GFP) and mechanical tests.

RESULTS:

Operative procedure: surgery and anesthesia were well tolerated.

Arthro CT-scanner: At 3 and 6 weeks, graft orientation was similar to that of native ACL, thus validating the surgical technique. Corticalization was seen around the drill holes 3 weeks after surgery (Fig 1).

Gross morphology: Six failures occurred. No infection occurred. Tendon grafts have a good aspect at 3 and 6 weeks, they were white and firm at palpation. At 12 weeks, the graft appeared weaker, less robust but still continuous (Fig 2). The infrapatellar fat pad was adherent to the graft suggesting its potential role as a source of blood supply.

Histology: The sections were stained with hematoxylin/Safranine/Fast Green stain. At 3 weeks, the graft was peripherally acellular in its intra-tunnel part and interposition of scar tissue was seen between the graft and the bone tunnel. In its intra-articular portion, the graft was acellular at the center, but a viable rim of cells was consistently observed at the periphery of the graft. At 6 weeks, Sharpey’s-like fibers anchored the graft to the bone walls and endochondral ossification was seen close to the growth plate.

Immunohistochemistry: Immunohistochemical localization of collagen type I showed altered collagen fibers within the bone tunnels. However, in the intra-articular part, graft remained intact and unaffected (Fig 3). Immunohistochemical localization of GFP demonstrated the presence of host cells within the grafts, thus confirming the process of repopulation (Fig 4), but interestingly donor cells could be still identified at the periphery of the graft in its intra-articular portion at 6 weeks.

Mechanical Testing: Operated knee and their control were tested to failure with an Instrom model 4505 testing machine, at a strain rate of 2% per second. After surgery, allografts significantly lost their mechanical properties (Fig 5). There was no difference at 3 and 6 weeks, with a maximal load reaching 20% and stiffness 15% of control group. Ruptures occurred either within the bone tunnels at 3 weeks or at the new enthesis at 6 weeks.

DISCUSSION:
To date, attempts to monitor cell survival and repopulation in a reconstructed ACL have had limited success, mainly because they relied on the DNA-probe technique which has inherent limitations. The GFP transgenic animal model presented in this study is a valuable, efficient, and reliable method to evaluate cell fate after transplantation. We demonstrated that donor cells were replaced by host cells in the intra-tunnel portion, but did survive within the intra-articular portion 6 weeks after transplantation. Further studies are required to evaluate tissue remodeling in the long-term.