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Research Paper

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Integration of the patellar tendon during the reconstruction of the anterior cruciate ligament – IN VIVO experiment on common European rabbits *(Oryctolagus cuniculus)*

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ABSTRACT

Anterior cruciate ligament (ACL) ruptures are one of the most common injuries of the knee joint. These injuries occur mostly during contact sporting activities (such as ice hockey or football).

Whatever the case, the elective therapy is to reconstruct the ACL by means of implanting a graft.

During our experiments we wanted to study the integration of the patellar tendon, which we implanted in the distal femur of 4 common European rabbits.

The rabbits where sacrificed after 1–3–4,5 and 6 moths post op, and their distal femur removed for histological examination.

After one month we could see an aseptic inflammatory process, which meant that the graft had begun integrating. After 3 and 4.5 months respectively we could see host fibroblasts taking over the extracellular matrix left after the aseptic necrosis of the graft, and after 6 months, the graft had been totally integrated.

KEYWORDS: Common European rabbit, ACL injury, press fit fixation.

Introduction

In the present we see the sudden increase of professional and/or amateur sporting activities and as a result the number of injuries affecting the knee joint is on a continuous rise. As such, this group of injury is responsible for the most days a patient is hospitalized and is missing from their work, generating great socio-economic costs in Europe [5][6].

The rupture (partial or otherwise) of the anterior cruciate ligament *(ACL)* is one of the most common injuries of the knee joint. These injuries occur mostly during sporting activities (such as skiing or football), and can often be accompanied by other soft tissue injuries of the knee as seen in O'Donoghue's "unhappy" triad, when the internal collateral ligament and the internal meniscus also suffer injuries.

The injury of the ACL can occur by means of two main mechanisms:

1. landing while the knee is hyper extended and the tibia is in an internal rotation position, or
2. a sudden deceleration while changing direction.

In any case, the elective therapy is to reconstruct the ACL by means of implanting a graft (taken either from the patient him/herself – autograft – or from a donor – allograft [7]).

There are many things to decide and discuss before an ACL reconstruction surgery, one of them being the type of tissue we wish to implant. Studies made by dr. Marinescu in Bucharest show that: "(…) Choosing the right type of graft is based on the age, sex and the athletic activities of the patient, as well as passed surgical interventions. Thus for the male sex, it is wise to choose a graft made from the median third of the patellar tendon, because it is more durable and it integrates more quickly, and for the female sex it is preferred to use the hamstring tendons for its elasticity and the moderate post-operative scaring (…)" [10].

Post-op the graft undergoes a three step process of incorporation:

1. first, by an inflammatory response, the graft suffers an avascular necrosis. The donor fibroblasts die, and the remaining tissue matrix serves a blueprint for the migration of the host cells, and thus a formation of a new collagen matrix. [10]
2. next, there is revascularization process which includes the migration of host fibroblasts. This happens cca. 20 days after surgery, and ends between 3 and 6 moths. During this time, the strength of the graft is decreased, and is easily influenced by stress. [10]
3. finally, because of the tension undergone by the graft, the collagen fibers reorganize in a functional structure similar to the original. [10]

During our experiments we wanted to study the integration of the median third of the patellar tendon, which we implanted in the distal femur of common European rabbits.

Material and technique

In order to do this, we operated on 4 rabbits in a specialized animal experimentation lab at the University of Medicine and Pharmacy Targu – Mures.

General hygiene and anesthesia

After the administration of a general anesthestetic **(fig. 1)** (1.5 ml *ketamine* + 1.1 ml *xylazine* in the case of 4.4 kg rabbits), we performed local shaving and disinfection **(fig. 2)** of the knee.

fig.1 administrating the anesthetic

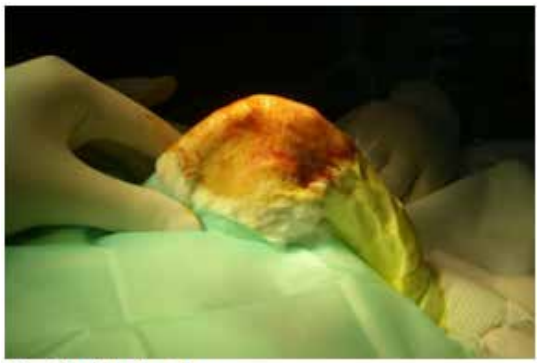

fig. 2 disinfection

Graft preparation and fixation

We sectioned the skin and subcutaneous tissue along the mid-line of the knee and after reaching the patellar tendon, we removed the mid one-third part **(fig. 3 and 4)**, then sutured the two remaining 2/3 parts together.

fig. 3 harvesting of the tendon

fig. 4 the graft

We then drilled a special, two part canal through **(see fig. 5 and 6)** the distal femur in the intercondylar notch [1]. The distal part was made to be greater in diameter in order to accommodate the graft more easily [2]. The proximal, smaller part was used to fixate the graft.

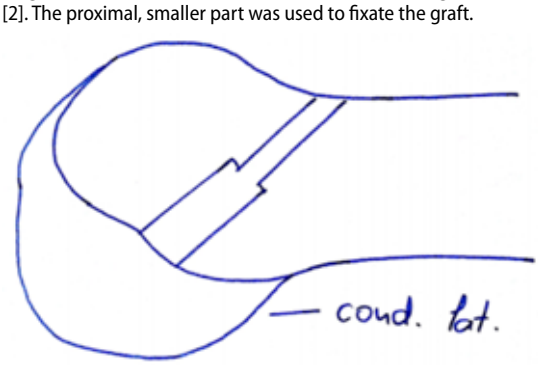

fig. 5 the tunel

fig. 6 drilling of the tunel

After running two to three sutures in one end of the removed tendon **(fig. 7)**, we used a special sterile wire to guide the graft through the bony canal **(fig. 8)**.

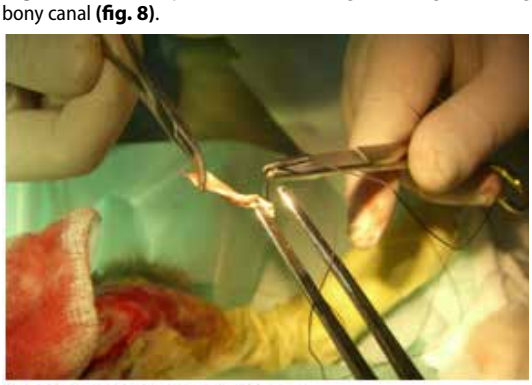

fig. 7 running of the wire

fig. 8 guiding of the graft

Special fixation was not necessary, as the canal was made to be smaller in diameter than the graft **(fig. 9)**.

fig. 9 the fixation resisted traction

Post-op these grafts where not put under any stress, we merely wanted to study the integration on a cellular level.

Results

The rabbits where sacrificed after 1 – 3 – 4,5 and 6 moths post op, and their distal femur removed for histological examination.

Using traditional hematoxylin and eosin stain, we were able to study the integration on a cellular level.

After one month **(see fig. 10 below)** we could see the graft in the bone *(left arrow)* surrounded by an aseptic inflammatory process *(right arrow)*. This was the first indication that the operation was a success, and that the rabbit's bone and immune system is actively trying to integrate the graft.

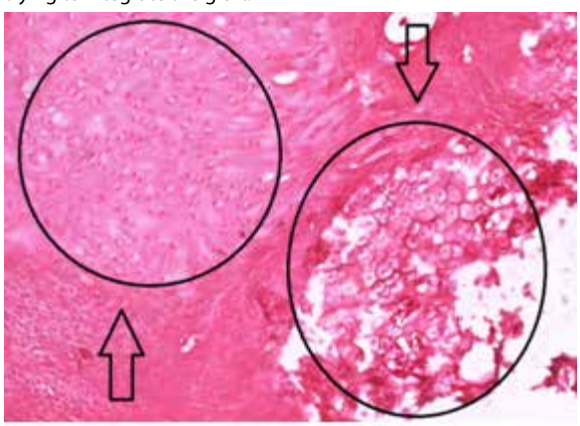

fig. 10 results after one month

As two months passed, we saw the necrosis of the graft. This process left behind it the extracellular matrix of the tendon, which, as we later saw, served as a kind of "scaffolding" for future host fibroblasts/ osteoblasts to use as a base to proliferate and successfully complete the integration of the graft.

After six months the graft was fully integrated, only clues of an inflammatory process could be seen.

Discussion

The purpose of this study was to investigate if the simple tendon could successfully integrate in bone tissue, and if so, then how long would it take.

We wanted to know this because the harvesting of traditional BTB (bone-tendon-bone) grafts are more invasive [14], and as such presume the appearance of more complications (ex. patella fractures or osteomyelitis).

Studies were conducted in order to determine which tissue type is more adequate for implant, the patella tendon [9] or the hamstring ligaments [4]. These studies concluded that for humans, the hamstring ligaments are a much better choice because of their elasticity [8] and their anatomical size (the operating surgeon has a much bigger piece of tissue to work with than the middle third of the patellar tendon) [11] [12]. Also, from a histological and biomechanical point of view, the hamstring ligaments show a much greater resemblance to the ACL than the patellar tendon, because of the trajectory of the fibers that make them up. The hamstring ligaments fibers are mostly parallel to one another which is as important aspect, because their trajectory determines their elasticity and durability against strain [3][13].

Despite the fact that the hamstrings are much suitable for implant and integration, in our experiments, we knowingly chose the patellar tendon and the rabbits for the following three reasons:

a. rabbits are much more attainable as experimental subjects than any other animal: they are not too big, and expensive to acquire and care for post op. (like pigs), and not too small to handle safely (like mice)
b. the patellar tendon was chosen because it was much more easier to locate, harvest and manage than the fragile hamstring ligaments
c. it also proved to be a great reference when it came to sacrifice the animals and harvest their femur for histological examination

Further research is necessary with a larger group of experimental subjects in order to better study and understand the integration process, and to properly correct the aleatory complications and develop special instruments (ex. a specially shaped drill bit with which the drilling of the femoral tunel would be more easily accomplished).

Conclusion

As our study shows, simple ligamentous grafts implanted in bone can, and do integrate within a relatively short period of time. We saw that after implanting the graft, the host organism reacted with an inflammatory process, during which the ligament cells suffered aseptic necrosis. Later their places where taken over by bone cells, completing the integration. We have not seen any signs of lysis in, or around the graft, which also indicates that the immune system recognized its own tissue and did not react against it, resulting in a perfect integration without any infection or pyogenic granuloma formation.

Aknowledgement

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