

Fresh autogenous and allogenous tendon graft in rabbit model

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Abstract Tendons are soft connective tissues consisting of parallel collagen fibers embedded within an extracellular matrix. Blood supply to the tendon is reported to be poor and, as a result, healing often progresses slowly. Autogenic, allogeneic, and xenogeneic tendon transplantations have been performed in reconstructive tendon surgery; however, there is little information on fresh allogeneic tendon transplantation experimentally. The aim of this study is to evaluate fresh autogenous and allogenous tendon graft in the rabbit to determine which gives the better result, clinically and histopathologically. Ten 1-year-old male New Zealand Albino rabbits weighing 3.5 ± 0.5 kg were used in this study. In five rabbits (group I), about 3 cm of the superficial flexor tendon was resected and a created defect was filled with the same 3-cm harvested tendon and

sutured with 2/0 polypropylene in a single modified Kessler suture pattern and the tendon sheath was sutured over the transplanted tendon completely. In the remaining five rabbits (group II), the harvested segment was changed between rabbits and created defects were filled with changed segment allogenous tendon, all other procedures were identical to group I. The main histopathological and gross evaluation showed graft acceptance by recipients in both groups. Our results showed that no significant tissue reaction or graft rejection was observed when fresh allogeneic tendon was transplanted in a rabbit model.

Keywords Fresh allogeneic · Fresh autogenic · Tendon transplantation · Rabbit model

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Introduction

Tendons are soft connective tissues consisting of parallel collagen fibers embedded within an extracellular matrix (Lin et al. 2004). Tendons connect muscle to bone and transmit tensile force generated by muscles to move and stabilize joints. They must be capable of resisting high tensile forces with limited elongation (Best et al. 1989; Buckwalter and Hunziker 1996). However, as tendons are subjected to repeated motion and degeneration over time, they are prone to both acute and chronic injuries (Lin et al. 2004). Barfred (1973) suggested that Achilles tendon ruptures can occur in a normal tendon if an excessive load is applied. Blood supply to the tendon is reported to be poor and, as a result, healing often progresses slowly (Clancy 1983; Harner et al. 1995; Kuo et al. 2003). The healing process in tendons results in the formation of fibrotic scars. The structural, organizational, and mechanical properties of this healed tissue are inferior to normal tendon (Frank et al. 1999).

Patellar tendon autograft has been used for anterior cruciate ligament reconstruction in the rabbit and it has been reported that the appearance of this graft had some general histological similarities when compared with the native anterior cruciate ligament. However, these similarities did not extend to the functional properties of the autograft (Amiel et al. 1986; Ballock et al. 2005). Totally autogenous tendon transplant and allogeneic transplantation of the tendon have been performed with varying degrees of success (Birch et al. 1999; Taniguchi and Tamaki 2000). Although these properties improve over time, they do not return to normal levels, even after long periods (Frank 1996; Frank et al. 1999). Rogers et al. (1995) proposed that xenografts are highly attractive as they carry a small risk of infectious disease, do not compromise the patient's remaining tissues, and may have the "correct" structure with regard to the component being replaced. Autogenic, allogeneic, and xenogeneic tendon transplantation have been done previously (McMaster 1985; Rodeo 1993; Strickland et al. 2003). Also, artificial tendon has been transplanted in man (Dong and Sheng 1988). Hamstring tendon graft was used for anterior cruciate ligament reconstruction (Gordia and Grana 2001). Transplantation of bovine fetal tendon in the rabbit model has been performed successfully (Dehghani et al. 2005). Repair of tendon gap by bovine fetal tendon transplant in the horse has been also done before (Dehghani and Varzandian 2007). Although autogenic tendon grafts have the minimum risk of antigenicity interaction, allogeneic tendon grafts are more accessible and popular. It has been reported that fresh allograft tissue is unsuitable for implantation because it is highly immunogenic and tissue typing is impractical (Fu et al. 2000). The aim of this study is to evaluate fresh autogenous and allogenous tendon graft in the rabbit to determine which is superior both clinically and histopathologically.

Materials and methods

Animals

Ten 1-year-old male New Zealand Albino rabbits weighing 3.5 ± 0.5 kg were used in this study. The university research committee approved the research protocol for this experiment.

Surgical technique

Animals were anesthetized with ketamine (40 mg/kg, IM) and xylazine (5 mg/kg, IM). The left or right hind leg (chosen randomly) was shaved, prepared aseptically with povidone iodine, and the limb draped with sterile drapes.

The skin was incised on the lateral part of distal third of the tibia over the tendons; the fascia and tendon sheath were incised. About 3 cm of the superficial flexor tendon was resected and the created defect was filled with the same 3-cm harvested tendon (group I, $n=5$) or with 3 cm of allogeneous tendon harvested from another rabbit in the same group (group II, $n=5$). The transplanted tendon was then sutured with 2/0 polypropylene in a single modified Kessler suture pattern (Fig. 1). The tendon sheath was sutured over the transplanted tendon completely in both groups.

Postoperative evaluation

After surgery, the operated leg was bandaged and the rabbits were individually housed in a restricted area to limit their movement. During the postoperative time, clinical parameters including appetite, activity, infection, bleeding, and wound dehiscence were evaluated daily.

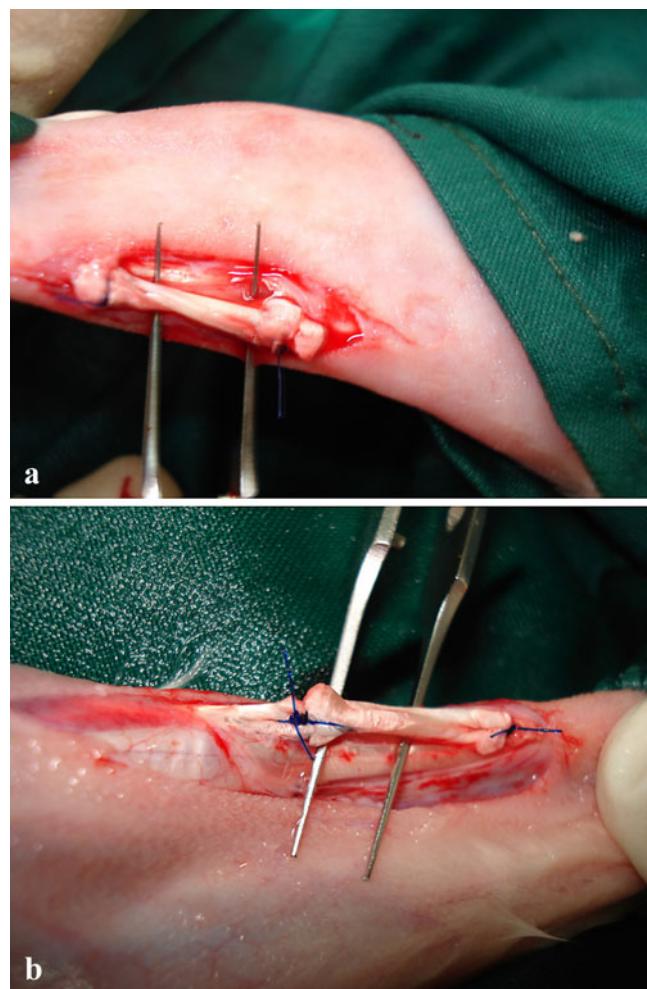


Fig. 1 Autogenous tendon graft (**a**) and allogenous tendon graft (**b**) in the rabbit model

Histopathological evaluation

Fifteen weeks after the operation, all rabbits were euthanized pharmacologically and tissue was taken for histopathological evaluation. The graft–tendon unit was resected and fixed in 10% formalin. Two 5- μ -thick sections were cut from the centers of each specimen and were stained with hematoxylin and eosin. A pathologist, blinded to the individual treatments, evaluated the sections.

Results

Clinical evaluation

All rabbits showed normal activity and appetite and there was no evidence of clinical complications such as local infection or wound dehiscence. In all rabbits, the skin was freely movable across the implant site.

Gross evaluation

A thick scar of the graft and a thin portion of the tendon proximally and distally to the graft were easily palpable on clinical examination in group II. In the allograft group, the graft appeared, by visual inspection, to be slightly thicker when compared with the autograft group (Fig. 2); however, the boundary between the graft and native tendon were difficult to determine, with good continuity and solid integration in both study groups.

Histopathological finding

The grafts were well integrated into the native tendons (Fig. 3) in rabbits in both groups and no evidence of immunological rejection was noted. A dense, regular collagenous connective tissue with many fibroblasts distributed between the collagen fibers was noted across the autogenic and allogeneic grafts (Fig. 4). The collagen disposition was oriented along the longitudinal axes of the tendon and an increase of fibroblast-like cells or myofibroblasts in a linear fashion (Fig. 5) was also observed during healing process of the tendon in all groups. Clusters of chondrocyte-like cells were found in an acellular collagenous area in one autogenic tendon graft (Fig. 6).

Discussion

In this study, a superficial digital flexor tendon defect model was created to evaluate fresh autogenous and

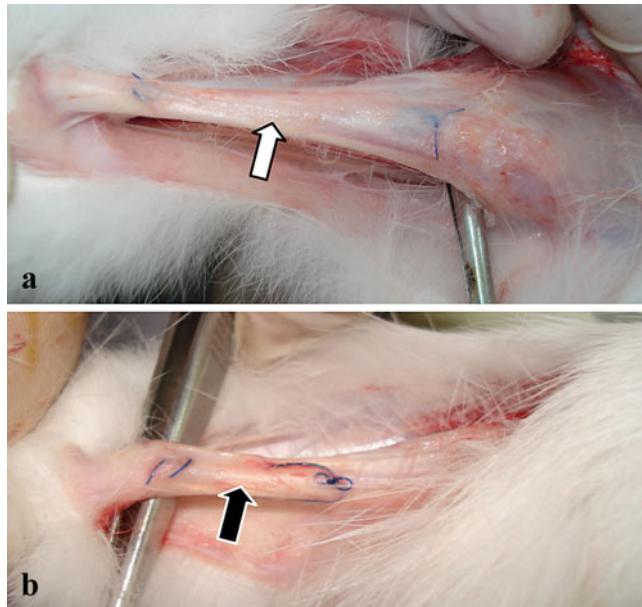


Fig. 2 White arrow shows autogenous tendon grafting area (**a**) and black arrow shows allogenous tendon grafting area (**b**) after 15 weeks

allogenous tendon transplantation in the rabbit model. The Achilles tendon or common calcaneal tendon has its origin in five muscles that give rise to three tendinous components: the gastrocnemius, superficial digital flexor, biceps femoris, gracilis, and semitendinosus (Dyce et al. 1996). Complete rupture of the Achilles tendon is rare in domesticated animals. Rupture of the gastrocnemius tendon and the superficial and deep flexor tendons are more common in small animal and large animal practice (Bertone 1995).

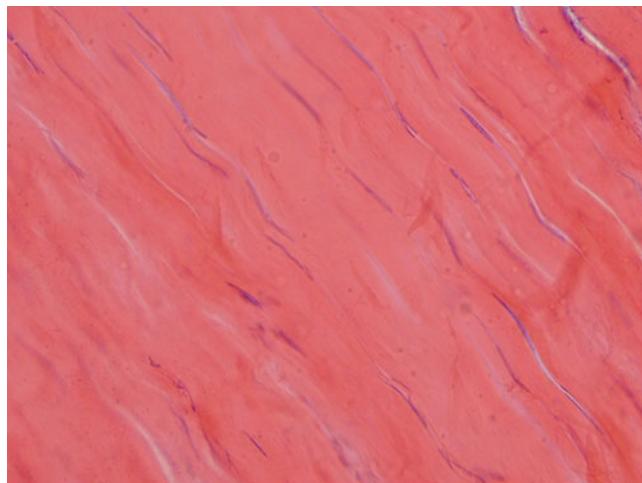


Fig. 3 Longitudinal section of a normal tendon (hematoxylin and eosin, $\times 400$)

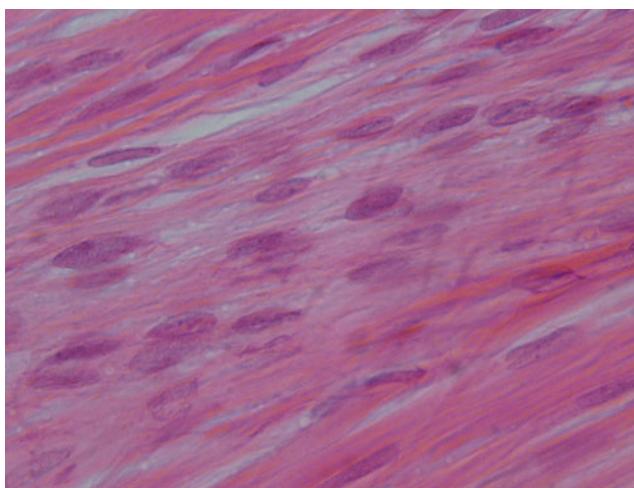


Fig. 4 A dense, regular collagenous connective tissue with many fibroblasts in an allogeneic graft (hematoxylin and eosin, $\times 1,000$)

The main objective of this study was to determine whether a fresh allogenous tendon graft could provide a grafting structure that enhanced the surgical repair of a tendon defect. The gross and microscopic observations indicated that all five recipients accepted the fresh allogeneic tendons. There was neither clinical evidence of gross infection nor microscopic evidence of significant tissue reaction in the rabbits with grafts. Although there was some scarring at the tendon junctions, the grafts remained relatively free of adhesions to their sheaths throughout the entire healing process.

It has been reported that fresh allograft tissue is unsuitable for implantation because it is highly immunogenic for recipients (Fu et al. 2000); in our study, fresh allogeneic tendon was transplanted in a rabbit model and

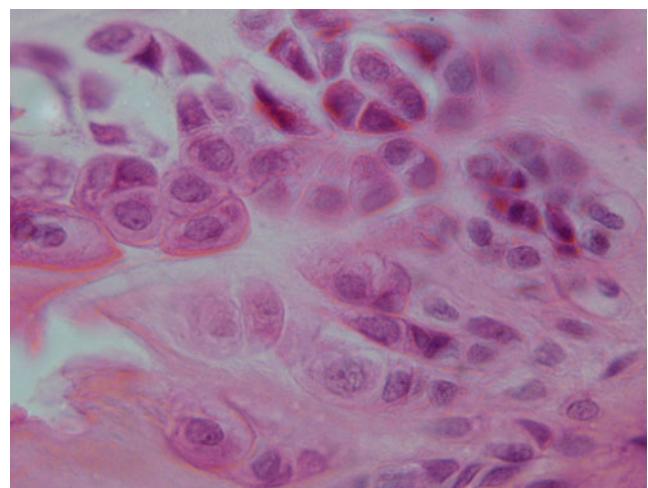


Fig. 6 Clusters of chondrocyte-like cells at 15 weeks (hematoxylin and eosin, $\times 1,000$)

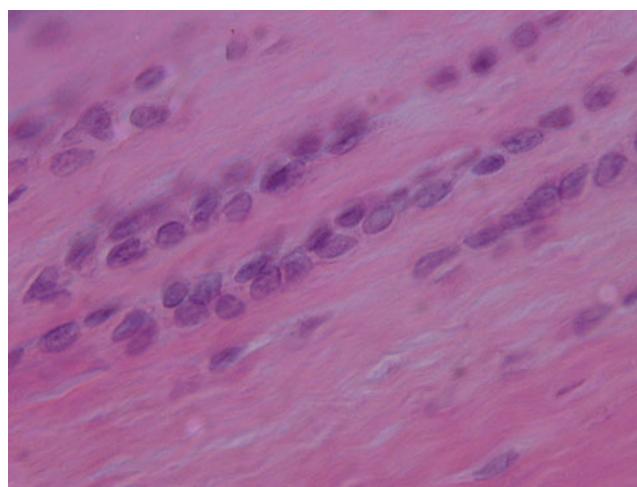


Fig. 5 Photomicrograph showing fibroblast-like cell proliferation in a linear fashion (hematoxylin and eosin, $\times 1,000$)

significant tissue reaction and graft rejection were not observed. Although early studies by Peacock (1959) and Peacock and Petty (1960) suggested that tendon allografts, which are really collagen transplants, can be transplanted without regard to antigenicity. The results of the present study support this belief as, despite the delay in healing, the transplanted collagen bundles were not rejected and the tendon tissue eventually resembled normal tendon both grossly and microscopically. However, it has been recommended that allograft tissue-preserving methods such as fresh-freezing, freeze-drying, and cryopreserving significantly reduced the tissue immunogenicity by killing fibroblasts within it (Arnoczky et al. 1986).

Tendon allografts play an important role in tendon and ligament reconstruction, particularly where there is a shortage of available local tissue (Harner et al. 1996; Noyes and Barber 1993).

Overall, the advantages of using allograft tissue include a lack of donor site morbidity, high tensile strength, decreased surgical time, smaller surgical incisions, and low risk of arthrofibrosis. The disadvantages include their limited availability, susceptibility to rejection due to immunoincompatibility between the donor and recipient, and potential risk for bacterial, viral, and prion disease transmission (Harner et al. 1996). Our results showed that fresh allogeneic tendon has no antigenicity and does not elicit an inflammatory reaction in a rabbit model. Further investigation in other animal models is required.

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