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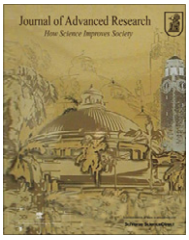

ORIGINAL ARTICLE

Clinical and biomechanical evaluation of three bioscaffold augmentation devices used for superficial digital flexor tenorrhaphy in donkeys (*Equus asinus*): An experimental study

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KEYWORDS

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Abstract The present study was designed to carry out an *in vivo* and *in vitro* comparative evaluation of three bio-scaffold augmentation devices used for superficial digital flexor tenorrhaphy in donkeys. Twenty-four clinically healthy donkeys were assigned for three treatment trials ($n = 8$) using one of three bioscaffold materials (glycerolized bovine pericardium xenograft, tendon allograft and allograft shielding with glycerolized by bovine pericardium). In addition, eight clinically healthy donkeys were selected to serve as control. Clinical signs of each animal were scored and the sum of all clinical indexes was calculated at each time point of the experiment. Four donkeys from each group were euthanized at 45 and 90 days postoperatively, respectively, for biomechanical and histopathological evaluation of treated superficial digital flexor tendon (SDFT). The failure stress in allograft shielding group significantly increased compared to the corresponding values of the other groups at 45 (62.7 ± 6.5 N mm^{-2}) and 90 (88.8 ± 3.5 N mm^{-2}) days postoperatively. The fetlock angle in the allograft shielding group at both 45 ($112.8° \pm 4.4$) and 90 ($123.8° \pm 1.1$) days postoperatively showed a significant increase ($p < 0.05$) relative to the values of the other groups and a significant decrease ($p < 0.05$) when compared to normal angle ($125° \pm 0$). However, the histomorphological findings

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revealed no remarkable changes between the treatment groups. In conclusion, the failure stress, fetlock angle and histomorphological findings may provide useful information about the healing characteristics of SDFT tenorrhaphy. The bio-scaffold augmentation devices, either xenogenic or allogenic, provide good alternative techniques accelerating SDFT healing with minimal adhesions in donkeys.

Introduction

Tendons are extremely complex in terms of their structural, functional and biomechanical characteristics [1]. Mechanical factors are important in the etiology of tendon and ligament lesion. The distribution of loads among several tendons in the equine limb have been studied extensively *in vitro* and *in vivo* [1,2]. Lacerations of the digital flexors are of traumatic origin and their effective treatment requires basic knowledge of the principles involved in tendon healing and their application [3,4].

Clinically, flexion or hyperextension of the fetlock during weight bearing indicates a lameness [5,6]. Moreover, the fetlock angle has been reported by Butcher and Ashley-Ross [5] to reflect the extent of maturity of the suspensory apparatus tissues at various ages. Clinical cases with tendon or ligament injury require a minimum of 3–6 months of restricted athletic activity to allow sufficient time for healing and consequently, for the biomechanical properties to recover (e.g. failure stress) [4,7].

Ideal tendinous repair must morphologically reconstitute the injured tissue and preserve the gliding function of the tendon, thus helping to maintain its movement capacity [8]. Tenorrhaphy, when possible is the most advantageous treatment for transected flexor tendons in equines that provide robust tendon anastomosis with minimal gap formation and increase the likelihood of returning horse to riding status [9]. These include tendon allograft [10], bovine pericardium xenograft [11], tendon shielding [12] and tissue engineering [13].

Manufactured form of collagenous materials from bovine or equine origin which chemically treated by glycerol or glutraldehyde usually has a popular starting point for development of graft prosthesis for tendon repair. It provides a strong collagenous non-stretch bio-integrate for tendon and ligament augmentation [12].

Histologically, the graft function as an organizer of tendon healing, is known to increase the rate of maturation of tendon repair in comparison to spontaneous healing or synthetic materials repair [14]. Natural bioscafold augmentation devices yielded histologically superior healing by improving fibroblast and collagen fiber orientation and enhancing vascularity, which serve as a barrier to the formation of extrinsic adhesion, and act as a guide for remodeling tendon and improving tendon gliding and movement biomechanics [14,15].

For assessment of the potency of the bioscafold materials used in tenorrhaphy, the biomechanical parameters including ultimate tensile stress, ultimate tensile strain and modulus of elasticity should be examined. [16]. The tensile stress of tendons is related to thickness and collagen content. For example, a tendon with an area of 1 cm^2 is capable of bearing 500–1000 kg of load [17,18]. In ponies, strains of the SDFT, deep digital flexor tendon (DDFT), inferior chick ligament (ICL) and suspensory ligament (SL) measured by mercury-in-silastic strain gauge showed non-significant changes between gaits [1]. The tensile strain was defined as the change in length of a substance normalized by the original length [1]. The failure stress is fundamentally simple to measure in N mm^{-2}, where a constant load is applied to a tissue and the progressive time dependant elongation is measured [16]. However, the load to failure represent the continuous loading of a tendon tissue sample till complete rupture [19].

The present investigation was designed to evaluate the clinical and biomechanical outcomes of reconstructed SDFT in donkeys using bovine pericardium xenograft, tendon allograft and allograft shielding with bovine pericardium. Also, it was extended to include the histopathological investigation of repaired SDFT with these bioscafold augmentation devices.

Material and methods

Donkeys

A total of 32 adult donkeys (24 in tenorrhaphy trials and 8 in control group) at age of 6–10 years with body weight of 140–180 kg, were used for this study. Donkeys were purchased from different localities of Dakahlia Governorate. These animals were examined clinically, radiographically and ultrasonographically (8 MHz liner transducer, Mindray, DP-2200Vet, China) to exclude any bony, joint abnormalities and/or tendinous lesions. Animals were kept in the animal house of veterinary teaching hospital at Mansoura University and fed on a maintenance balanced mixed ration containing chopped wheat straw ad libitum, 1–2 kg of bran and 2–3 kg of whole corn. Ration was supplemented by minerals and mixture of trace elements (MUVCO – Egypt). Two weeks before the start of the experiment, all animals were dewormed and vaccinated against tetanus. During the entire experimental period all animals were kept under similar management and feeding practices.

Study design

The present experimental study was approved by the Committee of Animal Welfare and Ethics, Faculty of Veterinary Medicine, Mansoura University. Donkeys were divided randomly into four groups (eight of each), one of them was used as a control group while the others were classified into three treatment groups according to the type of bio-scaffold material used for tenorrhaphy of SDFT. Glycerol preserved bovine pericardium (GBP) xenograft was applied to the first treatment group [20], preserved SDFT allograft from freshly euthanized donkeys to the second treatment group [21] and SDFT allograft shielding with GBP to the third treatment group [22].

Anesthesia and surgical procedure

Sedation was inducted via intravenous injection of xylazine HCl (Xylaject- ADWIA Co., Egypt) at 1.0 mg kg^{-1}. Then, the animals were generally anaesthetized using modified triple

drip regimen of xylazine (500 mg L^{-1}) and thiopental Na (NOVARTIS, Egypt) (4 mg L^{-1}) at infusion rate of 2 ml/kg^{-1} per hour.

The anaesthetized animals were positioned in lateral recumbency with the limb selected for tenorrhaphy uppermost and fixed in extension position to obtain the correct angle for the introduction of the instruments. The metatarsal region of the limb was aseptically prepared for surgery. A tourniquet was placed above the tarsus to minimize hemorrhage. A 10–12 cm mid metatarsal linear skin incision was made over the planter aspect, then the paratenon was longitudinally incised for exposure of SDFT, which completely transected with full thickness tenoectomy of 1–2 cm in both ends using scalpel blade. In animals of group I the ends of transected tendon were reapposed with a single locking loop suture pattern using No.1 polypropylene suture material (ETHICON LTD/UK) leaving 0.5 cm gap maintained between the two cutted ends after suturing. An appropriate piece of GBP was wrapped in the form of sleeve around the two cutted ends of incised tendon in continuous stitch. Glycerol preserved bovine pericardium was sutured to the cutted tendon ends with interrupted stitches using the fore-mentioned suture material No. 3/0 (Fig. 1). Whereas, in group II, the same technique as xenograft was performed except for a length of tendon graft two times equivalent to the removed part of transected tendon was grafted in place to fill the gap and sutured to each end by a single-locking loop tendon suture technique through the graft using No.1 polypropylene suture material (Figs. 1 and 2). In group III SDFT allograft shielding was performed with the same technique as mentioned above. Adequate single layer of GBP

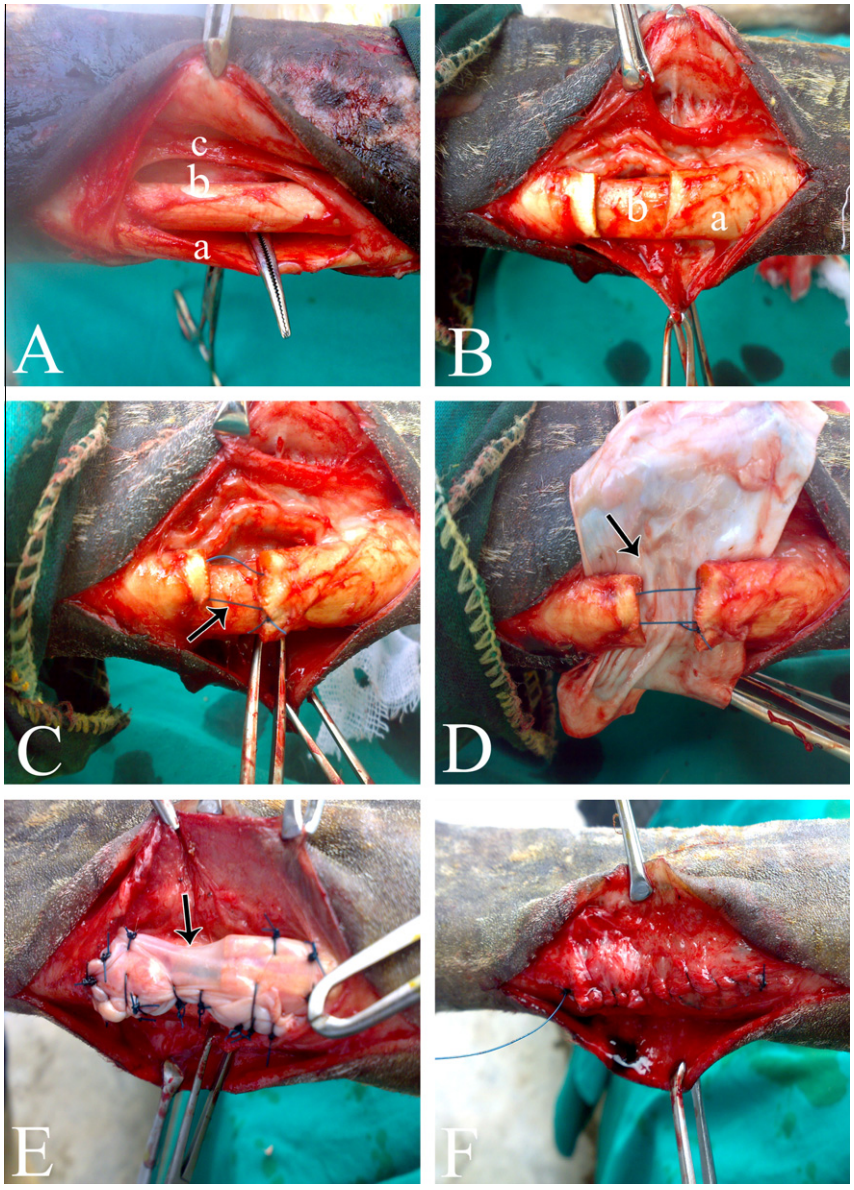

Fig. 1 SDFT xenograft with GBP: A – Mid metatarsal incision including the skin, s/c and paratenon(c) for exposure of the SDFT (a) and DDFT (b). B – Incidental full thickness defect of the SDFT. C – Stay stitch of the tendon ends using a single looking loop suture (arrow). D – Application of the GBP xenograft around the tendon gap (arrow). E – The tendon gape completely encased by the GBP with interrupted suture. F – Closure of the tendon paratenon above the graft bed of GBP.

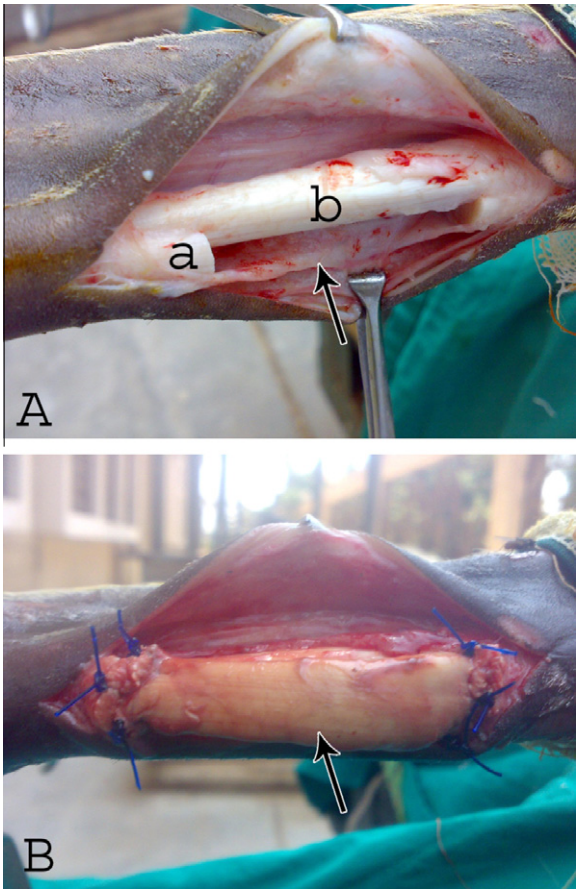

Fig. 2 SDFT allograft: A – Incidental full thickness defect of the SDFT (a) as a tendon gape (arrow) with the DDFT (b) exposure. B – Full thickness allograft fixed to the tendon ends using prolene suture (arrow).

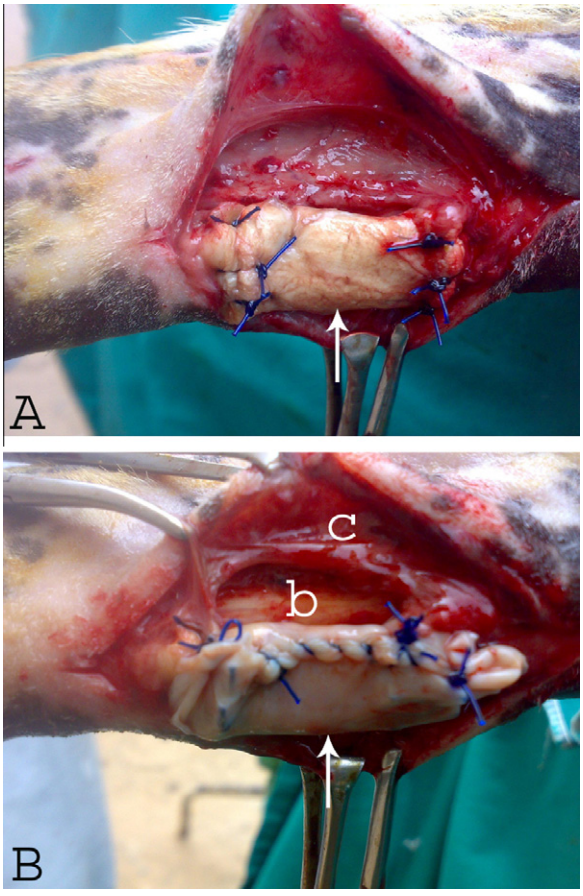

Fig. 3 SDFT allograft shielding with GBP: A – The allograft sutured to the tendon ends by prolene suture (arrow) B – The allograft completely encased by the GBP (arrow). b – DDFT; c – Paratenon.

was wrapped firmly around the grafted tendon. All implanted grafts were covered by paratenon which was sutured in continuous pattern using the same suture material (Fig. 3). Subcutaneous tissue was closed separately using polypropylene suture material No.1 with simple continuous pattern. Skin closure was accomplished using silk or polypropylene No.1 in a simple interrupted pattern. The operated left pelvic limb was immobilized using Plaster of Paris from the hoof up to a point proximal to the tarsus maintaining the fetlock joint in slight flexion. The cast was applied for four weeks postoperatively and changed regularly each 10 days within this period for the removal of skin suture and assessment of clinical parameters. After final cast removal, an extended heel shoe was applied to the operated hindlimb for another month to provide fetlock support and prevent tearing of reconstructed SDFT.

Clinical index score assessment

Subjective assessment of clinical signs, visual and palpable abnormalities of flexor tendons, fetlock joint angle and circumferential measurements of the left pelvic limb at the repair site were recorded and scored at 45 and 90 days postoperatively. The fetlock joint angle was measured in the standing position of donkeys by using a scaled malleable ruler (goniometer) and calculated graphically from the digital video recording by a line drawing laterally connecting between the three land marks of the angle from the mid tarsal passing through the fetlock joint and ended by the hoof quarter [5]. Clinical index scores, for each treated donkey, were evaluated and compared at different time points with scores of the control animals. Clinical index scores are reported in Table 1.

Donkeys were examined for lameness at each time point of the experiment. Lameness was graded on a scale 0–3, with 0 being no lameness, and 3 being unable to bear weight [23,24]. Pain was closely monitored during the postoperatively period by reluctant or difficult ambulation, prolonged recumbency and elevated pulse rate, respiratory rate, or rectal temperature [23,25]. While, discomfort was closely assessed by alteration in normal activities and appetite of the operated donkey with counting the number of limb hanging. Also, by the alertly stat and change in normal attitude with prolonged recumbency [25]. The limb circumference was examined by using a measured tape in the mid metatarsal region in control donkeys and operated donkeys pre and postoperatively at 45 and 90 days in each treatment group.

Biomechanical testing

In order to evaluate the biomechanical properties of the tendons, particularly failure stress, strain and load failure, four donkeys

Table 1 The clinical index score for subjective assessment of clinical parameters in donkeys subjected to SDFT tenorrhaphy at 45 and 90 days post-operative.

Clinical index	Description and level
Lameness	0 = negative; 1 = mild; 2 = moderate; 3 = severe
Discomfort	0 = comfort; 1 = discomfort;
Pain	0 = negative; 1 = mild; 2 = moderate; 3 = severe
Tissue reaction	0 = negative; 1 = mild; 2 = moderate; 3 = severe
Graft survival	0 = survived; 1 = rejected
Limb circumference	0 = 15 cm; 1 = 16 cm; 2 = 18 cm; 3 = 20 cm
Fetlock angle	0 = 125°; 1 = 120°; 2 = 110°; 3 = 105°

from each group were euthanized at 45 and 90 days postoperatively, respectively, by an overdose of intravenously administered barbiturate (Thiopental Na; Novartis Pharma- Egypt) and SDFT specimens were collected from each operated and control limb. Tendon specimens were collected by transecting each tendon 3 cm above and below the reconstructed tendon of each operated limb. However, the tendon specimens were collected from the freshly euthanized control donkeys in the mid metatarsal region in length equal to 10–15 cm. Biomechanical properties of both normal and surgically treated tendons were examined at the Laboratory of Biomechanics, Faculty of Engineering, Mansoura University. All collected specimens were packed in containers of normal saline and tensile testing was done within three hours of tissue collection. Each specimen was loaded in a hydraulic tensile testing device (LLOYD, Germany) by securing its proximal and distal portions to two metal clamps of the tensometer. The clamps were coated from inside by a piece of felt and tightened to avoid slipping of the tendon specimens. All specimens were loaded to failure (complete rupture of the tenorrhaphy) with a 1000 kg load cell moving at a crosshead speed of 500 mm min^{-1}. Load trials to failure were recorded using a digital monitor connected to the load frame and graphically by a digital camera focused on the tendon repair site. Tendon strains (%) were constantly monitored during loading trials and calculated graphically from the digital video recording.

Histological examination

Tendon specimens collected at each time point of the study were immediately fixed in 10% buffered formalin, routinely processed, sectioned at 6 μm and stained with Hematoxylin and Eosin (H&E) as well as Masson's trichrome [26]. Each specimen of treatment group were histomorphologically analyzed qualitatively using the following parameters: vascularization, cellularity, collagen fibers alignment, inflammatory cells and granulation tissues [27]. Histomorphological scores are shown in Table 2. The graft survival/rejection was examined during the early post-operative period (each 10 days) by hand controlled loading (extension and flexion) of the operated limbs with graft manipulation at the tenorrhaphy site. Palpable abnormalities and presence or absence of gape defect of the repaired flexor tendons were recorded [28]. Also, the healing properties of the lacerated tendon and the skin wound are indicators for the presence or absence of tissue reaction. The degrees of tissue thickness or adhesion of the repaired SDFT in euthanized donkeys were grossly evaluated at each time point in each treatment group. Also, the vascularization, cellularity, collagen fibers alignment, inflammatory cells and granulation tissues were microscopically evaluated by a professional pathologist [14].

Table 2 The histomorphological index score for subjective assessment of SDFT tenorrhaphy healing properties in donkeys at 45 and 90 days postoperatively.

Index	Description and level
Adhesion	0 = No adhesion; 1 = Thin adhesion (25% of traumatized area); 2 = Thick adhesion (50% of traumatized area); 3 = Thick wide spread adhesion (25% of traumatized area)
Granulation tissue	0 = No fibrosis; 1 = Only a few granulation; 2 = Some proliferative granulation; 3 = Abundant proliferative granulation
Neovascularization	1 = quiescent neovascularization; 2 = active neovascularization 3 = Highly active neovascularization
Inflammatory cells	0 = No inflammatory cells; 1 = Few number of inflammatory cells infiltration; 2 = Some inflammatory cells infiltration; 3 = Abundant inflammatory cells infiltration
Fiber alignment	0 = 75–100% parallel longitudinal alignment; 1 = 50–75% parallel longitudinal alignment; 2 = 25–50% parallel longitudinal alignment; 3 = 0–25% parallel longitudinal alignment

Statistical analysis

The obtained data were statistically analyzed with statistical software program (Graph pad prism version 5.0, USA). At each time point, the mean and standard deviation (SD) were calculated for tendon biomechanical parameters, whereas the median and range were assessed for the clinical index scores. Repeated measures MANOVA (with repeated measures on treatment and time) was used to determine the main effect of graft and time. Wilks' Lambda test was used to determine the within all interaction. Where Wilks' Lambda indicated a statistically significant difference between groups, one way ANOVA with HSD Tuky-Kramer *post hock* multiple comparison test was used to identify which group was statistically different from the rest. Differences between means at $p < 0.05$ were considered significant.

Results

Clinical index score

The clinical index scores of treated SDFT with the three bioscafold augmentation devices showed non-significant variations between treatment groups. Thus, no tissue reaction, no rejection or discomfort were noticed in all treated groups. At 45 and 90 days post-operatively, clinical parameters (clinical index scores) revealed no remarkable changes among the three treatment groups.

As reported in Table 3, fetlock angle showed a significant increase in all tenorrhaphies groups with time (MANOVA fit, $p < 0.0\ 047$, Time: $p < 0.001$, Wilks' Lambda for treatment xtime interaction: $p < 0.01$). In the group subjected to allograft shielding with GBP, the Mean ± SD value of fetlock angle at 90 days postoperatively (123.8° ± 1.1) was higher than that recorded at 45 days postoperatively (112.8° ± 4.4). Additionally, the mean fetlock joint angle in the allograft shielding group at both 45 and 90 days postoperatively showed a significant increase ($p < 0.05$) compared to the other treatment groups and a significant decrease ($p < 0.05$) compared to the fetlock angle of the control group (Table 3). Moreover, by the end of 90 days postoperatively, all animals regained the normal range of motion.

Biomechanical properties

Statistical analysis concerning the biomechanical properties including strain and load failure showed no significant differences between control (Fig. 4) and the three treatment groups of donkey SDFT (Figs. 5–7) except the failure stress ($p < 0.05$). Table 4 shows that the failure stress in all three treatment groups has been found to increase significantly with time (MANOVA fit, $p < 0.01$ Time: $p < 0.0001$ Wilks' Lambda for treatment x time interaction: $p < 0.001$). However, the mean ± SD of failure stress in allograft shielding treated donkeys was found to be higher than the corresponding values of both xenograft and allograft treated donkeys at 45 ($62.7 \pm 6.5\ N\ mm^{-2}$) and 90 ($88.8 \pm 3.5\ N\ mm^{-2}$) days postoperatively. At 45 and 90 days postoperatively there was a significant difference in the failure stress between allograft treated donkeys and both xenograft and allograft shielding treated animals (Table 4). Moreover, the mean ± SD of failure stress in allograft shielding treated donkeys was the most similar to that of the control animals (Table 4). Statistical analysis showed no difference between mean ± SD of both load failure and strain among all treatment groups.

Tissue morphology

In Hematoxylin and Eosin stained sections at 45 days postoperatively, SDFT reconstruction in the form of randomly distributed active fibroblasts as well as collagen deposition were encountered at the proximal and distal graft interface in all treated tendons (Fig. 8). Moreover, perivascular leukocytic infiltrations (mononuclear cells) were also observed in between cut ends of the original tendon. Furthermore, at 90 days postoperatively, the fibroblasts showed broad distribution with parallel wavy bundles of densely packed, well organized collagen fibers (Fig. 9). The tendon tissue architecture of repaired sites in the grafted tendons was difficult to distinguish from that of the normal tendon except for slight hyper cellularity. In Masson's trichrome stained sections, the repaired SDFT in all treatment groups showed homogenization of the collagen fibers between the original tendon and implanted device (Fig. 10). The mature wavy collagen bundles of the newly formed tendon were aligned in a longitudinal direction and showed bluish coloration with the Masson's trichrome stain (Fig. 10). Although the histomorphological healing properties showed non-significant variations among treatment groups, the allograft shielding group recorded the best results.

Discussion

In the present study, the normal biomechanical properties of SDFT in donkeys were found to be widely different from the

Table 3 Mean ± SD of the fetlock angle (in degrees) of the hindlimb in control and SDFT tenorrhaphies donkeys at 45 and 90 days postoperatively.

Technique	Control	Post-operative (day)	
		45	90
SDFT xenograft	125° ± 0.095	111.6° ± 3.7[a]	120.5° ± 1.8[a,b]
SDFT allograft	125° ± 0.062	105.8° ± 3.1[b]	117.3° ± 2.5[b]
SDFT allograft shielding	125° ± 0.031	112.8° ± 4.4[a]	123.8° ± 1.1[a]

MANOVA fit, $p < 0.0\ 047$.
Time: $p < 0.0001$.
Wilks' Lambda for treatment xtime interaction: $p < 0.0124$.
[a,b] Means with different superscript letter at the same column are significantly different at $p < 0.05$.

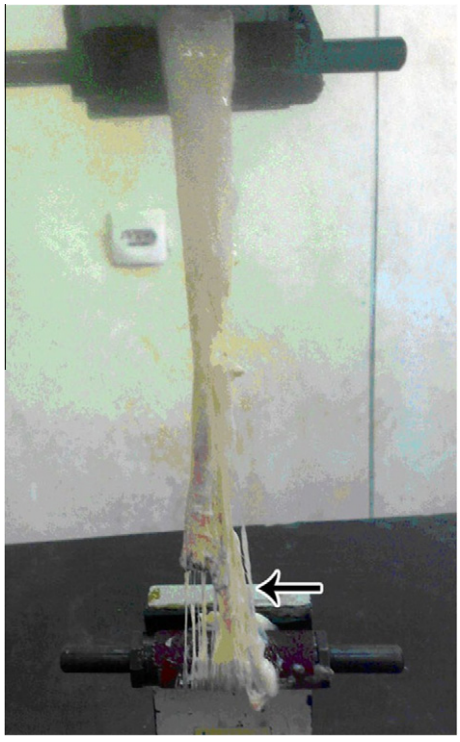

Fig. 4 Failure of a normal SDFT at the point of specimen gripping to the LLOYD arm. Arrow indicates the failure point of the tendon.

corresponding properties previously reported in horses [1]. This finding was demonstrated in the present data which showed the values of absolute load, strain and stress at tendon failure of donkeys as 5200 ± 860 N, 10 ± 1. 2% and 103.66 ± 4.1 N mm^{-2}, respectively. Therefore, it can be concluded that the values of biomechanical properties in horses are threefold greater than the corresponding values in donkeys. Our finding could be attributed to the biomechanical and/or functional differences between donkeys and horses. Difference between members of equidae should be considered for clinical evaluation during load bearing of donkeys in comparison with horses [29]. Also, it seems possible that there is a proportional relationship between the load and thickness of the tendon; the larger the thickness the greater the load it can bear. The present findings coincide with the suggestion reported by Cohen et al. [2] and Kane and Firth [8] that determination of the *in vivo* tendon biomechanics is very important since overload of a tendon is best expressed in terms of overstrain that can be clinically avoided by a good understanding of these parameters.

The present investigation of tendon biomechanical properties showed no significant differences between control and treatment groups of donkeys except the property failure stress ($p < 0.05$). Moreover, in all treated groups, the failure stress was found to increase significantly with time. The present data are in agreement with those reported by Masuda et al. [30], Dehghani and Varzandian [31] and Lin et al. [32] Smith et al. [33]. The difference in failure stress between control and treatment groups could be attributed to full loading of the tendon with incomplete collagen fibrils alignment at 45 days postoperatively, which is progressively improved with time since remodeling of tendon needs at least 3–6 months to have a complete healing.

The mean value of failure stress in allograft shielding treated donkeys was found to be higher than the corresponding values of both xenograft and allograft treated donkeys at 45 and at 90 days postoperatively. Also, the mean of failure stress in allograft shielding treated donkeys was similar to that of the control group. These results represent the first report on the use of allograft shielding with GBP in SDFT tenorrhaphy in donkeys. Other studies in this field were focused on the use of this device in other animals as sheep [10,22]. Therefore, it can be suggested that the use of allograft coupled with an adhesion barrier (GBP), in the present study, can stimulate proliferation of collagen fibrils into longitudinally aligned

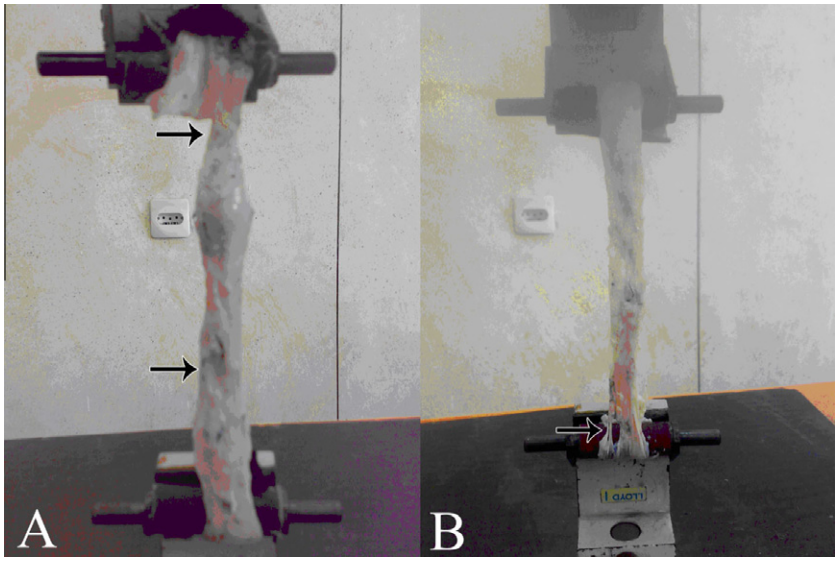

Fig. 5 Failure of SDFT xenograft with GBP specimen at 45 days postoperatively (A) and 90 days post-operative (B). Arrow indicates the point of implant failure proximally and the other refers to the graft bed (A). Arrow indicates the point of implant failure distally (B).

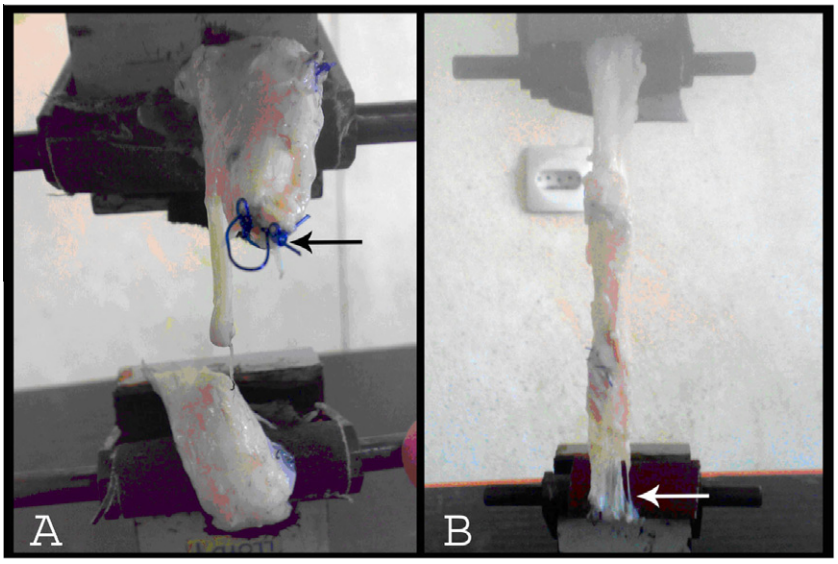

Fig. 6 Failure of SDFT allograft specimens 45 days post-operative (A) and at 90 days postoperatively (B). Arrows indicate the point of implant failure.

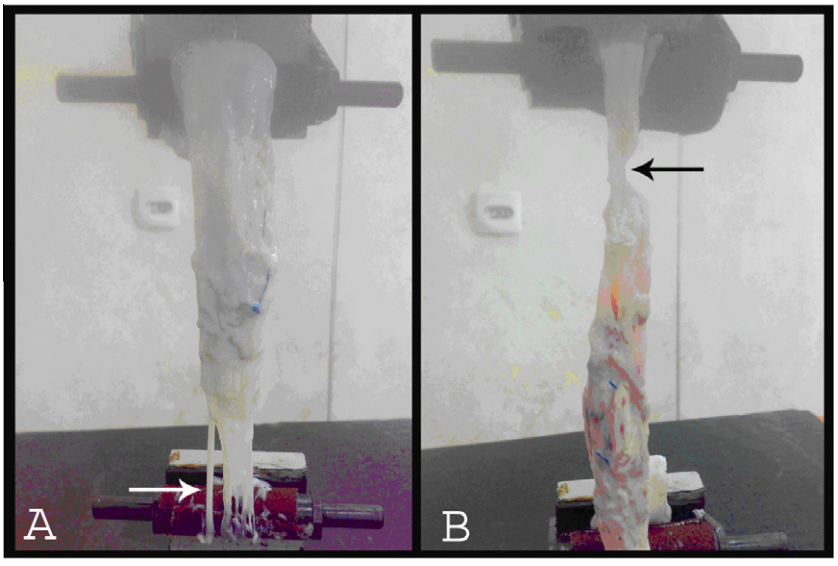

Fig. 7 Failure of SDFT allograft with GBP specimens at 45 days post-operative (A) and at 90 days postoperatively (B). Arrows indicate the point of implant tearing.

Table 4 Mean ± SD values of the biomechanical measurements of failure stress (N mm^{-2}) in the SDFT of the hind limb in control and treated donkeys at 45 and 90 days post-operative.

Technique	Control	Post-operative (day)	
		45	90
SDFT xenograft	103.66 ± 4.1	50.4 ± 5.7[b]	84.3 ± 4.1[ab]
SDFT allograft	103.66 ± 4.1	32.3 ± 4.6[a]	74.2 ± 7.1[b]
SDFT allograft shielding	103.66 ± 4.1	62.7 ± 6.5[c]	88.8 ± 3.5[a]

MANOVA fit, $p < 0.0065$.
Time: $p < 0.0001$.
Wilks' Lambda for treatment xtime interaction: $p < 0.0001$.
[abc] Means with different superscript letter at the same column are significantly different at $p < 0.05$.

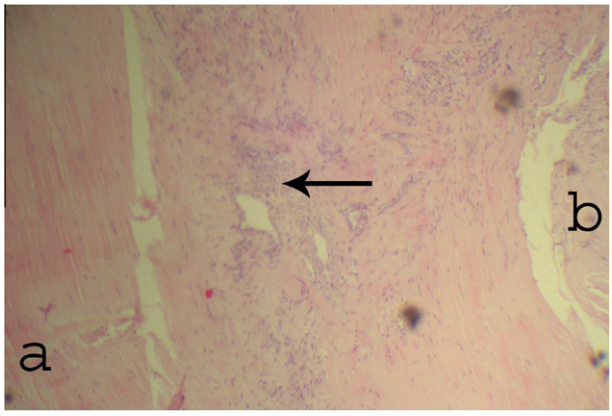

Fig. 8 Photomicrograph of grafted SDFT at 45 days postoperatively showing tendon reconstruction consisting of newly formed blood vessels (arrow), fibroblasts collagen fibers with leukocytic infiltrations in-between the two cutted ends of the original tendon (a and b). H&E; x130.

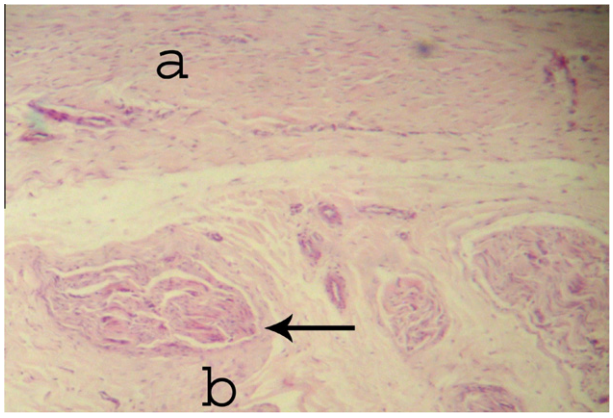

Fig. 9 Photomicrograph of grafted SDFT at 90 days postoperatively showing SDFT repair in the form of mature collagen fibers and normally aligned fibroblast with little vascularization (arrow) homogenize with the original tendon (a and b). H&E; x130.

bundles along the lines of tension that provide higher initial reconstruction strength and improve mechanical performance and tissue function of the repaired tendon with minimal degree of adhesion. The use of banked bioscafold augmentation devices significantly reduces the immunogenicity of the tissue by killing fibroblasts within the graft [21]. Also, it can survive for up to 2 months and would solve the problem of needing a donor as well as reducing the surgical time [34,35]. Moreover, the conjunction between the allograft with pericardial adhesion barrier (allograft shielding) would strengthen tenorrhaphies, mechanical performance and tissue response to healing without peritendinous adhesion [36,37]. Therefore, the success of using allograft shielding with GBP in SDFT tenorrhaphy in donkeys may in the future encourage other scientists to apply it in repairing SDFT in horses and other members of equidae.

The present histopathological investigation in all treated groups revealed a gradual improvement with time in the SDFT reconstruction, which initially appeared in the form of randomly distributed active fibroblasts as well as immature

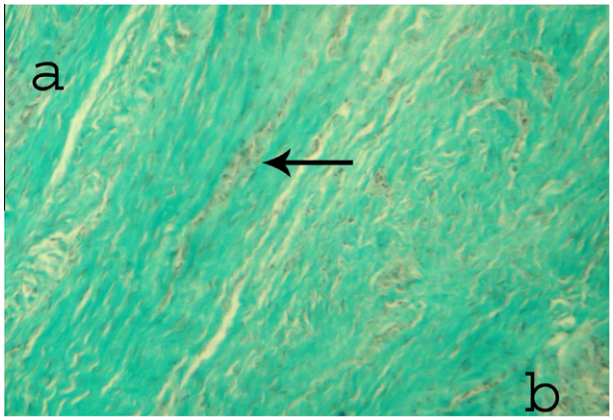

Fig. 10 Photomicrograph of the repaired SDFT showed homogenization of the collagen fibers (arrow) between the original tendon and implanted device. The mature wavy collagen bundles of the newly formed tendon appeared bluish with the Masson's trichrome stain x520.

collagen fibers at 45 days postoperatively and then become well organized with parallel wavy bundles of densely packed, collagen fibers at 90 days postoperatively. These healing properties were confirmed with Masson's trichrome stain, where the mature wavy collagen bundles of the newly formed tendon showed bluish coloration. These results coincide with those findings reported by Kumar et al. [14], Kummer et al. [15], Saini et al. [21] and Rogers et al. [22] and support the suggestion that the graft materials used in SDFT tenorrhaphy of the present study may act as a connecting device providing flexor support until complete tendon healing. Abundant fibrous tissue and vascular growth present in and around the graft bed form a fibrous bridge for tendon regeneration and a scaffold for laying down fibroblasts with newly formed collagen relatively as normal ones [11,12,38].

There was no significant variation in the clinical index scores in the treatment groups of the present study. Reaction, nor rejection or discomfort were noticed in all treated groups. At 45 and 90 days postoperatively, the median and range values of clinical index score were similar and measured 0.5 (0–1), 0 (0–1) and 0 (0–0) for xenograft, allograft and shielding, respectively. The clinical index score revealed that the grafted tendon is strong enough to tolerate the projected forces during active motion without dehiscence or gap formation at the repair site. The clinical recovery represented by normal weight bearing without apparent lameness and tissue reaction of the operated donkeys.

Statistical analysis of the mean fetlock joint angle showed a significant increase in all tenorrhaphies groups with time. In the donkeys subjected to allograft shielding with GBP, the Mean ± SD of fetlock angle at 90 days postoperatively (123.8° ± 1.1) was higher than that recorded at 45 days postoperatively (112.8° ± 4.4). However, mean values showed be lower case when compared with the corresponding normal angle of 125° which is thought to reflect the immaturity/maturity of the tendon fibers [4,39]. Moreover, by the end of 90 days postoperatively, all animals regained the normal range of motion. Therefore, it is reasonable to suggest that the repaired SDFT with variant augmentation devices at 45 days postoperatively may be immature and have not completely adapted to

strain loading. Our data are in accordance with Riemersma et al. [1] and Sharifi et al. [29], that reporting higher tension in the digital flexors reduces overextension of the fetlock joint.

Conclusion

In conclusion, evaluation of the clinical and biomechanical properties of repaired SDFT with different bio-scaffold augmentation devices either xenogenic or allogenic in comparison to normal ones may provide a useful information about the healing characteristics of SDFT tenorrhaphy in equidae. Moreover, the use of augmentation device coupled with an adhesion barrier (allograft shielding with GBP) is a good alternative technique for repairing of superficial digital flexor tendon in donkeys.

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