Surgical management of patellar ligament rupture in dogs using a prosthetic woven fabric: Experimental study.

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Abstract: A new synthetic fabric composed of a mixture of two biomaterials, polyamide 6.6 and polyester, was manufactured with specific tensile characters to serve in the reconstruction of the patellar ligament rupture in dogs. Twelve skeletally mature mongrel dogs with no evidence of clinical signs of lameness were used in the present study. Patellar ligament rupture was induced by severing the mid portion of the right ligament of each limb. Surgical intervention was performed by primary suturing of the severed patellar ligament ends and applying a synthetic fabric to act as a supportive internal splint. Satisfactory results were obtained concerning the tendon healing and the return to limb normal function without complications. It was found that the polyamide polyester fabric proved to be a suitable reconstructive biocompatible material that allowed primary ligament repair with adequate support by and giver an excellent outcome in cases of patellar ligament ruptures in dogs.
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1. Introduction

Patellar tendon rupture is a rarely reported injury in both veterinary and human medicine. In dogs it generally occurs as a result of direct trauma, or fall and was also associated with some systemic diseases (Quintero *et al.,* 2003). There are predisposing factors similar to those in the human literatures and others have not been identified (Culvenor, 1988 and Gemmil, 2003).

Recently, surgical inoculation of the new synthetic fabrics for reconstruction of biological tissues had become one of the things that attract attention, since they give very satisfactory results regarding the strengthening of living tissues (Kasten *et al.,* 2001). Different pathomechanisms have been postulated, and multiple techniques for repair have been described in the literatures (Smith, *et al.*,2000).

Diagnosis and treatment is time sensitive and should be achieved before more complications. Current recommendations for the repair of patellar ruptures included suturing of the severed ends and the protection of the repair with an interior splint (Vasseur,2003 and Moretti *et al.*,2008).

Nylon leader, orthopedic wire and fascia lata have been tried (Shipov,2001).They should accommodate the reconstruction and give enough support . Such implanted materials are applied to give strength to the cut tendon ends and act as a scaffold for the growth of the repairable tissue (Bushnell *et al.*,2008). Many techniques have been applied for the implantation including the transpatellar fixation, the suture anchor techniques associated with protective stainless wire, or associated with quadriceps tendon flap. (Bushnell *et al.,* 2008).

Nowadays, biomaterials and tissue engineering play an important role in the biological tissue repair to develop new approaches for augmenting and replacing damaged body parts (Kammula and Morris, 2001). When the tissue damage is so extreme, the difficulty is in finding a scaffold of special characteristics that the cells can grow and organize on (Moretti *et al.,* 2008). The key requirement of any material used in the body is that, in addition to providing mechanical support or repair, it should be biocompatible. Such materials can generally be produced either in nature or synthesized in the laboratory using a variety of chemical approaches (Vasseur, 2003).

There are many applications for biomaterials such as joint replacements, bone plates, artificial ligaments and tendons (Stupp and Braun,1997 and Wikipedia the free encyclopedia).

Of the most important industrial polymers that produce fabrics commonly used in the medical field are polyester and polyamide (Cozma, 2000)**.** The basic raw materials for polyester fiber production are petroleum, coal, air & water. Due to good extension, strength and functional property of polyester, it is widely used as sewing thread in the garment industries, its fibers do not shrink or extend and have very low moisture content which ranges from 0 – 0.4%. Polyester fibers are easily cleaned and resistant to mildew, moths and insects (The Textile blog., 2010).

Polyamide 6.6 has the property of tenacity-elongation at break ranges from 8.8g/d-18% to 4.3g/d - 45%, 100%, It is elastic under 8% of extension, of melting point 263^{0}c, it is chemically stable. It has a bactericidal effect and 4 - 4.5% of moisture regain and abrasion resistant. (Raghavendra *et al.*, 2004). The applications of textile materials as reconstructive devices for compensating the defects in the living tissues in the veterinary practice were previously discussed (Gadallah, El-Husseiny, 2000 and Smith, *et al.*,2000).

The aim of the present study was to develop a specification for producing a new synthetic fabric that should be available and cheap with special characters regarding the biocompatibility and the tensile strength that acts as an appropriate internal splint for the reconstruction of the patellar tendon ruptures in dogs.

2. Materials and Methods

This experimental work was carried out on 12 dogs apparently healthy and without any apparent signs of lameness. Animals were of different weights and ages. The mean body weight was 20.3± 2.38 kg and the mean age was 18.2± 4.6 months.

All dogs were operated under general anesthesia through intravenous injection of atropine sulphate (0.05 mg/kg); diazepam (1 mg/kg); xylazine (1mg/kg) and ketamine Hcl (10mg/kg). The anesthetic depth was maintained with intravenous injection of 2.5% thiopental sodium and general inhalation anaesthesia via the semi-closed technique (Vet Surg. model 2005) induction using oxygen and nitrous oxide and maintenance with Flothan gas 1.5: 0.5: 2%, respectively. The dogs were restraint in dorsal recumbence.

Characters of the used fabric :

This work was carried out in the Textile Division at the National Research Center in Cairo**.** In the present study, the textile sample was synthesized from polyester material for warp and polyamide 6.6 for weft with woven structures plain 1/1, using monofilament microfibers for each of the warp and weft yarn.

Physical and mechanical tests were carried out on the warp and weft directions after conditioning the fabric for 24 hours under the standard atmospheric conditions (20±2°C temperature, 65 ± 2% relative humidity).

All samples were used raw without treatment. The fabric was tested for the following parameters: tensile strength and elongation, weight and fabric thickness. Load to failure was examined before sterilization of the implantable sample.

The tensile strength and elongation measurements of the plain structure 1\1 fabric was done at the Instron 3345® England. The size of the sample was 5 cm width × 20 cm length for each warp and weft directions this is in accordance with ASTM (2006) ; and the weight tests were performed on Balance the capacity of 0.001 for sample size 5 cm width × 10cm length accordance with ASTM (1996). Frazier Thickness Gauge was used for the measurement of the fabric thickness without load with capacity of 0.01 according to ASTM (1996).

Table (1): indicates the specification of the implanted fabric:

Parameters	Value
Mixing Ratio of fabric	50:50
Density of polyester	1.38
Density of polyamide	1.14
Diameter of Warp yarn	55micron
Diameter of Waft yarn	58 micron
warp yarn Count	40 dtex.
weft yarn Count	40 dtex.
Warp density	36 ends / cm
Weft density	32 picks / cm

The surgical operation:

The right hind limb of each experimental animal was prepared for aseptic surgery. The left hind limb was considered as a control. Lateral parapatellar skin incision was performed and the patellar ligament was severed transversely. The prosthetic fabric, polyamide 6.6 – polyester was twisted and introduced through a 3 mm hole drilled in the tibial tuberosity (from lateral to medial), just behind the insertion of patellar ligament. The fabric encircled the patella and passed through the musculotendinius portion of the quadriceps muscle (from medial to lateral), just proximal to the patella (Fig.1). The stifle was flexed to a normal standing angle (~135^o) and the two ends of the prosthetic fabric were pulled in an opposite direction until the 2 ends of the severed patellar ligament were approximated. The two ends of the prosthetic fabric are tied together and knotted at the lateral side of the patellar ligament (Fig.2). The approximated ends of the ligament were then sutured with 2-0 polydioxanon in an interrupted suture pattern (Fig.3). Extreme flexion and extension of the operated stifle was performed to ensure a physiological smooth range of motion without failure of the stiches. The subcutaneous tissue and skin were routinely closed after thorough irrigation of the joint with sterile normal saline. Dogs were exposed to restricted activity for 2 weeks postoperatively followed by a leash walk until they were euthanized. All dogs underwent a complete physical examination, including orthopedic, neurologic examinations, and radiographic assessment. Dogs were euthanized 6 months postoperatively for histopathology and biomechanical testing. Radiology was performed with the limbs at dorsopalmar and lateromedial views using 60 K.V.P. and 220 m.A.s at 80 cm- F.F.D.to detect any abnormal changes.

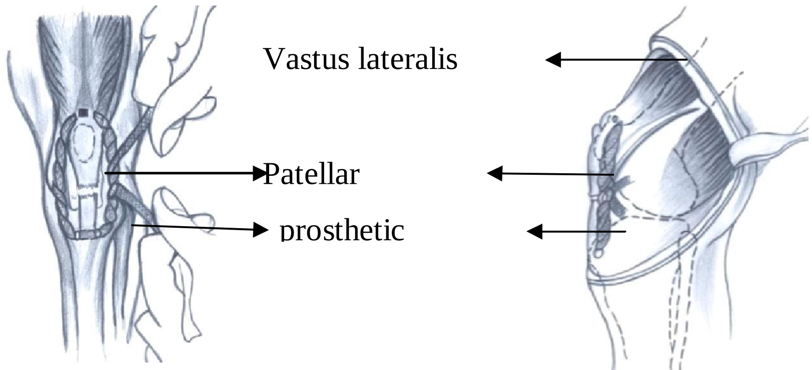

Fig.1: Diagram showing the cranial view of the stifle showing the application of the twisted synthetic fabric through the quadriceps tendon proximally and tibial tunnel distally "note the severed patellar ligament".

Fig. 2: Diagram showing the lateral view of the stifle showing the approximated and sutured patellar ligament after tensing and knotting the two ends of the prosthetic fabric.

Fig. 3: The prosthesis was twisted on both sides of the patellar ligament , the two ends of the fabric are tied at one side. (c) is the cut in the patellar ligament

Biomechanical evaluation:

This was carried out by collection of the specimen after euthanasia of the experimental animals, 180 days postoperatively. Samples were wrapped in saline soaked towels and immediately examined for biomechanical analyses. Biomechanical studies were carried out at The Textile Division– National Research Center, using a tensile testing machine (Instron 3345®, England).

Biomechanical parameters were measured and were represented in table (1).The examined samples were evaluated in the operated and control groups.

Histopathological studies:

Autopsy samples were taken from the healed patellar ligaments after euthanasia, 180 days after implantation. Samples were examined macroscopically then fixed and stained with hematoxylin and eosin stains for microscopical examination (Banchroft *et al.*, 1996).

3. Results:

Clinical observations:

Experimental animals began to ambulate and bear weight with mild to moderate limping on the operated limbs within the first 2 weeks. Progressive improvement occurred after this period. No patient had rerupture of the patellar ligament after surgery.

30 days postoperatively, there was a complete return to limb function and the operated stifles showed a limited degree of stifle flexion. A marked thickening of the periarticular soft tissues was noticed.

180 days postoperatively, animals were fully weight bearing with a full range of motion. There was an obvious reduction in the periarticular thickening. During the evaluation period (6 months), the patella was palpated as discreet, firm structure which maintained its integrity throughout the range of stifle motion in all dogs.
Radiographic findings revealed, that 30 days postoperatively the femoropatellar joint showed minimal periarticular soft tissue swelling with no evidence of intraarticular degenerative changes or effusion (Fig.4), 180 days showed no evidence of postoperative intraarticular changes (Fig.5).

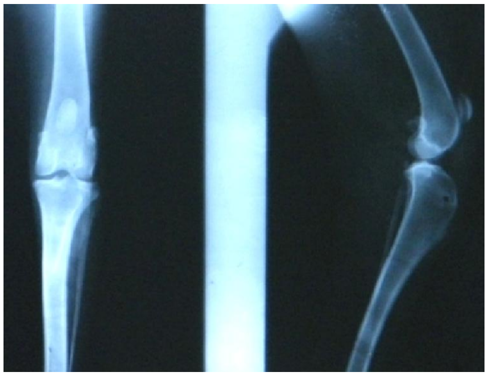

Fig.4: An experimental case just after surgery, Notice, the normal articular joint.

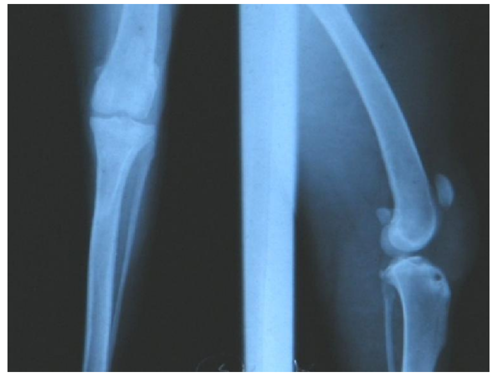

Fig. 5: 180 day's postoperation. Notice, the stifle joint was free from intraarticular changes.

Evaluation for the mechanical properties of the used textile fabric before implantation (Table 2):

Table (2): Result of load testing (M±SD).

	Load at max. (N)				**Load at break (N)**			
	warp	Warp twist	weft	Weft twist	weft	Weft twist	weft	Weft twist
Mean	236.7	120.7	254.5	139.5	216.3	56.5	248.4	126.9
±S.D	0.80	0.028	0.88	0.80	0.69	0.90	0.42	0.37
warp, weft twist means the normal sample after 20 twist\10cm.								

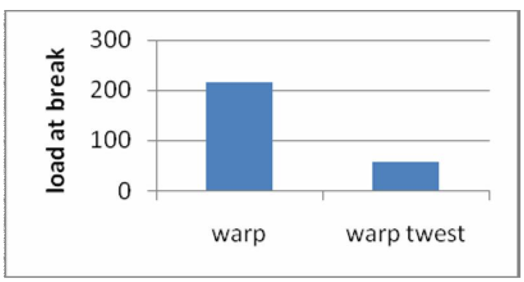

Fig.6 load at break for warp

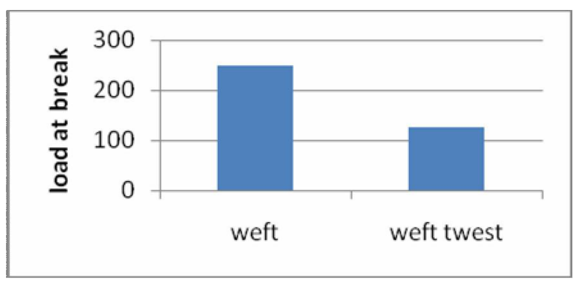

Fig.7 load at break for weft

Figs. (6,7) clear that the value of the tensile strength of directional warp and weft in the fabric product, without giving twist higher than the case of a twist, We also find that the value of tensile strength generally in the direction of weft is higher than the warp.

Table (3): Result of strain testing (M±SD).

	Stain at max. (N)				**Strain at break (N)**			
	Warp	Warp twist	Weft	Weft twist	Warp	Warp twist	Weft	Weft twist
Mean	21.35	43.58	35.10	56.14	21.8	36.16	36.20	56.50
±S.D	1.104	0.58	0.73	1.11	1.06	0.42	0.42	1.06
warp, weft twist means the normal sample after take 20 twist\10cm								

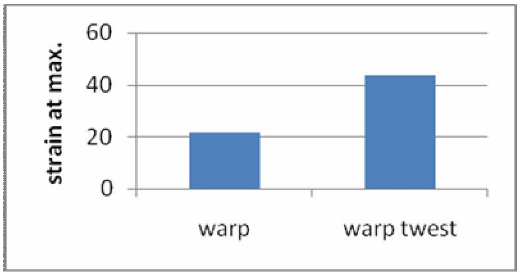

Fig.8 Strain at max. for warp

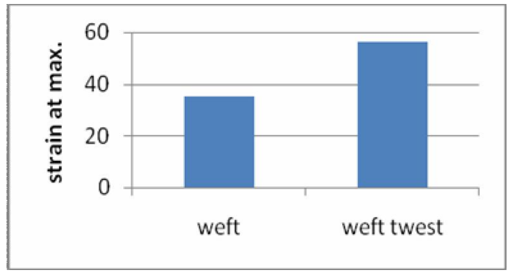

Fig.9 Strain at max. for weft

Figs. (8,9): show that the value of the strain of the fabric produced in the directional warp and weft are higher in the case of a twist of the non-existence. Also find that the strain in the case of weft is higher than the warp.

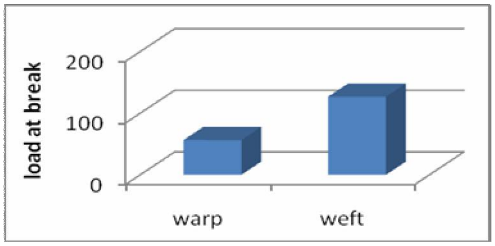

Fig. 10 Load at break for fabric after twist

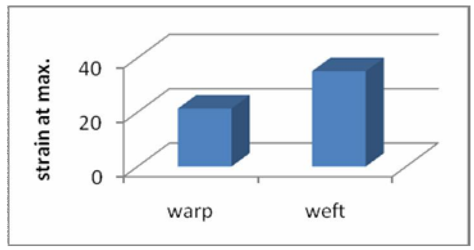

Fig. 11 Strain at max. For fabric after twist

Fig.(10): Shows that the value of tensile strength necessary to break the sample in the direction of weft is higher than warp after conducting the test on the fabric in the warp and weft and give the two directions 20 twist\10cm, As (Fig.11) shows that the maximum strain of the sample in the direction of the weft is higher than the warp with twisting.

From the table (4) we find that the thickness of producing a few, and interfaces with spaces accurate and reflected the value of weight.

Table (4): Result of weight and thickness for fabric sample.

Weight (gm\m^2)	Thickness (mm)	Opining size (Cm^2)
26.0	0.13	52.14

Table 5: The values of the biochemical tests applied on the samples of the normal control and healed tissues

Case No.	Strain at max. load (mm.mm)	Load at max. load (KN)	Load at break (KN)	Average Thickness of tissue
Normal(mean value)	1.400	624.26	331.85	7.45mm
1	0.408	1267.13	510.91	
2	1.367	1176.73	1072.02	
3	0.617	1171.39	520.27	
4	0.770	1205.09	701.07	
5	0.797	1176.26	1060.18	
6	0.610	1217.01	703.56	
7	0.380	1213.20	524.15	
8	1.210	1257.18	1070.01	
9	1.200	1202.16	522.01	
10	0.789	1253.70	515.01	
11	0.800	1170.30	701.12	
12	0.616	1151.013	515.02	
Mean	0.797	1205.09	701.27	
±S.D	0.504	5.49	32.76	
C.V	63.225	4.46	45.83	

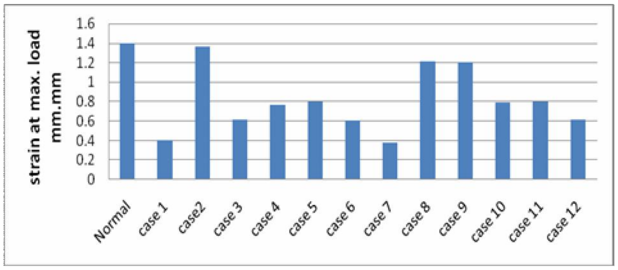

Fig.12:The diagram demonstrates the values of strain at max. load of normal and operated cases

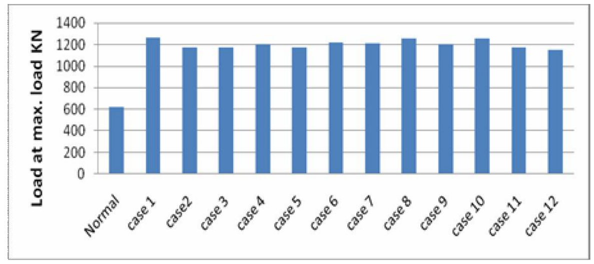

Fig.13:The diagram demonstrates the values of load at max. load of normal and operated cases

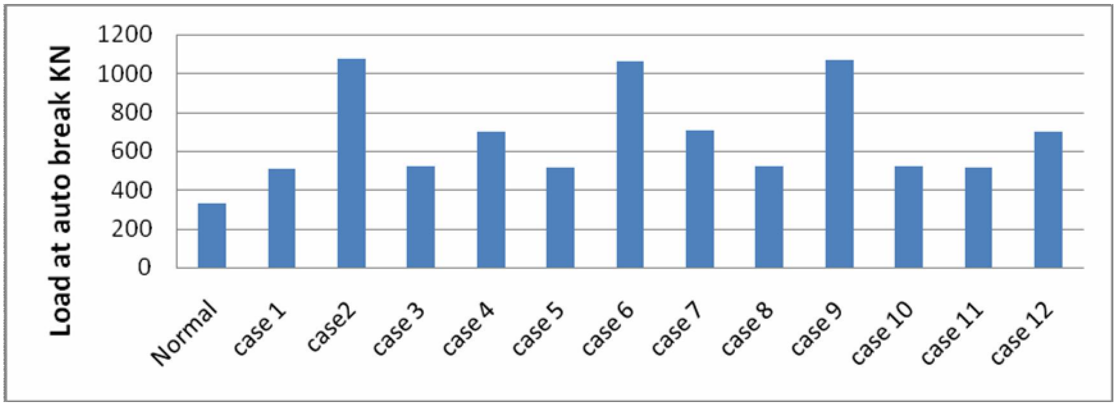

Fig.14:The diagram demonstrates the values of load at break of normal and operated cases.

Histopathological results:

Microscopically, complete fibrous healing appeared after 180 days post implantation which appeared in the form of dense bundles of collagen fibers. There was no evidence of infection or body rejection. Remnants of the implant were still present infiltrated and surrounded by mature fibrous connective tissue. The predominant cells were fibroblasts along with little numbers of macrophages and mast cells indicative of active matrix synthesis of collagen with the presence

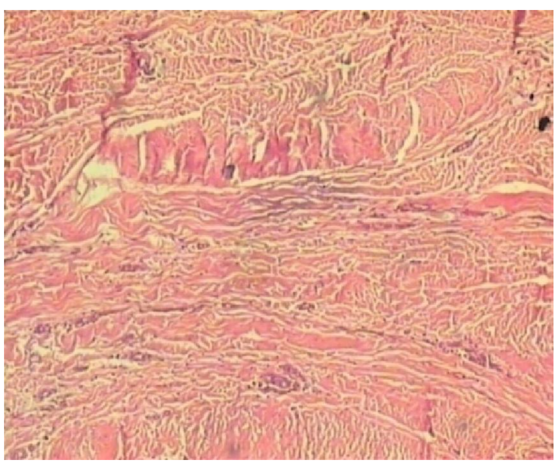

Fig.15: longitudinal section in a normal case, demonstrating, long bundles of dense collagen fibers closely packed together (H&E X50).

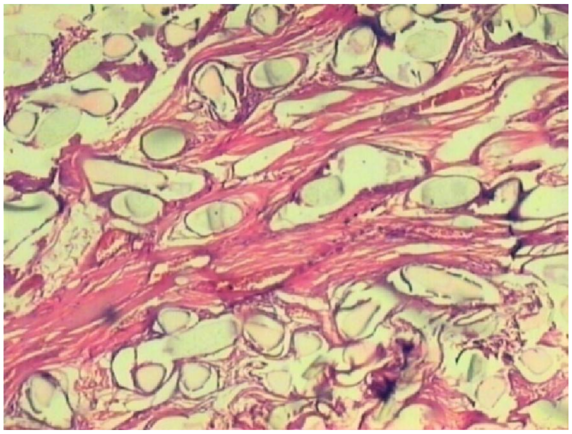

Fig.16: longitudinal section of the patellar ligament showed dense connective tissue with foreign material in multiple rounded spaces with small focus of calcification (H&E X50).

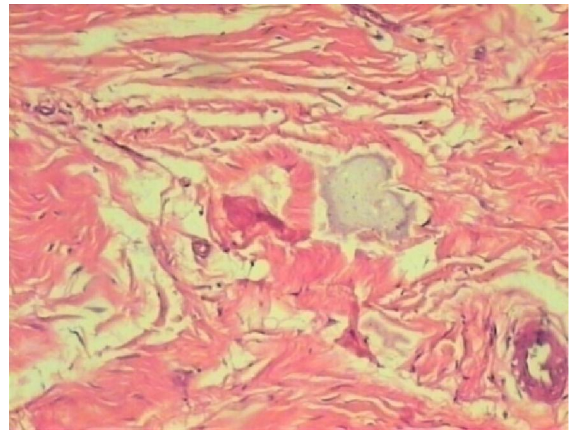

Fig.17: Section of tendon, formed of bundles of dense collagen fibers with intervening blood capillaries. There are remnants of foreign material (P) not surrounded with inflammation or foreign body reaction (H&E X50).

4. Discussion

The patellar ligament in dogs transmits the contraction of the quadriceps muscle to the tibia, and causes extension of the knee. It is present to prevent the patella from being dislocated upward Gibbons *et al.* (2006): Rupture of this ligament usually occurs as a result of severe trauma, chronic local stress and local or systemic administration of steroids (Culvenor, 1988):. Adequate support and stability are essential factors for better outcome. Best treatment was achieved by primary suturing and application of an internal splint(Gemmil & Carmichael,2003)

Massoud (2010) reported the importance of the support use of reinforcement device in cases of ruptured patellar ligament for tension regulation at the suture line. Kasten *et al.*(2001) added that,many augmentation techniques have been tried after end-to-end sutures for reinforcement such as wire cerclage or PDS cord. Reliable results were obtained and experimental cases did not sustain reruptures.

As was mentioned by Alexa and Cozma (2009) treatment of ruptured patellar ligament necessitates immediate repair with a synthetic material that allowed immediate mobilization and decreased the recovery period which improved the outcome of rehabilitation.

In the present study, A textile fabric was used for the treatment of patellar ligament ruptures. The fabric was synthesized at the Textile Division at the National Research Center which composed of a combination of two synthetic materials polyamide 6.6, a material which possesses a high tensile strength and elongation and polyester which is a strong , inert material. The prosthetic fabric was made in a special manner to fulfill the demanded strength and flexibility for the internal support of the ruptured patellar ligament in dogs .The implanted fabric would be biocompatible within the living tissues (Bushnell *et al.*,2008)

The structure of the prosthetic textile was composed of monofilament microfibers which gave advantage for the properties of the textile fabric to be strong enough to withstand the loads and the tensile forces to compensate the cut ligamentous tissue. The material also was not water absorbent and was resistant to the ordinary chemical and biological agents (Raghavendra *et al.*,2004).

Twisting of the prosthetic material during the surgery was applied as 10 twists / cm in order to gave success in using the material in a flexible manner and be capable of being a solid internal splint.This helped in the normal biomechanical movement of the joints(Phelps & Dormer, 1986).

Clinical results demonstrated that the operated animals were moving normally on the operated limbs after 15 days postoperatively and at the end of the experiment they were running freely without obvious signs of complications .At 30 days ,all animals recovered from surgery in which animals walked and ran normally on the repaired legs . At the end of the experiment ,180 days postoperatively, the dogs were fully weight-bearing.Same results were obtained by (Kasten *et al.*,2001 and Archer *et al.*, 2010**)** who stated that full range of motion was achieved at 3 months.

Our clinical and histopathological results proved that the prosthetic material did not show any signs of rejection or septic inflammatory reaction.

Clinical examination revealed to the presence of local periarticular soft tissue swelling at the seat of operation which decreased gradually till the end of the experiment seen as focal swelling, this was attributed to fibroplasias and healing around the synthetic fabric Same results were seen by Bushnell *et al.*(2006) . Radiographic results, 180 days postoperatively revealed no intraarticular changes a result which advocates the use of this technique as other treatment techniques were usually accompanied by intraarticular degenerative changes (Alexa and Cozma, 2009).

Macroscopic examination after euthanasia and exploration of the seat of the operation indicated healing of the ruptured ligament ends was evident indicated by the formation of new tissue greatly resembling the original ligament. Microscopically, results proved-primary ligament repair characterized by formation of bundles of dense collagen fibers running parallel to each others . At 180 days,The fabric disintegrated to remnants which appeared as foreign vacules not surrounded by inflammation or body reaction indicating biocompatibility with the body tissue. Healing by tissues greatly resembling the structure of the normal ligamentous tissue proved the efficiency of the fabric as an internal splint for immob ilisation and maintaining the normal physiological function of the joint without any adverse reactions.

Biomechanical results indicated that the newly formed ligamentous tissue at the end of healing 180 days postoperatio not only a good support against the loads applied on the joint but exceeded the values of the normal tissues needed to support strenuous loads applied on the joint during extension and flexion . This was proved by finding out that, the force values needed for complete cut of the newly formed tissue were exceeding those for the normal tissue of the control group , this was a considerable result regarding the tendon reconstruction using the implant material.

On the other hand biomechanical evaluations proved that the new healed tissue possessed nearly the same tensile strength of the normal ligamentous tissue but of less elasticity. This is attributed to the new replacing tissue which is fibrous in nature and don't identically resemble the pure collagen type 1 of the healthy ligamentous tissue. Same results were mentioned by Bushnell *et al.*(2006).

The biomechanical tests for the textile sample, found that the values of the tensile strength for the direction of the weft is higher than that of the warp , this is attributed to the material used which is the Polyamide 6.6 with its advantage of a tensile strength and strain higher than that of polyester. It was also noticed that, the tenacity-elongation at break ranges from 8.8g / d - 18% to 4.3 g/d-45%,100% elastic under 8% of extension for polyamide 6.6 While tenacity varies from 4.5 to 5.0 g / d for the polyester textile. It is known that, high tenacity fiber reaches up to 8.0 g/ d and extension at break varies from 20% to 30 % (Raghavendra *et al.*,2004 and The textile plog. 2010).

A slight proximal displacement (patella alta) was radiographically observed in most cases which may later on predispose to patellar luxation , a point which need more further studies.

In conclusion, we recommend the polyamide 6.6 polyester fabric as an internal synthetic supportive splint for acute patellar tendon ruptures in dogs The product has the advantage of mechanical and biological properties in the medical field.The implant also allowed immediate rehabilitation without the need for prolonged immobilization .

Twisting of

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