A comparative biomechanical study of repaired tendons wrapped with two biological matrices in Bucks

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Summary

This study is planned to evaluate the efficacy of two biological matrices represented by autologous platelet rich fibrin matrix, as well as a cross linked decellularized caprine pericardial extracellular matrix on enhancing healing of the experimentally severed superficial digital flexor tendon in a goat model. It was carried out on 48 adult apparently healthy bucks, which were divided randomly into three equal groups. Under the effect of sedative and local ring block anesthesia, superficial digital flexor tendon was severed at the mid metacarpal region of the right forelimb. In the first control group, tenorrhaphy was performed and left without additives. While in the second group the tenorrhaphy site was wrapped with a previously prepared autologous platelet rich fibrin strips, as well as in the third group the tenorrhaphy site was wrapped with a cross linked decellularized pericardial extracellular matrix strip which was prepared from the whole fresh caprine pericardium obtained from the slaughter house. Both matrices were fixed in their position at the tenorrhaphy site by few interrupted stitches. The biomechanical evaluation of the operated tendon indicated an increase in tensile strength with time in all groups, but the comparisons among groups showed a significant ($P \leq 0.05$) increase at day 15 in both treated as compared to control animals. On day 45 the pericardial extracellular matrix group showed a significant increase in tensile strength as compared to platelet rich fibrin matrix and control groups, but at day 75 there were no differences among groups, at day 180 the pericardial extracellular matrix group showed a significant increase in the tensile strength as compared to platelet rich fibrin matrix and control groups. In conclusion, both biological matrices led to improvement in the biomechanical properties of the operated tendons with time.

Keywords: Tendons, Buck goat, Biological, Biomechanical.

Introduction

Tendon injuries are a clinical problem for orthopedic surgeons and investigators, maintaining approximation of the severed flexor tendon ends is critical after repair to achieve healing and there have been multiple techniques and extensive research to identify the optimal tenorrhaphy method (1 and 2). An ideal tendon repair would ensure a sufficient breaking force with a minimal deformity in the tendon repair site to allow early passive and active motion so as to reduce tendon adhesions and improve the functional outcome. In a conventional tenorrhaphy, knots are the weak point of tendon repair which decreased the tendon apposition (3). To reduce the rate of re-rupture and accelerate rehabilitation, primary suture repair is sometimes reinforced with biologic scaffolds or grafts (e.g., bovine pericardium, small intestinal submucosa (SIS), or a cellular human or porcine dermal matrix) (4 and 5). In addition to improved mechanical support, these biologic materials provide an extracellular matrix for the in-growth of tissue so they become well incorporated into the tendon. Tendons augmented with biologic grafts have been able to return to early activity without re-rupture or complications (6 and 7). Many studies revealed the use of tissue engineering technologies and detected its beneficial effects in full-thickness injuries of tendon and ligament; therefore, various types of biomaterials have been used as development technologies (8).Tissue engineering techniques have been developed as advancing strategies that aim to induce repair and replacement or regeneration of tissues and organs. A collagenous material is considered as an excellent biomaterial which gives promising effects on tissue regeneration and physical function of the injured tendons and ligaments (9-16). Other researchers attempted to develop alternative non-toxic, easy to prepare, and economically cheap therapeutics that lead to

the local release of growth factors which accelerate hard and soft tissue healing. Platelet- rich fibrin (PRF) is an autologous platelet concentrate in a natural fibrin- based biomaterial prepared from autologous blood without anticoagulant to allow obtaining fibrin membranes concentrated with platelets and growth factors that play a potential role in tissue engineering (17). The aim of this study is to evaluate the tensile strength of the repaired superficial digital flexor tendon experimentally underwent tenorrahphy wrapped with two different biological matrices in bucks.

Materials and Methods

Forty eight apparently healthy adult bucks, aged 1-2 years weighed 20- 25 kg, were used in this study, they were examined clinically and ultrasonographicaly for any abnormalities of the superficial digital flexor tendons pre surgery. During the trial interval all animals were kept under same circumstances and dewormed with Ivermectin (Chongqing, china) administrated subcutaneously at a dose of (0.2 mg/Kg B.W.) Caprine pericardium was obtained from the local abattoir, immediately after slaughtering. The pericardium was submerged in saline solution in order to be transported to the laboratory; the tissue was gently rinsed with saline to get rid of the adhered blood. Mechanical cleansing was performed manually to eliminate all unwanted fat and connective tissues from the pericardium using dry gauze. The tissue was cut into 1×3 cm size pieces (Fig. 1), and were decellularized with 0.1% peracetic acid and 4% ethanol combination for two hours and cleaned with phosphate buffered saline (PBS) and deionized water for 15 min. (18 and 19), then crosslinked using 0.5% Glutaraldehyde (GA) in PBS for 72 hrs. The crosslinking was done at room temperature, washed in PBS. The prepared acellular cross linked tissue matrices were stored at 4 °C in PBS containing 1% gentamycine (20). Specimens from native and decellularized pericardium matrices were obtained and fixed in 10% buffered formalin, examined histologically by staining the sections using hematoxylin– eosin and Van-Gieson's stains to check the cellularity and the collagen component of both pericardium Specimens.

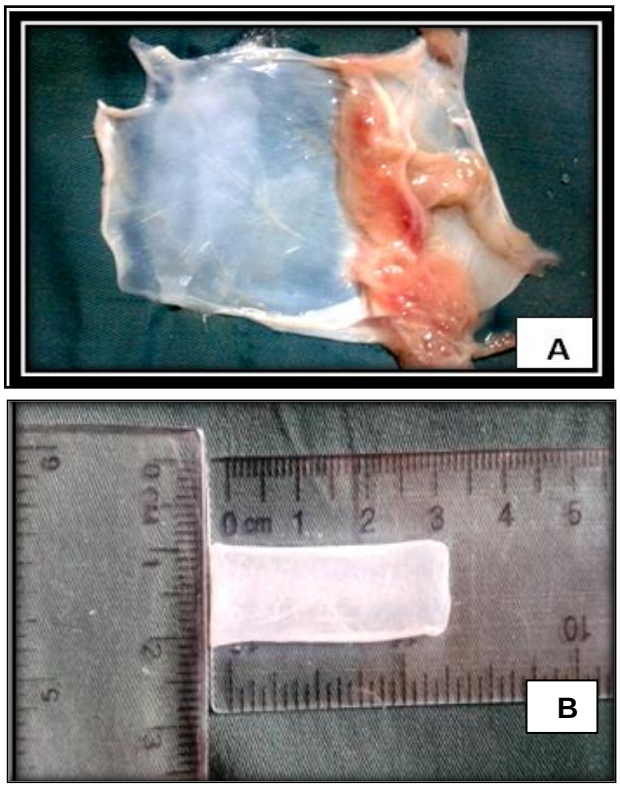


Fig.1: Shows steps of pericardial extracellular matrix preparation. (A) Manually cleansing of pericardium. (B) Trimmed pericardium (1x3 cm).

10 ml of blood samples were taken without anticoagulant in tubes, then immediately centrifuged at 3000 rpm for 10 minutes (21). Three separated layers resulted after centrifugation: the lower layer represented the red corpuscles, PRF matrix as a fibrin clot presented in the middle, and the superficial layer represented acellular platelet poor plasma (PPP). the matrix was withdrawed with forceps from the tube and cutting off the red blood corpuscles, then squeezing of platelet rich fibrin matrix from the fluid to obtain a fibrin membrane, then trimmed in a piece size of approximately (1×3 cm). Specimens from platelet rich fibrin matrix were taken and fixed in 10% buffered formalin, examined histologically by staining the sections using hematoxylin and eosin stain to observe the platelets and fibrin network.

In first group (control group), the surgically severed SDFT was immediately repaired by suturing (tenorrahphy) and in the second group the tenorrahphy site was wrapped with previously prepared PRFM. While in third

group, SDFT tenorrahphy site was wrapped with previously prepared PECM.

Food was withheld for 24 hrs. and water restricted 12 hrs. prior to surgery. The animals were controlled in lateral recumbency after light sedation by using Xylazine ((Bayer-Germany) in a dose of 0.2 mg/Kg B.W. I/M (22) and the metacarpal region of the right forelimbs (between the carpal and fetlock joint) was prepared for aseptic surgical operations, tourniquet was applied above the carpal joint to control bleeding during operation. Ring block was performed in the fore limb using 2% lidocaine hydrochloride (Jayson Pharmaceutical Ltd, Bangladesh) at dose rate of 4 mg/Kg body weight (22). Then Slightly lateral to site of superficial digital flexor tendon a straight 5 cm incision was made, including the skin, subcutaneous fascia and tendon paratenon, to expose the dorsal surface of the tendon. Blunt dissection was performed to separate the superficial digital flexor tendon from deep digital flexor tendon, then two needles were placed at the proximal and distal side of the superficial digital flexor tendon to prevent tendon slipping, the SDFT was severed transversely with the scalpel, then approximated by Bunnell suture using polypropylene (3-0).

These steps were followed in control group, while in treatment groups; the same steps were performed in addition to wrapping the site of tendon anastomosis with PRFM in the second group, and with PECM in the third group. (5-0) USP polydiaxinone was used to secure membranes in their position by interrupted stitches. (2-0) polydiaxinone was used for subcutaneous fascia closure, finally the skin was closed using interrupted horizontal matters with silk (0). In all groups the site of operation was bandaged, and the operated limb immobilized using plaster of Paris cast (with window) for two weeks, postoperatively pencilline - streptomycin in a dose of 20000 I.U. and 10 mg/kg. B.W. respectively was administered intramuscularly for five days.

Specimens of operated tendon were collected at 15, 45, 75 and 180 day postoperatively for biomechanical assay, each tendon was transected for approximately (5cm) above and (5cm) below the anastomotic site. However, the tendon specimens were collected randomly from the mid-metacarpal region of the contra lateral limb in length approximately equal to 10cm and considered as a standard control. Biomechanical properties of both normal and operated tendons were examined at the Laboratory of directorate of materials research in Ministry of science and technology in Baghdad-Iraq. All collected specimens were packed in containers of buffered normal saline and tensile force test was done within three hours of tissue collection. The test was done using tensile testing machine (Tinus Olsen model H50KT-English) by securing its proximal and distal portions to two metal clamps of the tensometer. The specimen's ends were wrapped by a piece of gauze and tightened to avoid slipping of the tendon specimens. All specimens were loaded to failure at speed of 5 mm/ min. Load trials to failure were recorded and calculated graphically using a monitor.

The Statistical Analysis System- SAS (2012) was used to influence of different aspects in study factors. Least significant difference –LSD analyze was used for significant compare between means.

Results and Discussion

Biomechanical properties of the operated tendons in this study indicated an increase in tensile strength with time in all groups, but the comparisons among groups showed a significant increase ($P \leq 0.05$) at day 15 in both treated groups (100 N) in PECM and (75 N) in PRFM groups, as compared to control group (40 N). At day 45 the PECM group showed a significant increase in tensile strength (120 N) as compared to PRFM and control groups, 111.50 N and 106.50 N respectively, but on day 75 there were no differences among all groups 161.50 N in PECM, 155 N in PRFM and 150.75 N in control groups. At day 180 the PECM group showed a significant increase in the tensile strength (261.37 N) as compared to PRFM group (217.50 N) and control group (193.25 N), as shown in (Table, 1 and Fig. 2).

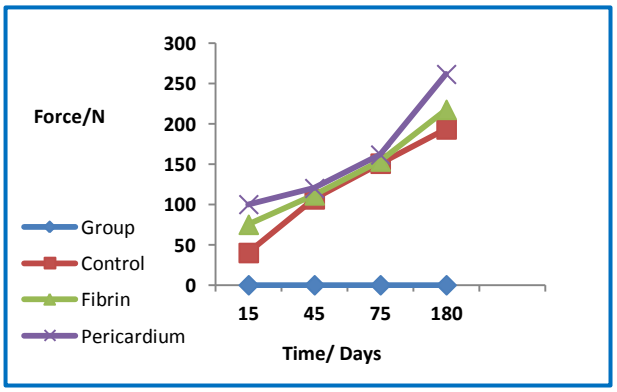
Results of biomechanical assay for PECM group in this study were agreed to the results of (23) who indicated that the failure stress increases with time when using pericardium for tendon graft; they indicated that the use of scaffolds restores the biomechanical properties

of tendon that provide exceptional support for tendon repair and made the tendon strong enough to tolerate the force during active motion without dehiscence or gap formation at the repair site. Also the current results are supported by (24) who indicated that the use of collagen and collagen with polydioxaynone implant sheath on the healing of a large defect in the Achilles tendon in rabbits increased the biomechanical properties of the lesions compared to the control tendons at day 60 post implantation. They explained that the collagen implant has improved new tendon structural and functional properties.

Table, 1: Mean values of tensile force test in goat.

Group	Mean			
	15 day	45 day	75 day	180 day
1st (control)	40.00 b	106.50 b	150.75	193.25 b
2nd (PRFM)	75.00 a	111.50 ab	153.50	217.50 b
3rd (PECM)	100.0 a	120.00 a	161.50	261.37 a
LSD value	25.48 *	11.74 *	15.09 NS	27.61 *

***(P<0.05). NS: Non-significant. a–b: letters in same column indicate the means significantly different at (P<0.05). Number of animals (4/group). The normal failure force value for 10 animals= 307.15**



Figure, 2: Mean values of tensile force test for experimental groups in differently analyzed time in goat.

While the results of biomechanical assay in PRFM group showed increased tensile strength as early as 15 days, then became at the same level with the control group; these results were supported by (25) who noticed that the PRFM enhanced mechanical properties as compared to other platelet products. Also, present results were in line with the results of (26) who indicated that the modulus of elasticity and hardness were less for PRF membrane as compared to collagen membranes. This is related to the PRF membrane which is an autologous membrane without any external additives to cross linked and enhanced its physical properties that lead to faster degradation and not maintain for adequate time to strengthen the injured tendon and improve its mechanical strength.

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دراسة مقارنة لبايوميكانيكية الأوتار الملتئمة الملفوفة بمصفوفتين إحيائيتين في ذكور الماعز

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الخلاصة

صممت هذه الدراسة لتقييم فعالية مصفوفتين إحيائيتين المتمثلتين بمصفوفة الفايبرين الذاتي الغني بالصفيحات الدموية ومصفوفة شغاف القلب اللاخلوي المترابط على تسريع التئام وتر القابضة الإصبعية السطحية المقطوع تجريبيا في ذكور الماعز. أجريت الدراسة على 48 حيوان سليم وبالغ، قسمت عشوائيا إلى ثلاث مجاميع متساوية، تحت تاثير المسدر والتخدير الموضعي، قُطِعَ وتر القابضة الإصبعية السطحية للقائمة الأمامية اليمنى وخيط بطريقة بانيل في المجموعة الاولى (مجموعة السيطرة) في المجموعة الثانية لفت منطقة التفمم بغشاء الفايبرين الذاتي والغني بالصفيحات الدموية المحضرة مسبقا، أما المجموعة الثالثة فقد لفت منطقة تفمم الوتر بغشاء شغاف القلب اللاخلوي المترابط المحضر مسبقا والمأخوذ من حيوانات الماعز المذبوحة حديثا. كلا الغشائين تم تثبيتهما في مكان العملية بغرز من البسيط المتقطع. أظهرت نتائج الفحص البايوميكانيكي في المجاميع المختلفة زيادة في قوة شد الأوتار المعالجة بمرور الوقت و بمقارنة المجاميع أظهرت الدراسة فروقا معنويا ($P\leq0.05$) في اليوم 15 بعد العملية في المجموعتين المعالجتين مقارنة مع مجموعة السيطرة، في اليوم 45 بعد العملية أظهرت المجموعة الثالثة فرقا معنويا في قوة الشد مقارنة مع باقي المجاميع أما في اليوم 75 بعد العملية لم يظهر فرقا معنويا بين المجاميع بينما أظهرت المجموعة الثالثة فرقا معنويا في اليوم 180مقارنة مع باقي المجاميع. تستنتج الدراسة أن كلا الغشائين الإحيائيتين لهما تأثير في زيادة قوة الشد للأوتار المعالجة بمرور الوقت.

الكلمات المفتاحية: الأوتار، ذكر الماعز، إحيائية، بايوميكانيكية.