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ASSESSMENT OF AUTOGRAFT AND XENOGRAFT TRANSPLANTATION ON TENDON HEALING IN A RABBIT ACHILLES TENDON MODEL

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ABSTRACT

The extra cellular network got from bovine small intestinal submucosa (BSIS-ECM) is used for the rehabilitation of musculotendinous tissues. Preclinical appraisal and clinical use have suggested that little intestinal submucosa extracellularmatrix corrupts rapidly after implantation and can be supplanted by having tissue that is practically and histologically like the regular tissue. The current investigation wanted to assess the effects BSIS-ECM on the fix of full-thickness Achilles tendon imperfection. Consequently, this examination was guided on twenty adult and clinically healthy males' rabbits. Under sterile condition and general anesthesia; A 1.5-cm portion long segmental resection was made in the mid substance of the Achilles tendon, in autografts gathering (n=10) the section was inverted and re-implanted between the distal and proximal tendon stumps. While, in scaffold gathering (n=10)the tendon defect, was bridged the stumps with BSIS- ECM implanted constructs shaped to fit in the defects with a 4-0 polypropylene thread by utilizing the modified Kessler technique; that connected the stumps of the native tendon and the specific implanted graft BSIS- ECM. The histopathologicale valuation of the picked biopsies at 30th, 60^{th,} and 120th days post-surgical treatment, which that BSIS- ECM treated reveled the implanted graft supported upheld fast cell invasion and host tissue in growth. After sixty days, the remodeled BSIS- ECM comprised of dense collagenous tissue with organization, cellularity, and vascularity similar to that of a normal tendon. However although, that the neovascularization and cellularity were significantly increased on 30 post-operation in the scaffold group and then decreased with time, while they were showed lesser scores in the autograft group, the collagen fibers alignment scores were significantly increased at days 30, 60, and 120 post-operative days, in scaffold group as compared to autograft group. Likewise, the scaffold group indicated well fiber alignment on day 60 post-operative; while, the autograft group was still at the same score level on day 120 postoperative day. In conclusion, BSIS- ECM is rapidly degraded after implantation, for Achilles tendon repair model and is substitute by the deposition and organization of host tissue that is histologically identical to that of normal tissue and gives a microenvironment favorable to the growth and differentiation of cells and positive improve the recuperating of tendon defect. Subsequently, this examination estimates that the use of this biological material may consider as a novel strategy for injured tendon repair.

Keywords: Achilles tendon, tendon-healing; a cellular bovine SIS, Implantation, rabbit's model.

Introduction

The Achilles tendon break can be credited to theblend of tendon debasement and frustrations inthe inhibitory segment of the musculotendinous unit (Ho *et al.,* 2019 and Buschmann *et al.,* 2014). The normal degenerative histological features of tendon split recollect changes for fiber structure, fiber plan, vascularization, cell morphology and cell development (Zange and Kissel, 1997). These changes are progressively expressed with serious breaks than with interesting tendinopathies (McNally and Marcelli, 1998). Despite the way that development or reconstructive frameworks are used for tendon fix after injuries because of the wide debridement required, exceptional Achilles tendon splits can moreover be opportunities for clinical technique due to essential tissue injury, sore extension, and the development of the impacted populace (Yang *et al.,* 2010). It is generally felt that tendon have some reparative limit, anyway when the tendon is either completely destroyed or the flaw site is too gigantic to even consider evening consider taking into account reposition of the completions, by then tendon replacement is commonly crucial. Starting at now, the remedial decisions to fix tendon wounds are autograft and allograft (Yuen and Nicholas, 2001). Notwithstanding, there are issues related with the usage of natural associations, for instance, provider site horridness, supplier lack, tissue dismissal. As for the use of tendon prostheses, there is unfathomable worry over the drawn out display of such gadgets (Milankov *et al.,* 2007). In this way, elective tendon fix decisions ought to be examined, including tissue-fabricated associations. Generally, tissue-planned associations are made out of cells cultivated on a cross section or structure either with or without dissolvable regulators. All things considered, there are two fundamental approaches to tissue building; erroneously produce ligament tissue and an in vitro condition for coming about implantation, or develop an install to empower recuperation in an in vivo condition (Kocabey *et al.,* 2006). Basic

common biomaterials determined extracellular grid (ECM) items as platforms for tissue remaking and wound administration have been considered in both the *vitro* and *vivo* (Badylak *et al.,* 2009). Gilbert *et al.* (2006) referred that ECM has been isolated from different organs, including the lung, heart, windpipe, and liver, of various species. In any case, only ECM from the porcine small digestive tract, porcine urinary bladder, equine pericardium, and human dermis has expanded clinical broad acknowledgment (Wainwright *et al.,* 2009). The current examination was directed at assessing the viability and destiny of a cellular BSIS-ECM for the speeding up and remaking of Achilles tendon full crosscut contrasted with standard autografts as the control in rabbits models.

Materials and Methods

Experimental animals

Research consents to animals' use and care committee for College of Veterinary Medicine, along the time of the study from 10 November 2019 to 10 June of 2020. Twenty adult healthy males' were used in this investigation. They were erratically separated into two gatherings; in autografts group the segment was inverted and reimplanted between the distal and proximal tendon stumps. In the scaffold group the tendon defect was bridged the stumps with BSIS- ECM implanted constructs shaped to fit in the defects by utilizing the modified Kessler technique that connected the stumps of the native tendon and the specific implanted graft BSIS. The rabbits were sacrificed at 30th, 60thand 120th post-operative days for histopathological and immunohistochemistry evaluation.

In vitro study

Fabrication of Small Intestinal Submucosa Extracellular Matrix

Bovine Small Intestinal Submucosa (BSIS-ECM) was set up according to as of late depicted shows (Freytes *et al.,* 2004). The bovine small intestinal was acquired from the abattoir in Babylon following slaughtering. Briefly, Then, the small intestinal was lowered in phosphate buffered saline (PBS) to be transported to the laboratory. The tissue tenderly flushed with PBS to dispose of the adhered blood, and afterward precisely rubbed to eliminate the tunica muscularisexterna and the majority of the tunica mucosa (Fig. 1A below). The rest of the tunica submucosa and basilar portion of the tunica mucosa was then disinfected and decellularized in a 0.1% peracetic acid (PAA), and 4% ethanol combination for two hours and cleaned with PBS and deionized water for 15 minutes. Decellularized BSIS, linked tissue matrices were put away at 4°C in PBS containing 1% Gentamycin, (Fig. 1B below).

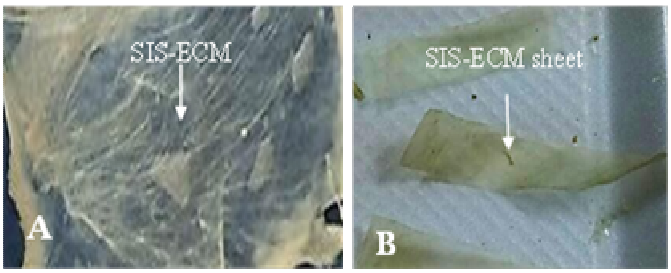

Fig. 1: Steps of BSIS-ECM, preparation
(A) Mechanical decisiveness of mucosal and seromuscular layers from the submucosa of bovine small intestinal
(B) Fabrication BSIS-ECM, sheet.

In vivo animal study

Surgical Protocol

Prior surgical procedures, the animals were first sedated with Diazepam, at a dose of 1mg/kg and a mixture of 5 mg/kg of xylazine hydrochloride, 35 mg/kg of ketamine hydrochloride, by intramuscular injection. The Achilles tendon complex was exposed by a midline skin and the fascia incision at (0.5cm) distal to the gastrocnemius muscle and (0.5cm)over the calcaneus (Fig. 2 below). A 1.5-cm portion long segmental resection was made in the mid substance of the Achilles tendon, including the medial and lateral gastrocnemius. In autografts group (n=10) the segment was inverted and re-implanted between the distal and proximal stumps (Fig. 2A below). In the scaffold group; the tendon defect was connected the stumps with BSIS-ECM, implanted constructs shaped to fit in the defects with a 4-0 polypropylene thread (Fig. 2B below). Byutilizing the modified Kessler technique that associated the stumps of the native tendon and the specific implanted graft BSIS. Then skin was sutured by interrupted horizontal mattress using (Silk No.1). At that point Plaster of Paris cast extending from the stifle joint to the end of the limb was applied. A window in the cast was made at the site of skin incision. A general clinical estimate was performed in all rabbits (body temperature, animal behavior, and cardiorespiratory activity). Inside the wounds, a daily macroscopic follow-up was completed during the entire time of experimentation.

Histopathological Evaluation

Biopsies were gathered on 30, 60 and 120 days postoperatively and immerged in 10% buffer formalin solution, for histopathological study. The formalin solution was changed after 72hr, dried out through graded alcohol series, cleared in xylene, embedded in paraffin, and sectioned longitudinally and transversally in (5-7) micron sections on a rotary microtome and staining with H&E (Luna, 1992). Examined semi-qualitatively according to Schon *et al.,* (2014) utilizing the specific parameters to observe the repair status of injured tendon site.

The Statistical Analysis

Adjectival statistical analysis was performed for each factor in this study. The Statistical Analysis System-SAS (2004); was used to impact on various factors (treatment and days) with in study parameters. The least significant difference (LSD) test was used to relative between percentages.

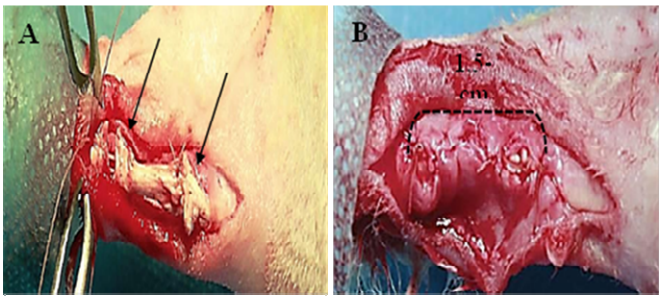

Fig. 2: Steps of surgical procedure. (A) The tendon segment was inverted and reimplanted between the distal and proximal tendon stumps (B) Tenorrahphy site is wrapped with SIS -ECM and fixed in position utilized Kessler pattern then sutured subcutaneous fascia and skin closure with interrupted horizontal mattress.

Results

Semi-quantitative Histopathological Examination

The histopathologicalareas of the autograft group on day 30 post-operative censured the high cellularity, sporadic collagen fibers with floods of fibroblasts, the moderate neovascular arrangement at the anastomotic site with mononuclear cells aggregation between the unpredictable collagen fibers (Fig.3A below). Tissues collected from the redesigning union in scaffold group, demonstrated that tendon fibers with marked proliferation tenocytes extending into injury site with moderate collagen fibers and mononuclear cells invasion (Fig.3B below). On 60 days post-operative; auto graft group demonstrated tendon fiber extending into the injury site infiltrated with marked mononuclear cells and irregular fibrous connective tissues (Fig. 4A below). While, scaffold group exhibited mature granulation tissue consisting of few congested and regular less cellular collagen fibers appended to tendon fibers (Fig. 4B below).Additionally, on 120thday's post-operative in the autograft group showed attachment between the newly formed tendon tissue with intact tissue by collagen fibers and dilated blood vessels with enlarged endothelial cell with sporadic barely any fibroblasts (Fig. 5A below). In contrast, scaffold group indicated new fibers with highly cellular identical to native tendon fibers connecting the anastomotic ends of the tendon and highly cellular new tendon fiber in contact with native tendon fibers (Fig. 5B below).

Histological investigation score were represented in (Fig. 6); at 30th days post-surgery exhibited that BSIS-ECM treated; which the granulation tissue with congested BVs were significantly increased ($P \leq 0.05$); graded (3), in examination with the autograft group which was graded (2-2.5), while the vascularization at this period was essentially diminished ($P \geq 0.05$), in BSIS-ECM group which was graded (0.5) and stay in this level till day 120th.While,autograft group were graded (1), the penetration of the inflammatory cell significantly increased ($P \leq 0.05$) as early as day 30 post-surgery procedure in BSIS-ECM group which graded(3), while in autograft group graded (2) and then decreased with time which graded (2.5), in SIS-ECM group and grade (1.5), in autograft group. Additionally, at days 60th and 120th, there were no significant differences ($P \geq 0.05$) between two groups. The collagen fiber deposition and direction were altogether expanded in BSIS-ECM group on day 30th which graded (2.5), while in autograft group was graded (2 and 1.5), and they totally oriented till day 120 post-surgery procedure which evaluated (1.5), in BSIS-ECM group.

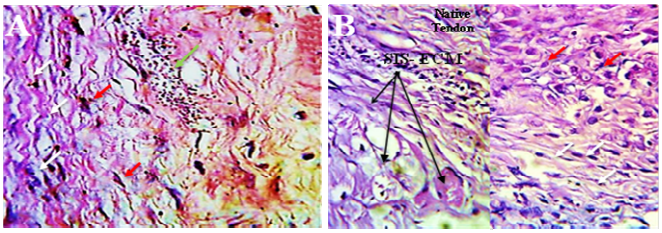

Fig. 3: Histopathological segment on day 30th post-operative "(A) Autograft group indicated the hemorrhage (green arrow) and an irregular wave of fibroblast (red arrows) and irregular collagen fibers in the injury site (white arrows) "(B) scaffold group shows tendon fibers with marked proliferation tenocytes extending into injury site (white arrows) with moderate collagen fibers and mononuclear cells infiltration surrounded by new host extracellular matrix (red arrows)" (H&E, × 400).

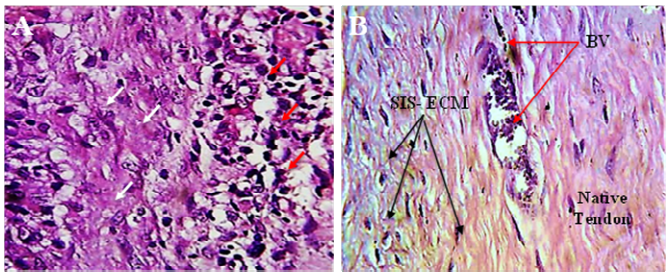

Fig. 4: Histopathological segment on day 60th post-operative "(A) Autograft group indicated tendon fiber extending into the injury site (white arrows) infiltrated with marked mononuclear cells and irregular fibrous connective tissues (red arrows) "(B) scaffold group shows mature granulation tissue consisting of few congested blood vessels and regular less cellular collagen fibers (black arrows) The graft was well integrated with the newly deposited host tissue (red arrows)" (H&E, × 400).

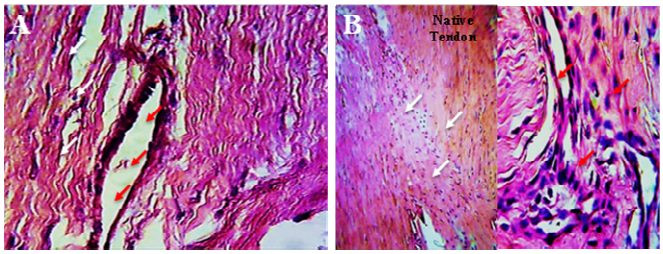

Fig. 5: Histopathological segment on day 120th post-operative "(A) Autograft group indicated attachment between the newly formed tendon tissue with intact tissue (white arrows) by collagen fibers and dilated blood vessels with enlarged endothelial cell (red arrows) "(B) scaffold group shows new tendon fibers similar to normal tendon fibers connecting the anastomotic ends of the tendon (white arrows) and highly cellular new tendon fiber in contact with normal tendon fibers (red arrows) "(H&E, × 400).

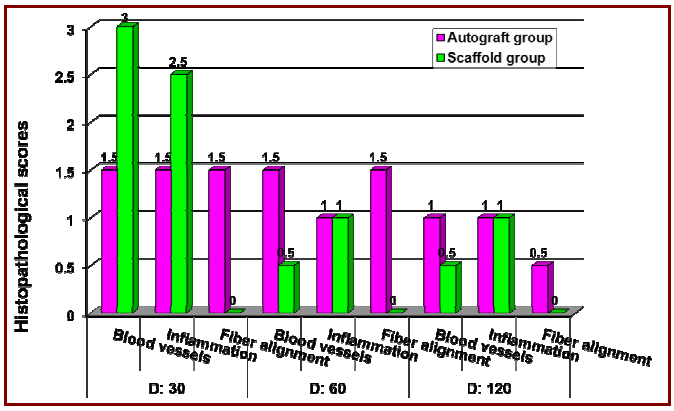

Fig. 6: The mean values of the histopathological analysis scores

Discussion

In the exploratory examination, demonstrated the (BSIS-ECM), when utilized as a biologic platform for Achilles reconstruction in this rabbit model; is quickly corruption after implantation, with around half of the mass debased and resorbed inside one month. Degradation of the (BSIS-ECM)gives off an impression of by three months this outcome are like to Record *et al.* (2001) who recommended the corruption rate after implantation; with around 60% when utilized (SIS-ECM), for reconstruction of the urinary bladder treated.

The current examination indicated no rejection, no infection and all tendons injuries were healed without any signs of complications. These perceptions were near these of Sarrafian *et al.* (2010) who contemplated the sheep model

augmentation of Achilles tendon utilizing the dermal patch as platform. Also, theywere shown absence of infection, inflammation or swelling at the site of surgical operation at the test time (24th weeks). Dohan *et al.* (2006) referred that composition of scaffold play role as pro-inflammatory cytokines and anti-inflammatory cytokines with angiogenesis growth factor (VEGF) and ability to manage the immunity and control the inflammation thus prevent infection at the site of the injury.

The decellularized embedded graft (BSIS-ECM), in to the deformity tendons as a biologic scaffold promote healing at early recuperating period in all animals. The bio-scaffold impact of on inflammatory response and maturation of healing the tendon was evaluation by histopathological. In scaffold group demonstrated less inflammatory response by the more modest number of both mononuclear cells and neutrophils at about fourteen days. Likewise, the arrangement of fibroblasts and collagen fibers in the (H&E) staining, which intently corresponds with development of maturation of tendon-healing, was more organized in the treated groups. These outcomes are like (Yuen *et al.,* 2001). The evidence of cellular penetration into the scaffold may demonstrate fibroblasts intrusion into the scaffold and incorporated the scaffold with the tendon and surrounding tissues before extreme crumbling which may limit fibrosis and tendon contracture (Sarrafian *et al.,* 2010).

The results of this assessment might be related to the effect of embedded BSIS-ECM which could be accepting a significant function in the overhaul and speeding up of tendon injury recuperating. This choice is in amicability with other numerous investigations, wherein the a cellular framework was utilized to fix tissue imperfection legitimately. In concentrate by Badylak *et al*. (2010) assumed that a cellular matrix could instigate explicit tissue recovery in *vivo*. Another study by Brown *et al*. (2010) assumed that embedded (ECM) exhibit tissue healing through promoted progenitor cell infiltration, adhesion and generation association with angiogenesis at the injuries site; just as, promotion of granulation tissue formation and deposition of host derived new matrix collagen content that result in tissue renovating with lessen scar tissue arrangement. The component activity of(BSIS-ECM) in elevate of tendon defect recuperating was pictorial by Massee *et al.* (2016) explained that the around '461 protein' biomolecules were recognized in ECM, with a critical number of development variables or cytokines that assume a part in physiological cycles embroiled in tissue rebuilding and homeostasis. These consolidate factors, (TGF-β1), (PDFG-AA), VEGF and 'angiogenin-4', which are known to be related with cell augmentation and angiogenesis (Sobolewski *et al*., 2005). In addition, Koob *et al.* (2014) demonstrated other recognized factors include inflammatory modulators and chemokines, just as proteases and inhibitors. Inflammatory modulators, such (IL-1β) and (MCP-1), are known to stimulate macrophages and recruit neutrophils to injury site. However, in study by Schultz and Wysocki, (2009) delighted the proteases and inhibitors recognized incorporate serine and cysteine proteases that can alter proteins to their active form, as well as matrix proteases and their inhibitors, for example, (MMP-1), MMP-3 and (TIMPs 1-2) that are significant controllers of ECM turnover during the redesigning stage, as reflected in the solvent protein analysis, (BSIS-ECM)is a biologically active tissue that contains numerous signaling factors capable of stimulating responses that occur during tissue recovery (Sato *et al*., 2000 and Serena *et al*., 2015). Also, Sheikh *et al*. (2014) delighted that comparable variables have additionally been recognized in placental-determined unites that has demonstrated clinical viability in the treatment of venous leg ulcers and unmanageable non-mending wounds. Consequently, it is acknowledged that placental-inferred unites can be incredible in advancing the conclusion of ceaseless injuries also.

In the present exploratory investigation dependent upon the clinical and histopathological perception during this assessment, it has been seen that (BSIS-ECM) was normally connected with tissue acknowledgment and no signs of immune rejection were detected despite the xenogeneic characteristic of the implant. This result might be related to the structure of the biomaterial implant which is formed mainly from acellular, non-immunogenic, resorbable collagen-based biomaterial. Numerous investigations examine the immunogenic reaction of the objective tissues after implantation of bio-inserts, in concentrate by Serena *et al*. (2015) showed that the implanted scaffold educe an immune lymphocytic reaction that is predominately T-helper lymphocyte-2-like which animates the production IL-4, IL-5, IL-6 and IL- 10, subsequently promote graft admission with prevent the activation of neighboring inflammatory macrophages cells.

Conclusion

(BSIS-ECM) as a novel and natural 3D scaffold that can be used for tissue planning and regenerative medication applications.

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