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Effects of a Cellular Camel Urinary Bladder Sub Mucosa on Chronic Full Thickness Skin Wound Healing in Equine Species

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ABSTRACT

This study is designed to assess the effectiveness of Camel sub mucosa which extracted from urinary bladder on healing of chronic cutaneous wound in Equine species. A 20 (5X4) cm of full- thickness wounds were induced in three donkeys, two wounds on each side of the adjacent pelvic region. Wounds were isolated into two equal groups (each 3wounds/ one group); treatment group included the wounds on the three side which were treated by covering the wound beds with strips of a cellular sterilized camel urinary bladder matrix. While the wounds on the left side were left without any treatment (control group). The results were evaluated clinically (along) and histopathologically on 20 days post-inducing of wounds. The clinical examination of treated wounds appeared that the wound healing process re-epithelization %, contraction% and total wound healing %were $P<0.05$ significantly than that of control wounds at 20 days of the study. The histopathological valuation confirmed that urinary bladder matrix treated wounds have improved cellularity, enhance vasculature, thick and large granulation tissue proposing improved cutaneous wound healing, comparison with untreated wounds. According to the clinical observation and histopathological results, this study concluded that a cellular camel urinary bladder matrix play an important role in stimulation of cutaneous wound healing of donkeys without signs of immune rejection.

Keywords: Skin, Chronic Cutaneous wounds, Urinary bladder matrix, Urinary bladder sub-mucosa.

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INTRODUCTION

The wound healing is a complicated process includes few overlapping stages that include inflammation, formation of granulation tissue, re- epithelialization, extra cellular matrix development and remodeling stage. The injuries of the skin due to trauma or disease, basically the chronic wound, considered a basic issue in healthcare field. Conventional wound care approaches attempt to control the unseen causes, such as ischemia and infection, while the use of wound dressings aims to alter a poorly wound healing environment into a micro-environment more closely approximating an acute wound allowing the body to recover the wound normally (1).

The goal of wound care is to encourage wound healing process in most brief time possible, with minimum pain, inconvenience and scarring to the patient and must be occur in a physiologic environment contributory to tissue repair and regeneration (2). The bio implant has been utilized to describe the process through which materials influence cell activity in a wound undergoing the reparation process. Xenografts (collagen-derived) are of significance particular as they may have a therapeutic effect on wounds, particularly those described by significant levels of chronic inflammation. A cellular biological tissue like; equine pericardium (EP), small intestine submucosa (SIS) or urinary bladder matrix) UBM have been proposed to be utilized as common biomaterials for a different tissue repair. Regular biomaterials are made out of extracellular matrix (ECM) proteins that are preserved and can be aid as scaffolds for cell attachment, migration and proliferation (3). The extracellular matrix (ECM) includes of a complicated mixture of functional and structural proteins and plays a significant function in tissue and organ morphogenesis, upkeep of cell and tissue structure and function, and in the host response to wound. Xenogeneic and allogeneic ECM has been utilized as abioscaffold for the recreation of. A wide variety of tissue types in both pre-clinical and human clinical examinations. normal highlights of extra cellular matrix that associated with tissue restoration implicate comprehensive angiogenesis enrollment of coursing ancestor cells, fast scaffold debasement and beneficial restoration of missing or damaged tissues. The extra cellular matrix that encouraged makeover reaction is a clearly not similar to the process of scar tissue arrangement (4).

The current research led to evaluate the effectiveness and fate of Equine a cellular UBM for the acceleration and reconstruction of skin defects in Equine species.

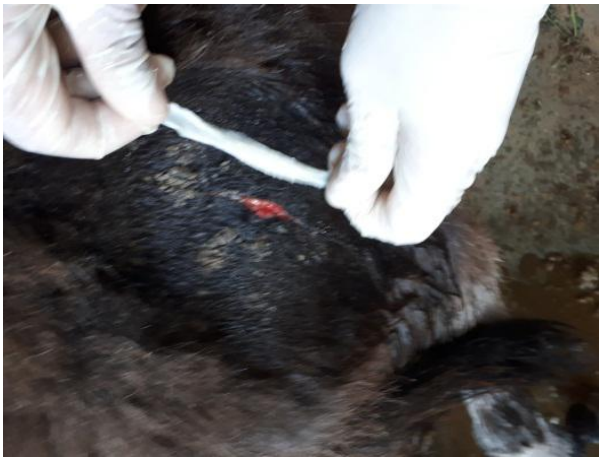
MATERIALS AND METHODS

The entire new urinary bladders were gathered from a butchered camel and UBM will be set up as a decellularized scaffold (5 and 6). The intraluminal water pressure was utilized to extend and stretch the bladder to encourage the expulsion of urinary bladder layers excepting the sub mucosa layer. The bladder was then dissected on one side from the opening to district framing a sheet. By using of a sharp knife, the tunica serosa, tunica muscular is and mucosal layers were removed by genital mechanical delamination, and finally prepared a flattened rectangular sheet. The residual tissue (sub mucosa layer) was then saturated in pH 7.4 phosphate buffered saline (PBS) containing streptomycin (100 ug/ml), penicillin 100 IU/ml. The risk of host rejection (immuno-rejection) of UBM was minimized by disruption of cellular and DNA materials. 0.1% per acetic acid (PAA) and 4% ethanol was used to treat the remaining tissue for two hours at

room temperature on a shaker. Remains of per acetic acid were cleared and the pH was restored to around 7.4 by washing the UBM at room temperature, with shaking, in PBS once, in water twice, and later in PBS once again. Each wash continued for 15 min. The resulting decellularized ECM scaffolds were terminally sterilized by immersion in 0.1% PAA solution titrated to pH 7.0 at room temperature for five hours and the disinfected and decellularized scaffold was maintain in sterile PBS containing antibiotics and antifungal drugs and preserved at 4 ºC (6). Three healthy local breed adult male donkey were used. After injected the animals with broad spectrum antibiotic, and deeply sedated with intramuscular injection of 2% xylazine hydrochloride at a dose of 0.2 mg/kg, B.W., the lateral pelvic sides were prepared aseptically for the creation of 2X10 cm four square full-thickness skin wounds under local anesthesia with 2% lidocaine hydrochloride, two wounds on each side, one right side and one left side. The wound left without treatment or disinfectant until it became infected. Enough quantity of sterilized a cellular UBM strips were covered the wound beds of the treatment group by one stich in proximal and distal of wound then wounds closed by non-absorbable silk suture of horizontal matters technique (Fig. 1), while the wounds of control group were left without treatment and closed by same technique. Post-operatively, all wounds were and bandaged which had been changed three times a week and wounds have been gently cleaned. The wound healing processes in treatment and control group were evaluated clinically, morphometric ally and histopathologically for four weeks study, as follows.

Evaluation: All animals were subjected to whole clinical examination every three days along the period of the study. To enhance visualization of the area of epithelialization and granulation tissue, the scab of each wound was removed carefully using saline. level of epithelialization, wound reduction and total wound healing (total reduction in open wound area from contraction and epithelialization) were calculated for each wound, depending on the parameters (7). The histopathological examination was performed on 21 post-creation of wounds for each treatment and control group. A full-thickness incisional biopsies were obtained 8-10 mm in width, and they included approximately 5-6 mm of unwounded skin on both side of the wound, and fixed in 10% neutral formalin solution, embedded in paraffin, sectioned in 5 micron sections on a rotary microtome and staining with hematoxylin-eosin stain (8).The Statistical Analysis System SAS (9), was performed to influence on different elements (treatment and days) in study parameters (percentage). The least significant difference (LSD) test was used to compare between percentage.

Figure, 1: The covering of wound of treatment group with strips of UBM



RESULTS AND DISCUSSION

Generally, the wounds of control and treatment group decreased in size along the study, but the inspection of wounds images exposed that the rate of wound closure in UBM treated wounds were significantly (P<0.05) more along the period of the study as compared to untreated wounds. The enhancement appeared in the UBM treated wounds on day 7th post-wounding and continuous until the end of the study.

Depending on the data in (Table 1), clinical morphometric investigation of wound healing process along 21 days of the study showed that wound re-epithelization percentages were increased in treatment group faster than control group, 75.61± 2.68in treatment group and 55.44± 1.59 in control group, on day 21 post-wounded, with a significant difference (P<0.05).The contraction percentages was noticed on day 7th post-wounding in both control and treatment group with no difference between them. After that these percentages were started to increase with significant difference (P<0.05) until the end of the study 65.43± 2.53in treatment group and 40.82± 1.64 in control group. While the percentages of total wound healing were 77.33± 2.70in the treatment group and 65.56± 1.95in the control group, with a significant difference (P<0.05).

Table, 1: The % of wound epithelization, contraction and total wound healing (%) in both treatment and control wound, equine species (M±S.E)

LSD Value	Control wounds	Treatment wounds	Days	Parameters

10.133 **7.343*** **7.551***	**19.35± 0** **30.67± 0.73** **55.44± 1.59**	**28.22± 0.52** **58.45± 1.25** **75.61± 2.68**	**7** **14** **21**	**Epithelization**
8.232 **7.788*** **6.790***	**15.87± 0.63** **35.65± 1.56** **40.82± 1.64**	**22.60 ± 0.81** **48.70± 1.68** **65.43± 2.53**	**7** **14** **21**	**Wound Contraction**
6.212 **5.888*** **4.890***	**20.34± 0.47** **45.23± 1.73** **65.56± 1.95**	**25.69± 0.64** **54.22± 1.84** **77.33± 2.70**	**7** **14** **21**	**Total Wound Healing**

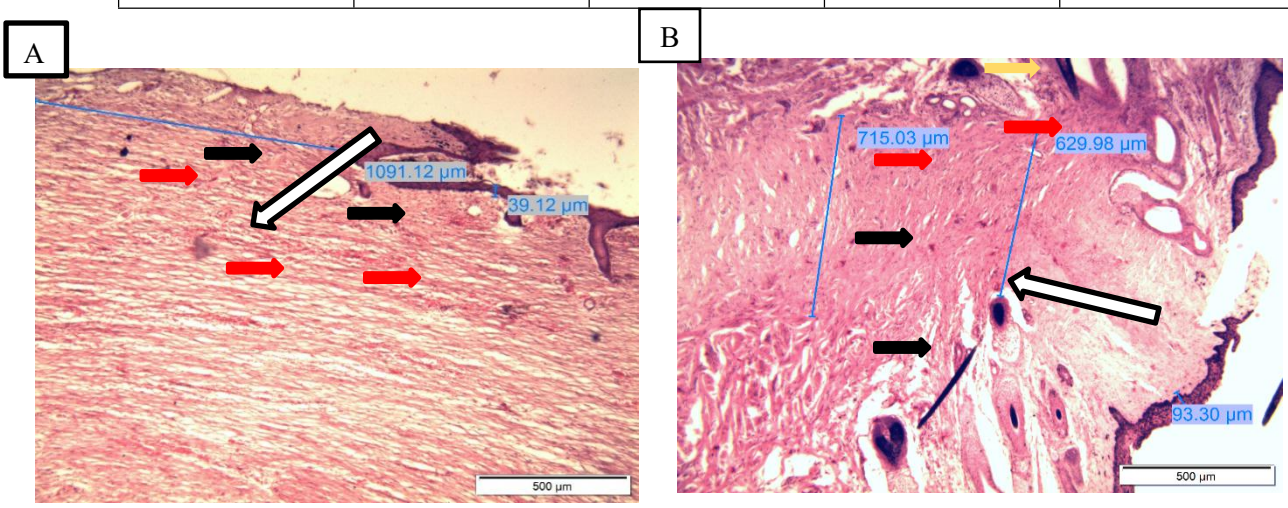


Figure 1: Photomicrograph of skin of control and treated donkeys.

A/ Skin of control donkey. The wound borders were not close, where the keratinized epithelial layer not totally attached. In addition, the width of fibrous scare tissue (white arrow) was measured as 1 mm with loss of fibrous tissue integrity, where certain spaces (red arrows) were observed. Absence of blood vessels in dermis layer with presence of hemorrhage (black arrows) within the collagen fibers was observe. **B/ Skin of treated donkey.** The wound borders were closed, where the keratinized epithelial layer was attached. In addition, the width of fibrous scare tissue (white arrow) was measured as 0.6 mm, and the fibrous tissue was appeared integrated and compact compared with control. The blood vessels (red arrows), hair follicles (black arrows) and sweat glands (yellow arrow) were observed in dermis layer of skin. H&E. A&B: X40.

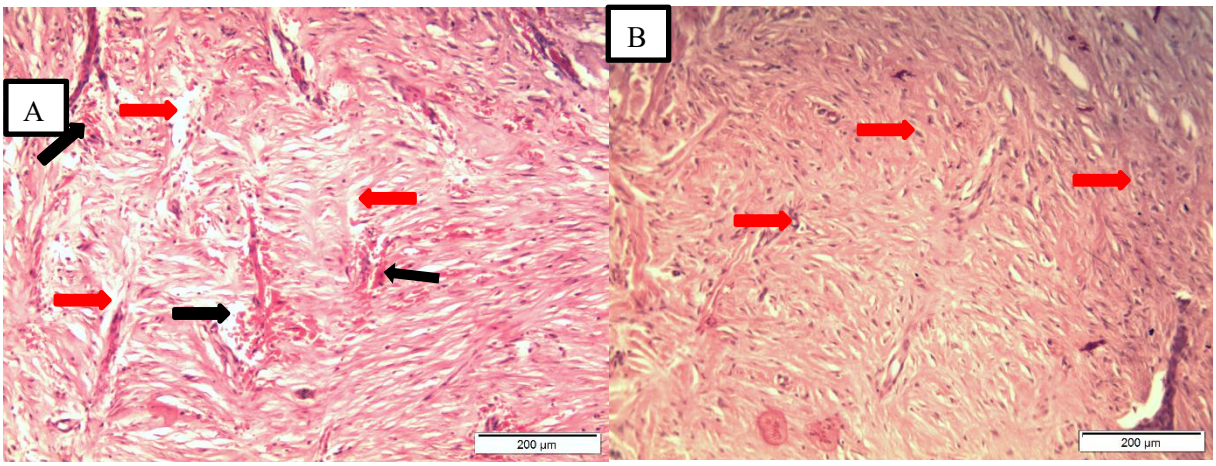


Figure 2: Photomicrograph of skin of control and treated donkey.

A/ Dermis layer of control donkey. Several spaces (red arrows) were observed within fibrous tissue led to loss of fibrous tissue integrity. Hemorrhage (black arrows) within fibrous tissue was observed. **B/ Dermis layer of treated donkey.** The fibrous tissue of dermis layer appeared integrated, compact and well orientated with presence of several fibroblast cells (red arrows) compared with control. H&E. A&B: X100

In addition of that the histological inspection appeared in control group wide scar tissue, weak proliferation of

epithelial cell and stratum basal of epithelium, high hemorrhage with profuse fibrosis , scattered inflammatory cell, mainly macrophage and formation of new B.V and there is absence of hair follicular and sweat gland. While the histological inspection revealed very narrow scar tissue and proliferation of both edge of epidermis due to proliferation of epithelial cell and stratum basal of epidermis, complete healing which characterized by profuse collagen, fibrosis and new B.V also there is formation of small hair follicular, very few hemorrhage also present

Along the period of the study, no signs of immune rejection were detected in all sections of treated wounds (no accumulation of inflammatory cells or immune cells (lymphocytes) at the site of implantation, no foreign giant cells and no fibrous encapsulation. In this study, a xenogeneic, collagen rich membrane scaffold derived from the bovine urinary bladder sub-mucosa has been used to evaluate the effectiveness of UBM on skin wounds healing. The clinical inspection wound healing along the study showed rapid significant decreasing in wound size with a minimum scar tissue formation in treated wounds compared to untreated once. While, the histopathological evaluation of treated wound sections appeared a high incidence of mature granulation tissue, myofibroblasts and new B.Vs, at the same time, few myofibroblasts were scattered through fibrous connective tissue containing congested B.Vs were notice in the sections of control wounds. The results of this study might be related to the effect of implanted UBM which could be play an important role in the enhancement and acceleration of cutaneous wound healing. This conclusion is in a harmony with other many studies, in which a cellular matrix was used to repair tissue defect directly. They have shown that a cellular matrix (in different forms) could induce specific tissue regeneration *in vivo*. They have reported that implanted ECM proved tissue healing through promote progenitor cell infiltration, adhesion and proliferation association with acceleration of angiogenesis at the wound site, as well as, enhancing of granulation tissue formation and deposition of host derived neomatrix (collagen contents) that results in tissue remodeling with minimal scar tissue formation (4 and 11).

The mechanism action of UBM in promote of wound healing was described (13) who explained that the positive effects of these bio implant could be obtained either directly by ECM molecules or indirectly by their bioactive signal molecules within the UBM; like growth factors, cytokines, chemokines and hormones.

(16) showed that ECM shows an attractive property towards circulating bone marrow-derived cells and they will remain in the remodeled tissue. It confirmed that ECM also helps in the stem cells differentiation and maintain the phenotype of the differentiated cell line in a tissue specific manner. As a result, these events or reactions have an important role in determining the eventual clinical outcome. Allogeneic and xenogeneic grafts are limited by the risk of immune rejection or infection diseases in comparison to autologous once (17). Depending on the clinical or

histopathological observation during this study, it has been noticed that UBM was

typically associated with tissue acceptance and no signs of immune rejection were detected despite the xenogeneic characteristic of the implant. This result might be related to the composition of the implant which formed mainly from a cellular, non-immunogenic resorbable collagen-based biomaterial. Previous studies discuss the immunogenic response of the target tissues after implantation of bio-implants and indicated that the implanted scaffold (18 and 19).

The absence of the infections during this study could be due to good and suitable pre

and post-operative care and may be connected to the characteristic of UBM to resistance of micro-organisms, as mentioned by many preclinical and clinical studies which explained that ECM scaffolds shown resistance towards deliberate and spontaneous bacterial contamination (20 and 21).

In conclusion, the qualified healing process of treated wounds comparison to untreated wounds confirmed that using of camel a cellular UBM promoted and enhanced skin wounds healing

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