Evaluation of the Effects of Biomaterial Scaffold for Healing Cutaneous Chronic Wounds in Dog Model
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ABSTRACT

Therapeutic assessment of the biomaterial scaffold utilized as a surgical adjunct to keep up the inflammatory process and to potentiate tissue healing, make the subject of ongoing research in regenerative medicine. This study was intended to assess the healing activity of effect of lyophilized bovine urinary bladder sub mucosa (BUBM) by topical application on the skin experimentally in chronic full-thickness cutaneous wounds in a dog model. For this reason, this investigation was conducted on twenty adult and clinically healthy males’ dogs, full-thickness square skin wounds (4x4 cm) and 10 cm apart were made on the back of each animal. After surgical creation these injuries were confronted every daily surgical scratching to interrupt healing process continuation to prolong inflammatory reaction to form chronic wound. The latter surgical procedure was continued for eight weeks to make certain of their change to chronic wounds. The animals were arbitrarily divided into two equal groups of ten dogs for each. In control group, the cranial injuries were left without treatment. While in scaffold group, and the caudal injuries were treated by implantation 0.08 mg (BUBM) powder. The clinical assessment of treated wounds demonstrated that the injury healing process contraction%, Re-epithilization % and total wound healing % were significantly P<0.05 than that of control wounds at forty five days of the investigation. The histopathological studying on seventh, fourteen, 28 and 45 days post-treatment demonstrated that treated wounds have reduces inflammation during 3 first days post-implantation and promotes epithelialization in 3 weeks of healing withincreased vasculature than those in untreated wounds. This study concluded the continuous mechanical irritation of wounds site may lead to form chronicity state for the effected wounds and topical administration lyophilized bovine urinary bladder sub mucosa (BUBM) enables us to increment and improve the therapeutic approach to the chronic cutaneous wound.

Keywords: lyophilized bovine urinary bladder sub mucosa powder, cutaneous wounds, dogs, chronic wounds healing

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INTRODUCTION

Broad skin loss, if whether secondary to trauma, congenital defects, infection or neoplastic resections, can present considerable challenges for surgical reconstruction[1]. Numerous techniques have been created to
achieve tension-free wound closure; including a variety of local and distant mobilization and reconstructive procedures. Size and location of the deformity and adjacent crucial structures, however, may constrain these strategies. Andreadis,[2] suggested that autografts, allo grafts or xenografts are interchange strategies for temporary or permanent closure of huge skin defects, but these can be correlating with important complications, such as donor site morbidity, rejection, infection, or transmission of infectious agents.

Predominating, chronic wounds stay in the inflammation phase of healing [3]. A wound is portrayed as chronic if standard medical procedures do not prompt to the expected healing, or if the wound does not heal within 6 weeks, which can be brought about autoimmune disorders, vascular diseases, infectious diseases, metabolic and genetic diseases, external factors and neoplasm [4]. Metcalfe and Ferguson[5] recommended that biological wound dressings aim to get incorporated in the wound bed, giving a scaffold that promotes adhesion and migration of fibroblasts and keratinocytes. Marks et al.,[6] demonstrated that in vitro and in vivo investigations have sought to develop an implantable wound dressing that can ideally integrate into the wound while optimizing dermal and epidermal restoration also the growth factors and cytokines retained in these dressings are also thought to enhance healing.

There are numerous experimental reports on the application of ECM biomaterial in the repair and reconstitution of many soft tissue structures with variable success, comprise the laryngeal cartilage [7], abdominal wall [8], esophagus [9], diaphragm[10] and lower urinary tract [4]. However, McPherson and Badylak, [11] showed that porcine small intestinal sub mucosa, porcine urinary bladder sub mucosa are decellularized matrices consisting of a complex array of collagens, glycosaminoglycans, proteoglycans and glycoproteins. In addition, Hodde et al.[12] answered that porcine urinary bladder sub mucosa contain active growth factors, such as TGFβ, FGF-2, and VEGF, making these materials bioactive builds that are able to promote tissue remodeling with initial investigation also demonstrated a possible antibacterial effect. Be that as it may, very few reports have thoroughly studied its application in clinical veterinary medicine with chronic wound healing. The purpose of this investigation was to assess effectiveness of lyophilized (BUBM) on healing of induced open chronic wound healing in dogs.

MATERIAL AND METHOD

Experimental Animals Design

Animal testing was conducted in accordance with the ethical standards of the Animal Experimentation Ethics Committee of the College of Veterinary Medicine, Al- Qasim Green University, along the period of the study from 4 March to 10 April of 2019. Twenty apparently healthy local breed dogs, weighing between 15 and 20 kg, aged 1–3 years were acquired. Physical assessment, complete blood counts were performed on each dog before study entry.

Preparation scaffold: The fresh urinary bladders were gathered from the sacrificed animals immediately after slaughter. To de-cellularized UBM-ECM tissue has done according to [13]. The de-cellularized BUBM sheets allowed to set slightly before being transferred to −20°C for 24 hours then transferred to the deepfreezer at −80°C for 5 days and the scaffolds was later freeze dried in a freeze drier at −56°C under 5 mm Hg in a lyophilizer (FTS Systems Bulk Freeze Dryer Model 8–54) for lyophilization till it is
completely dried for 4 days at -50 °C. The powder has been sterilized by 60°C in oven at 16 hours, later kept in a sterile container before use.

**Utilization of BUBM powder in deep full-thickness chronic wounds:** Our study utilized in eighty chronic wounds, (4X4) centimeters of square full-thickness cutaneous wounds were created in twenty dogs. Under the impact premedicated with atropine sulfate in dose rate of 0.03 mg/kg, then following 10 minutes the dog was anaesthetized by a mixture of ketaminexylazine in dose rate of 15mg /kg and 5mg/kg B.W intra-muscular respectively [14]. Full-thickness skin defects were created bilaterally on the trunk, each dog two wounds were made on one side of the back, the distance between the cranial and the caudal, the wounds were daily scratched and eroded to remove newly formed cells to prevent new tissues formation in wound bed and transform it to chronic wound, this surgical procedure was repeated for eight weeks to ensure chronicity state, with weekly measurement of wounds dimensions. These wounds were allocated, depending on the method of the treatment, into two groups; the cranial injuries were left without treatment, as a control group. The caudal injuries were treated by implantation 0.08 mg bovine urinary bladder sub mucosapowder as scaffold group.

**Healing follow-up:** A general clinical assessment was performed in all animals (animal behavior, cardiorespiratory activity and body temperature). Digital photographs of the wounds were obtained at day 0, 3, 7, 14, 28 and 45. A standardized ruler was included in each photograph for digital calibration of the photographs according to [15]. Gross evaluation of wound healing was performed based on the percent of wound contraction (WC %), area of epithelialization (AE) (cm²), and wound area (WA) (cm²). Wound Contraction was calculated using the formula: 

\[ \text{WC} \% = \frac{W_0 - W_t}{W_0} \times 100 \]

W₀ = the initial wound measurement; W₁ = the wound measurement on day of measurement.

**Biopsy Sampling:** The perception was performed at seventh, fourteenth, 28th, and 45th post-treatment with UBM and the same period was depended for the control group. A full-thickness incisional biopsy specimens were gotten (5-6 mm) in width and they included roughly (3-4 mm) of unwounded skin on two sides of the injury which were fixed in (10%) neutral formalin solution, and then embedded in paraffin which were trailed by sectioned in (5-7) micron on a rotational microtome and staining with Haemotoxylin-eosin stains (Luna, 1992).

**Statistical Analysis:** The Statistical Analysis System-SAS [17] was utilized to impact on various factors (treatment and days) in study parameters (percentage). The least significant difference (LSD) test was utilized to comparative between percentages in this examination.

**Results:**

**Chronic Wound Geometric Analysis:** all created wounds of treatment and control group diminished rapidly in size along the examination, yet the close inspection of wounds images indicated that the rate of wound closure in UBM treated wounds were significantly \((P \leq 0.05)\) more along the period of the study as compared to untreated injuries. Depending on the information in (Tab.1 below), the mean ± SD of total wound area for scaffold group in day 7 was recorded (27.74± 3.65) cm²; and Control group (13.19± 3.21) cm². There were
some significant differences between groups. However, total wound area in day 45 in scaffold group was significantly higher than in Control group ($P<0.05$) (92.75 ±4.07), (76.86 ±2.89) respectively. The mean ± SD of epithelialized area of each group in day 7 was as follows: in scaffold and control group recorded (32.98± 4.26)(19.11± 2.23) cm$^2$ respectively. There was significant ($P\leq0.05$) difference between groups. On day 45 there was significant ($P<0.05$) difference in scaffold compared than control group (96.38±2.76)(82.62± 0.07) cm$^2$ respectively (Tab.2 below). The mean ± SD of wound contraction percentage of each group in day 45 was as follows: in scaffold and control group recorded (92.20 ±3.83)(72.15 ±2.76) cm$^2$ respectively. Significant differences were determined between scaffold and control group ($P\leq0.05$) and showed better wound contraction compared with group Control.

**Tab. 1:** Shows the Rates of Total Wound Healing (WH %), (cm$^2$) in Scaffold and Control

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>Control Group</th>
<th>Scaffold Group</th>
<th>LSD   Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th</td>
<td>0.00 ± 00 a</td>
<td>0.00 ± 00 a</td>
<td>0.00 NS</td>
</tr>
<tr>
<td></td>
<td>3th</td>
<td>0.00 ± 00 a</td>
<td>0.00 ± 00 a</td>
<td>0.00 NS</td>
</tr>
<tr>
<td></td>
<td>7th</td>
<td>BC 13.19± 3.21b</td>
<td>B 27.74± 3.65a</td>
<td>7.103 *</td>
</tr>
<tr>
<td></td>
<td>14th</td>
<td>AB 29.84± 3.20 b</td>
<td>A 43.16± 5.55a</td>
<td>10.753 *</td>
</tr>
<tr>
<td></td>
<td>28th</td>
<td>A 38.86±2.13 b</td>
<td>A 58.48±4.21 a</td>
<td>8.036 *</td>
</tr>
<tr>
<td></td>
<td>45th</td>
<td>A 76.86±2.89 b</td>
<td>A 92.75±4.07 a</td>
<td>6.312 *</td>
</tr>
</tbody>
</table>

Values (Mean ± SD) having with the different small letters in same row and big letters in same column differed significantly at $p \leq 0.05$.

**Tab. 2:** Shows the Rates of Wound Epithilization (AE %), (cm$^2$) in Scaffold and Control

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>Control Group</th>
<th>Scaffold Group</th>
<th>LSD   Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th</td>
<td>0.00 ± 00a</td>
<td>0.00 ± 00a</td>
<td>0.00 NS</td>
</tr>
<tr>
<td></td>
<td>3th</td>
<td>0.00± 00a</td>
<td>0.00 ± 00a</td>
<td>0.00 NS</td>
</tr>
<tr>
<td></td>
<td>7th</td>
<td>BC 19.11± 2.23b</td>
<td>B 32.98±4.26a</td>
<td>8.107 *</td>
</tr>
<tr>
<td></td>
<td>14th</td>
<td>AB 32.84± 3.20 b</td>
<td>A 65.16±3.56a</td>
<td>8.316 *</td>
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<tr>
<td></td>
<td>28th</td>
<td>A 59.84±3.13b</td>
<td>A 85.25±3.13a</td>
<td>8.006 *</td>
</tr>
<tr>
<td></td>
<td>45th</td>
<td>A 82.62±0.07 b</td>
<td>A 96.38±2.76 a</td>
<td>8.123 *</td>
</tr>
</tbody>
</table>

Values (Mean ± SD) having with the different small letters in same row and big letters in same column differed significantly at $p \leq 0.05$. 

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differed significantly at p ≤0.05.

**Tab. 3: Shows the Rates of Wound Contraction (WC %), (cm²), in Scaffold and Control**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>Control Group</th>
<th>Scaffold Group</th>
<th>LSD Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th</td>
<td>0.00 ± 0.0a</td>
<td>0.0 ± 0.0a</td>
<td>0.00 NS</td>
</tr>
<tr>
<td></td>
<td>3th</td>
<td>0.00 ± 0.0a</td>
<td>0.0 ± 0.0a</td>
<td>0.00 NS</td>
</tr>
<tr>
<td></td>
<td>7th</td>
<td>BC 16.00 ± 3.21b</td>
<td>B 27.00 ± 3.11a</td>
<td>7.209 *</td>
</tr>
<tr>
<td></td>
<td>14th</td>
<td>AB 35.28 ± 3.27b</td>
<td>A 55.31 ± 3.72a</td>
<td>8.822 *</td>
</tr>
<tr>
<td></td>
<td>28th</td>
<td>A 43.57 ± 3.13b</td>
<td>A 78.03 ± 3.21a</td>
<td>8.011 *</td>
</tr>
<tr>
<td></td>
<td>45th</td>
<td>A 72.15 ± 2.76b</td>
<td>A 92.20 ± 3.83a</td>
<td>7.218 *</td>
</tr>
</tbody>
</table>

a, b, c Values (Mean ± SD) having with the different small letters in same row and big letters in same column differed significantly at p ≤0.05.

**Semi-quantitative histopathological evaluation,** all processing illustrated in Figs. (1 to 6) at day 7, the most cases received UBM scaffold implantation demonstrated immature granulation tissue described by dense less cellular regular collagen fibers, at day 14 post treatment indicated formation of thickened layer of epithelial cells extended over granulation tissue and under cellular debris and which was infiltrated by few mononuclear cells, likewise demonstrated dense thick mature granulation tissue and on the special stain appeared stained collagen fibers in the wound site. However, on day 28 post treatments, the histological section indicated develop of epidermal layer over mature granulation tissue and in the other section were indicated improvement of epidermal layer with rete ridge over mature granulation tissue. Moreover, on 45 days post treatment showed up the presence of thickened epidermal layer over dense collagen fibers and appeared dense collagen fibers.

While, in control group showed, neutrophils and mononuclear cells invasion in the immature granulation tissue in the injury site. On 14 days post-operatives indicated the presence of immature granulation tissues with moderate mononuclear cells aggregation around blood vessels; also other section indicated immature granulation tissue in the wound with moderate mononuclear cells aggregation around blood vessels. On 28 days post-operative demonstrated development of thin layer of epithelial cells extended over immature granulation tissue and under the cellular debris and which characterized by irregular cellular collagen fibers with numerous blood vessels as well as mononuclear cells infiltration and demonstrated mononuclear cells aggregation around blood vessels in the dermal layer. Moreover, on 45 days post-operatives was showed up the presence of dense collagen fibers in the injury site with mononuclear cells aggregation around B.Vs, in the dermal layer. Other section indicated vascular granulation connective tissue consisting from congested blood vessels with collagen fibers.
**Fig 1:** Histopathological segment of control group, at 7 days PO, indicated neutrophils and mononuclear cells infiltration in the immature granulation tissue (red arrow) in the wound (H&E stain 400X).

**Fig 2:** Histopathological segment of control group, at 14 days PO, indicated immature granulation with moderate mononuclear cells aggregation (yellow arrow) around blood vessels (H&E stain 400X).

**Fig 3:** Histopathological segment of control group, at 28 days PO, indicated thin layer of epithelial cells extended under cellular debris (red arrow) and over immature granulation tissue (H&E stain 100X).
Fig 4: Histopathological segment of control group, at 45 days PO, indicated mononuclear cells aggregation BV in the dermal layer (red arrow) (H&E stain 400X).

Fig 5: Histopathological segment of treated wounds, at 7 days PO, indicated granulation tissue in the dermis with complete epidermal layer (black arrow) (H&E stain 100X)

Fig 6: Histopathological segment of treated wounds, at 45 days PO, indicated thickened epidermal layer with dark nuclei of basal epithelial cells (yellow arrow) over mature granulation CT. (H&E stain 400X).

Discussion

The treatment of large chronic skin wounds can be extremely challenging, also healing by second intention may take several months, as occurred with the large chronic wounds created in this study by...
continuously irritated mechanically and left for two months to guarantee their chronicity. This reality confers with other searchers who talked about the time of the possibility of instigating chronicity stage in wound, according to Gurgen, [18] who alluded that the wound healing is a complex process which regulated by interactions between a large number of cell types, extracellular matrix proteins and mediators such as cytokines and growth factors. In addition, lack of balance between these interactions may result in a chronic wound. In our investigation by Fonder et al., [19] showed that a chronic wound takes more than six weeks in repair process to reflect chronic state of healing. While, Izadi and Ganchi [20] revealed that the wounds which fail to progress through a normal sequence of repair in 4 to about two months, are generally presumed to be chronic. However, tentatively instigated wounds that stopover with inflammatory reaction indicate chronicity in this period [21].

In current investigation, the clinical follow up in this period indications typical signs of chronic wounds, without showed signs of infections or systemic reactions and these results were related to the aseptic technique and healthy housing of animals, but local symptoms related to normal tissues reacted toward daily surgical irritation of wounds sites this result agree with [22]. The consequence of study, a xenogeneic, collagen rich membrane scaffold derived from the BUBM has been utilized to assess the effectiveness of UBM on skin wounds healing. The clinical investigation of wound healing along the examination demonstrated rapid significant decreasing in wound size with a minimum scar tissue formation in treated wounds compared to untreated once. These results are close to the results obtained by Kumar et al. [23] who used bovine collagen sheets with fibroblasts in the treatment of full-thickness cutaneous wounds in the rat. They investigated the presence of improvement in wounds healing potential compared to the standard dressing materials. Also, Shuklaet al. [24] studied the effects of acellular dermis as a dermal matrix on the healing of burns. They noticed that the using of ADM for treatment of deep burns played an important role in the acceleration of the healing of this type of wounds with minimum scar formation. However, the histopathological assessment in the sections in the present study at about two months post inducing open skin wound appeared the presence hemorrhage on the epidermis and irregular few cellular collagen fiber and mononuclear cells around blood vessels. This form expounds the chronicity state of the wounds in this group and revealed to presence of inflammatory reaction in wounded area. This outcome is in concurred with the investigation of Brown and Badylak, [25], [26].

Our outcome the histopathological assessment of treated wound sections seemed a high incidence of mature granulation tissue, myofibroblasts and new blood vessels, at the same time with few myofibroblasts were scattered through fibrous connective tissue containing congested blood vessels were notice in the sections of control wounds. The results of this investigation might be related to the effect of implanted UBM which could be played an important role in the enhancement and acceleration of cutaneous wound healing. This determination is in a harmony with other many studies, in which the acellular matrix was used to repair tissue defect directly. They have indicated that acellular matrix could induce specific tissue regeneration in vivo. In study by Joao et al., [27] detailed 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 neomatrixcollagen contents that outcomes in tissue remodeling with minimal scar tissue formation. Our study by Biellia et al., [28] showed that in tissue engineering and regenerative medicine applications, the cells that
participate in the processes of tissue reconstruction require 'instruction' for proliferation morphogenesis and differentiation. The sources of this 'instruction' are the cellular microenvironment and the scaffold or matrix surfaces with which these cells interact.

In general, the mechanism action of UBM in promote of wound healing was depicted by Brown and Badylak, [25] who referred 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; such as growth factors, cytokines, chemokines and hormones. However, in vivo studies, Clark et al., [29] showed that during the biodegradation process of ECM components, a peptides molecules like collagen, elastin, fibronectin and laminin will be released and participate in mellowprotease matrix metalloprotease (MMP) expression, cellular activity modulation, growth factor signaling and tumor vascularization and angiogenesis; therefore, they help in the recruitment of cells to the remodelling site, and help in the tissue specific differentiation. A similar notice by Frantz et al., [30] indicated that ECM shows an attractive property towards circulating bone marrow-derived cells and they will remain in the remodelled 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.

In outcome, 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 xenogenic characteristic of the implant. This result might be related to the composition of the implant which formed mainly from acellular, non-immunogenic, resorbable collagen-based biomaterial. In addition, Gilbert et al., [31] demonstrated that the implanted scaffold Small Intestine Submucosa (SIS) elicits an immune lymphocytic response that is predominately T-helper lymphocyte-2-like which stimulates the production of interleukins (IL-4, IL-5, IL-6, IL-10), and as a result promote graft acceptance and prevent the activation of neighboring inflammatory macrophages. However, 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 [32]. In study by Badylak and Gilbert, [33] who alluded that after the transplantation of scaffold into body, it will begin degradation and during the biodegradation process, small peptides (5 kDa to 16 kDa) will be released from the fibers of the scaffolds like collagen, these molecules mimic some peptides that inhibit the growth of gram positive and gram negative bacteria in vitro.

In conclusion, wound healing is a complex process, resulting from the interaction between a large number of cell types, extracellular matrix proteins, and mediators such as cytokines and growth factors. Contingent upon assessment, the UBM powder techniques enhancement healing of chronic wounds without adverse effect or complications. Also use topical of UBM as lyophilized powder allows us to increase and improve the therapeutic approach to the chronic cutaneous wound.

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References