

COMPARISON BETWEEN THE EFFECT OF MEBO AND ENDOFORM PATCHES TO REPAIR INDUCED SKIN WOUNDS IN DOGS

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ABSTRACT

This study aimed to compare the ability of mebo and endoform patches for restoring full-thickness cutaneous wounds in dogs. Twenty-seven local strain mature dogs were used. For all experimental animals, a 2x2 cm of square full-thickness skin defects were carried out on the lateral side of the radial area. Then, these animals were divided into three identical groups. In first group (control), these induced defects were left open to heal spontaneously without any kind of remedy, while for the 2nd and 3rd groups, mebo and endoform patches of the same defect dimensions were placed over the wounds and bandaged firmly. The evaluation of the outcome data was done at 7th, 14th and 21th days postoperatively according to the gross and histopathological examinations. The gross results exhibited that the start and progress of the healing process was much faster and better in both mebo and endoform groups since first week, compared to the control group, however, Endoform animals were even better. Furthermore, there was a gradual reduction in wound size of both treated groups, especially of Endoform, compared to the control group. Furthermore, the histopathological evaluation displayed extra and more mature granulation tissue formation with little or tiny inflammatory cell infiltration, good angiogenesis, re-epithelization and development of hair follicle and other skin appendage in both treated groups, compared to the control group. In conclusion, similarly mebo and endoform patches can be considered for hastening perfect healing of cutaneous full-thickness wounds in dogs, even though endoform is regraded the first top choice.

Keywords: Cutaneous Wounds, Mebo Patch, Endoform, Healing Process, Extracellular Matrix.

INTRODUCTION

Animal body consists of tremendous variety of cells, tissues and organs. Some of them are tiny while others, like skin, have the largest percentage to enable performing several vital tasks as being the first defense castle against any toxic, infectious, and harmful invaders and

factors, besides other related duties including thermoregulatory, electrolytes and water controller (Dabrowska *et al.*, 2018). Skin has several layers including epidermis, dermis, and subcutaneous tissue. All these tissues have extracellular matrix (ECM) and different cell populations (Ingegneri & Romanelli, 2020). Being the biggest and the upper most position is not always the most desirable choice especially for the skin as it makes it being the first shield facing any traumatic injuries particularly hug extensive full-thickness

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wounds which are still have impacts in both man and small animal veterinary therapies owing to their highly daily recurrence, prolonged healing time, need for excessive care and being more susceptible for various complications (Davidson, 2015; Cavanaugh, 2024). On the other hands, full-thickness skin injuries are still being the most driving factor for the innovation of new wound therapeutic alternatives that stimulate better and swifter healing process (Abd-El Azeem *et al.*, 2022; Al-Sabaawy & Al-Hyani 2022; Allawi & Saeed, 2023) either through mimicking their normal structures or stimulating their potential repairing abilities (Lucich *et al.*, 2018). Full-thickness wounds are greatly associated with damaging or missing of the extracellular matrix (ECM) which represents a reconstructive challenge to find out the proper complementary substitutions or even the efficient dressing in order to produce an appropriate condition that facilitate repairing initiation, inspire the start of curing, decrease pain, offer biomechanical funding, and prevent infection (Tan *et al.*, 2022; Aisa & Parlier, 2022; Rajab *et al.*, 2022; Mirani *et al.*, 2023). Skin available alternatives for treatment of full-thickness wounds can be simply categorized according to their anatomical origin into epidermal and derma skin alternatives that could be either cellular or acellular (Zaki *et al.*, 2024). Recently, numerous synthetic and viable epidermal and dermal renewing alternatives have been used (Nicholas & Yeung, 2017; Lucich *et al.*, 2018). One of the most advanced alternatives for repairing full-thickness wounds is by replacing or rebuilding them by exogenous sources especially decellularized ECM that could be gained from many biological sources including porcine, ovine, or bovine (Deng *et al.*, 2022; Kaur *et al.*, 2022; Motiea *et al.*, 2025). These ECM scaffolds derived from exogenous sources act as tissue biosimulation that can properly functionate ECM bio roles within the healing events (Raizman *et al.*, 2020). The

decellularization technique represents a new innovative technology that preserve the extracellular e architecture of any tissue without its cellular components to eliminate its immunogenicity (Lun *et al.*, 2010; Tan *et al.*, 2021). Endoform represents one of the advanced exogenous decellularized ECM. It holds the intact extracellular matrix with a structure and architecture that faithfully resemble soft tissues obtained from forestomach of sheep. The idea behind using of endoform for wound dressing belong to its epithelium which is composed of keratinized-stratified squamous that closely identical to that of skin histology making it perfectly suitable for skin wounds. Furthermore, the forestomach is one of the chief ECM products because of its highly stretching abilities that can produce up to 40 × 40 cm of ECM size (Simcock & May, 2013; Tan *et al.*, 2021). On the other hands, endoform has been noticed to hold the inherent ECM collagen construction that is entirely biodegradable into the regenerating soft tissue over time, with an open spongy construction to permit quick cell attachment. It has a huge number of materials and proteins like collagens, glycoproteins, growth factors and signaling molecules that arouses cellular distinction, immigration, and the swift progress of vasculature that are a significant regulator of any inflammatory response (Raizman *et al.*, 2020). Endoform might also aid modify the tiny environment within wound area and encourage conversion to the perfect healing events through shift wound healing from a nonhealing phase to a healing one (Lucich *et al.*, 2018). Furthermore, Endoform experiences swift angiogenesis that can, consequently transport seeding of autologous tissue. Undeniably, endoform upsurges blood vessel concentration, and proves more angiogenic possessions (Lucich *et al.*, 2018). While other innovative approach to deal with full-thickness wounds is through using various preparations that have the ability to stimulate their potential repairing abilities. MEBO (Moist exposed burn ointment) is

one of these most well-known traditional remedies for enhancing wound healing (Li *et al.*, 2017; El Charif *et al.*, 2021). It is kind of Chinese patent remedy inspired from the traditional Chinese medicine that can be used as topical ointment. It has an oily-texture nature consisting of sesame oil, beeswax, berberine, beta-sitosterol and small amounts of other herbal plants. These ingredient's formula has up to 18 amino acids, 4 critical fatty acids, polysaccharides and vitamins, however, the most important fatty acid is linoleic acid, which constitutes the cell membrane besides its crucial role as an essential fatty acid for proper cellular function (Zhan *et al.*, 2021; Najim *et al.*, 2022). The oils within MEBO formula dismiss pain, keep humidity, moistened the wounds. While beta-sitosterol exerts anti-inflammatory impact and provokes epithelialization besides, berberine displays analgesic and antimicrobial properties. Additionally, MEBO encourages epithelial repair and upgrade scar tissue quality. As well as, it plays an overall significant task within wound tissue repair and healing (Tan *et al.*, 2019; Mabvuure *et al.*, 2020; Najim *et al.*, 2022). The benefits of this easily applied traditional remedy for enhancing wound healing are countless such as promoting neovascularization and fibroblasts within granulomatous tissue and improving propagation of angiogenic endothelial cells (Li *et al.*, 2017; El Charif *et al.*, 2021). Furthermore, MEBO is believed to be used in shield wounds from being infected that in turn speed up wound healing process without less or no complications (Jewo *et al.*, 2009). Furthermore, many clinical and experimental researches have revealed that MEBO decreases water evaporation from the burned surface, encourages debridement and epithelial repair besides its antimicrobial and analgesic effects (Moustafa, 2016; Alshehabat *et al.*, 2020; Guanghuai *et al.*, 2022). This article is a trial to evaluate the efficiency of using two approaches to hasten healing events of full-thickness cutaneous wounds in dogs, one

based on using of biodegradable xenograft materials derived from ovine forestomach to mimic their normal structures and other inspired from traditional Chinese remedy to stimulating their potential repairing abilities basing on macroscopical and histopathological investigations.

MATERIALS AND METHODS

Ethical Approve

The whole adapted procedures associated with this work gained the institutional consent of the animal care and use commission ethics of Mosul University/ College of Veterinary Medicine. UM.VET.2024.067.

Animals and Experimental Design

A total of twenty-seven (N=27), 1-3 years old with an average weight of 12-20 kg local breed dogs, were acclimatized for seven days before the start of this experiment. All handling and operational protocols utilized for this experimental design were obeyed the standard morals of animal care and use commission.

A square of 2x2 cm of full-thickness cutaneous wounds were induced at the lateral aspect of radial region in all dogs. Then animals were grouped randomly into 3 equal sets of nine dogs for each. In the 1st group (control group), the induced wounds were left to heal spontaneously without applying any patch. While, for the 2nd (mebo) and 3rd (endoform) groups a patch of the same wound size of mebo and endoform were applied to cover the induced wounds.

Then, a piece of sterile plastic was applied over the dressed wounded area then bandaged. The induced wounds were examined grossly and subjected to histological examination on days 7, 14 and 21 following inductions.

Supplying of Mebo and Endoform Patches

Both Mebo and Endoform patches were purchased from the local pharmacy. While mebo is a dressing material made from non-woven fabric with an adhesive pad soaked with an ointment composed of beta-sitosterol 0.25%, sesame oil, beeswax, and nutritional elements (Gulf Pharmaceutical industries, Ras Al-Khaimah, UAE). On the other hands, Endoform™ antimicrobial restorative bioscaffold is a bio scaffold patch that has a unique extracellular matrix (ECM) products contains 0.3% ionic silver, manufactured by Aroa Biosurgery, Auckland, New Zealand.

Anesthetic Protocol

The dogs were fastened for 12 hours before surgery. All surgical procedures were accomplished with general anaesthesia using atropine sulfate of a dose 0.044mg/kg (Atropina Santisa Injetável. Santisa Pharmaceutical Laboratory. Bauru, SP) as a premedication, then followed by intramuscular injection of premixed blend of ten mg/kg of Ketamine Hydrochloride

drug (Narketan®-10, Troy Laboratories PTY Limited, Australia) and five mg/kg of Xylazine drug (ILium Xylazil-100, Troy Laboratories PTY Limitd, Australia) (AL-Qadhi *et al.*, 2022; Mohamed *et al.*, 2024).

Surgical Procedure for Creation of the Full-Thickness Defects

The ordinary preparative aseptic technique for skin surgery was applied on the lateral radial region. A full-thickness square skin wounds of 2x2cm² were made at the lateral aspect within radial region in all dogs. Then animals were grouped randomly into 3 experimental groups. In the 1st control group, the induced wounds were left to heal spontaneously without applying any patch (Fig. 1A). While in the 2nd experimental group a patch of the same wound size of Mebo was applied to cover the induced wounds (Fig. 1B). Whereas Endoform patch was used to cover the induced wounds in 3rd group (Fig. 1C). A piece of sterile plastic was applied over the dressed wounded area to fix the dressing patches in situ, then bandaged firmly for the three experimental groups.

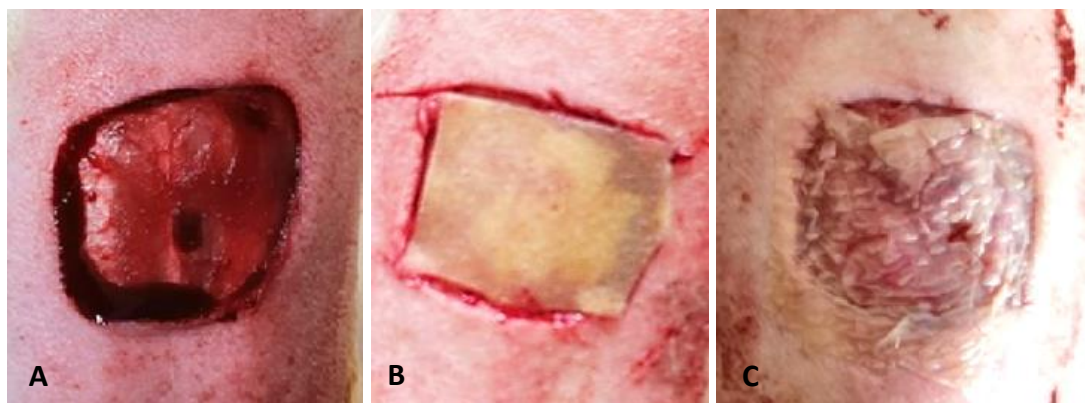


Figure 1: Photographic picture after creation of full-thickness cutaneous wounds. (A) 1st control group left to heal spontaneously. (B), 2nd group covered with Mebo patch and (C) 3rd group covered with Endoform patches.

Post-Operative Care

The dressing bandages of wounded area was exchanged every 48 hours during the whole period of study starting from the first 48 hours post operation, besides inspection of the skin wounds was done for any gross signs of wound complications or

dehiscence. All animals received intramuscular injection of penicillin-streptomycin (kanavet, Canadian), in a dose of 1ml/10kg and metagen in a dose of 1ml/day as a prophylactic and analgesic, respectively for five days (Al-Saiegh *et al.*, 2024; Khudhur *et al.*, 2024).

Assessment of Healing

Macroscopic Assessment

Microscopic examination was performed on 7th, 14th and 21th days following procedures. They were judged by gross physical check for the progress of the cutaneous wound healing, the decrease in size of wounded area.

Histopathological Assessment

At the end of days 7, 14 and 21 after wound initiation, experiment samples collection was achieved, the implanted tissues were

kept for 24 h for fixation in containers filled with neutral-buffered formalin of ten percent. Then, the samples were fixed using paraffin wax, sectioned, and finally stained with hematoxylin–eosin. The examination was done using light microscope. Each histological section was evaluated microscopically to assess the intensity of the healing features including granulation tissue, angiogenesis, inflammatory reaction and re-epithelization (Sultana *et al.*, 2009; Tsioli *et al.*, 2016) as shown in Table (1).

Table 1: Showing the histopathological parameters scoring

Criteria	1	2	3	4
Granulation tissue	Nil	Few	Moderate	Strong
Angiogenesis	Nil	Few	Moderate	Strong
intensity of inflammation	Nil	Few	Moderate	Strong
Re-epithelialization	Nil	Few	Moderate	Strong

Statistical Analysis

The obtained histopathological descriptive scores outcomes for the microscopic features were achieved by a blind pathologist and analyzed statistically by Kruskal-Wallis test and used Pairwise Multiple Comparison Procedures (Tukey Test) at $p \leq 0.05$. The Sigma Plot (version 12.5) software program analyzed the data for statistical analysis.

RESULTS

Macroscopic Results

All experimental dogs were survived the procedures and well recovered uneventfully. Firstly, there was good adhesion between both patches of Mebo and Endoform to the underling wound beds, then a well biodegradation of these patches was also observed. Furthermore, the macroscopic assessment of full-thickness wound healing on day 7 following operation showed that speed of healing process was much better in both Endoform

and Mebo groups as compared to control group however, Endoform animals group exhibited even better healing process than Mebo especially with the progress of healing tile day 14 (Fig. 2). In both treated groups, healing process was started earlier on day four after dressing and was more obvious after seven days with significant difference comparable to untreated wounds until the end of the experiment. Besides, the induced wounds of the three experimental groups had almost the same size at day seven (Fig. 2) but after one week, the Endoform and Mebo groups showed smaller sized wounds in comparison to control group although that size was even smaller for Endoform group along the whole duration of the study particularly on days 14 (Fig. 2) and 21 (Fig. 2) compared to those in mebo and control groups. The degree of wound contraction, reepithelization and total wound healing process progressed faster in both patched groups than in control group during the whole period of study.



Figure 2: Photographic picture of the gross appearance of the treated full-thickness wounds of the three experimental groups. The size of wounds at day 7 in 1st, 2nd and 3rd groups respectively (A, D and G). The size of wounds at day 14 in 1st, 2nd and 3rd groups respectively (B, E and H). The size of wounds at day 21 in 1st, 2nd and 3rd groups respectively (C, F and I).

Histopathological Findings

The microscopic manifestation of control group wound on day 7 post induction exhibited presence of severe inflammatory reaction that was composed of fibrinous exudate, intense presence of inflammatory cells, existence of granulation tissue and edema. While in both treated groups i.e.

Mebo and Endoform, their associated inflammatory reaction was characterized by less extent of fibrinous exudate with less infiltration of inflammatory cells, good established granulation tissue and presence of high newly blood vessels and re-epithelialization especially with Endoform patched group (Fig. 3).

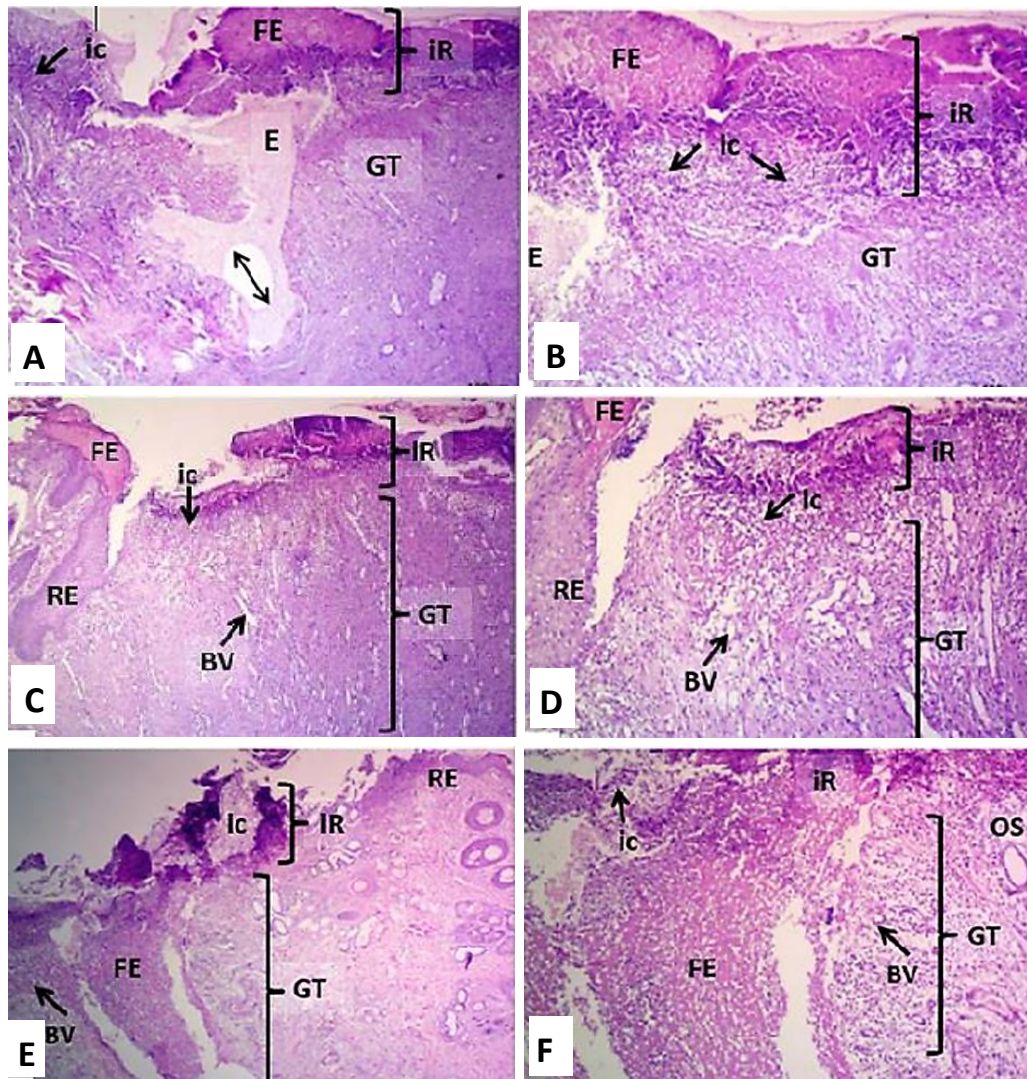


Figure 3: Histological sections of dogs' skin wound at 7th days. [A&B]: Control group demonstrates the site of wound with severe inflammatory reaction (iR) composed of fibrinous exudate (FE) and intense presence of inflammatory cells (ic), granulation tissue (GT) and edema (E). **[C&D]: Mebo group** demonstrates the site of wound with inflammatory reaction (iR) composed of fibrinous exudate (FE) and spread of inflammatory cells (ic), good established granulation tissue (GT) with high new blood vessels (BV) and re-epithelialization (RE). **[E&F]: Endoform group** demonstrates the site of wound with low inflammatory reaction (iR) composed of fibrinous exudate (FE) and presence of inflammatory cells (ic), good established granulation tissue (GT) with huge newly blood vessels (BV) and re-epithelialization (RE), old skin (OS). [A, C & E: 40X] [B, D & F: 100X]. H&E stain.

While the histopathology of the control group on day 14, exhibited the continues presence of fibrinous inflammatory exudate that was infiltrated with inflammatory cells, besides presence of granulation tissue, new blood vessels and re-epithelialization. On the other hands, the Mebo group showed low infiltration of inflammatory cells, good

established granulation tissue with hug newly blood vessels and re-epithelialization. While the Endoform group showed so low infiltration of inflammatory cells, good established granulation tissue with very high newly blood vessels and well-developed re-epithelialization (Fig. 4).

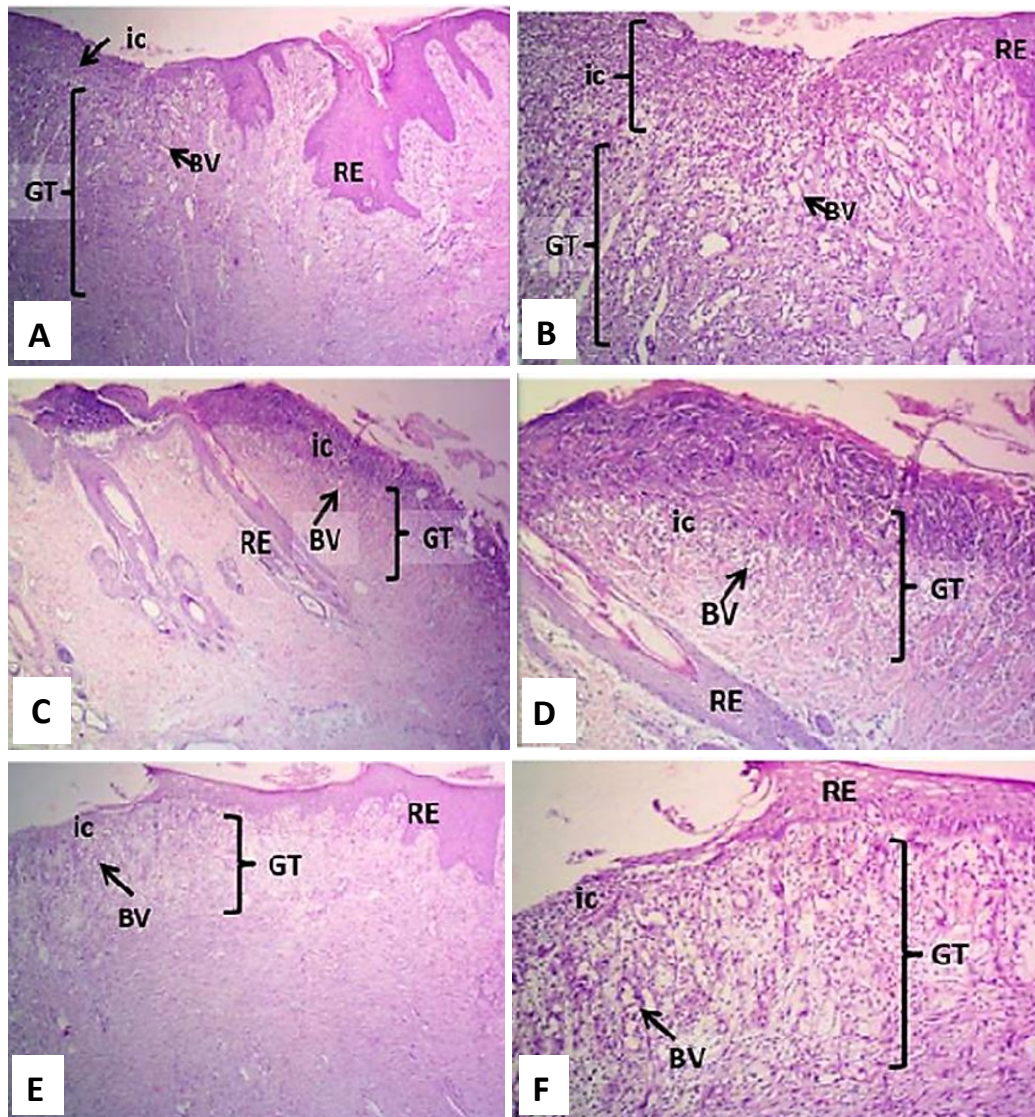


Figure 4: Histological sections of dogs' skin wound on 14th days. [A&B]: Control group demonstrates the site of wounds with inflammatory reaction (iR) composed of fibrinous exudate (FE) and presence of inflammatory cells (ic), granulation tissue (GT) with new blood vessels (BV) and re-epithelialization (RE). **[C&D]: Mebo group** demonstrates the area of wound with low spread of inflammatory cells (ic), granulation tissue (GT) with high new blood vessels (BV) and re-epithelialization (RE). **[E&F]: Endoform group** demonstrate the site of wound with very low and presence of inflammatory cells (ic), good established granulation tissue (GT) with very high new blood vessels (BV) and well-developed re-epithelialization (RE), and old skin (OS). [A, C & E: 40X] [B, D & F: 100X]. H&E stain.

Finally, on day 21 for control group, it exhibited the persistence of fibrinous inflammatory exudate associated with existence of inflammatory cells, granulation tissue and newly blood vessels and well-developed re-epithelialization. While in Mebo group displayed low spread of inflammatory cells, high recently blood

vessels, complete re-epithelialization with developed hair follicles, sweat gland and sebaceous gland. Furthermore, Endoform animals revealed absent of any inflammatory infiltration, high newly blood vessels, complete re-epithelialization and well-developed of hair follicles, sweat and sebaceous glands (Fig. 5).

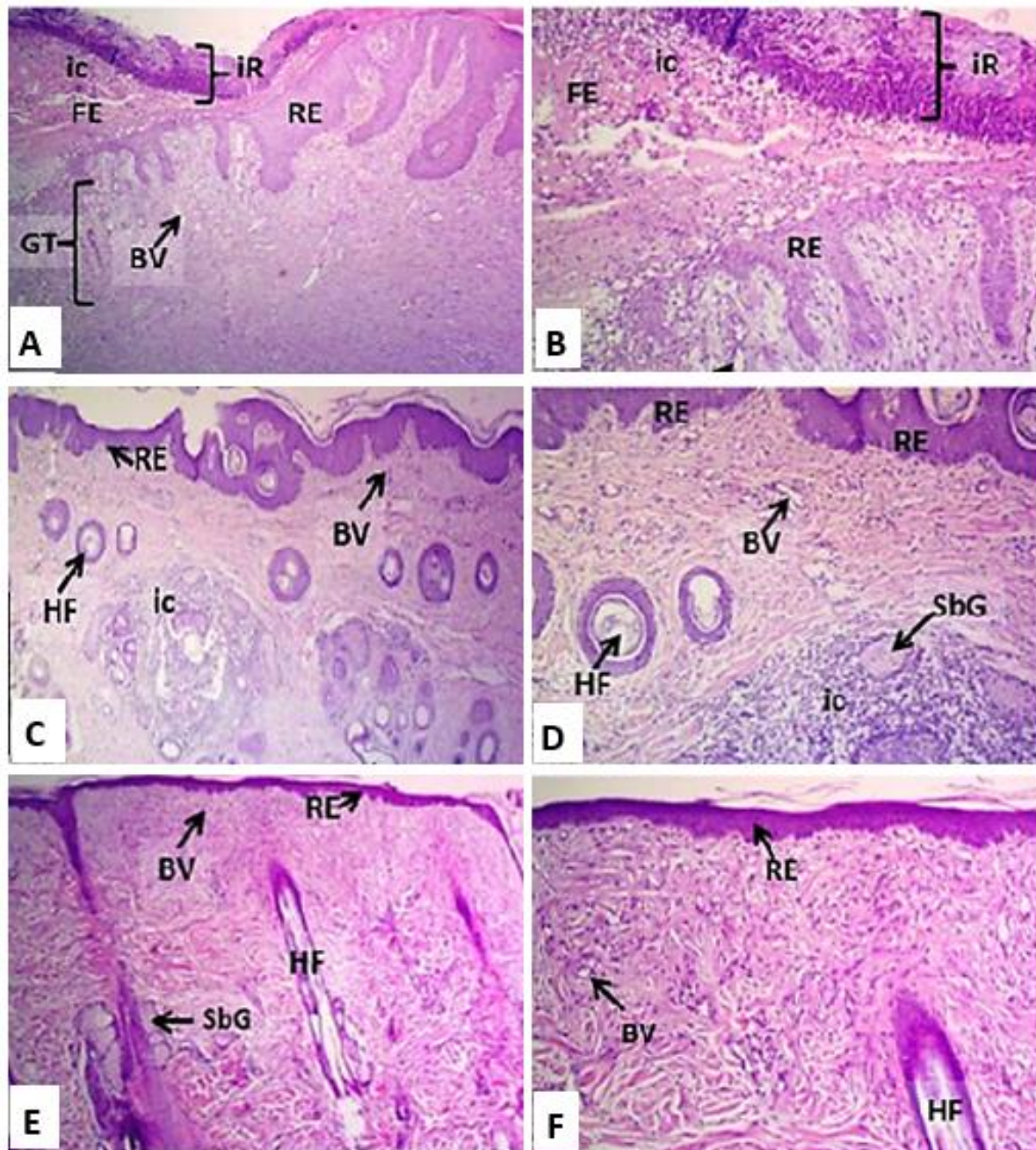


Figure 5: Histological sections of dogs' skin wound at 21th days. [A&B]: Control group demonstrate the site of wound with inflammatory reaction (iR) composed of fibrinous exudate (FE) and presence of inflammatory cells (ic), granulation tissue (GT) with newly blood vessels (BV) and well-developed re-epithelialization (RE). **[C&D]: Mebo group** demonstrate the area of wound with low presence of inflammatory cells (ic), high newly blood vessels (BV), complete re-epithelialization (RE) with developed hair follicles (HF), sweat gland (SG) and sebaceous gland (SbG). **[E&F]: Endoform group** demonstrate the area of wound without existence of inflammatory cells, high newly blood vessels (BV) complete re-epithelialization (RE), with developed hair follicles (HF), sweat gland (SG) and sebaceous glands (SbG). [A, C & E: 40X] [B, D & F: 100X]. H&E stain.

Furthermore, the statistical analysis of the most noticeable histopathological features among the three experimental groups

indicated presence of significant differences (Tables 2-5).

Table 2: Histopathological Scores of the of Granulation Tissue GT

Scores of GT	7 th D (N=3) Median (IQR)	14 th D (N=3) Median (IQR)	21 st D (N=3) Median (IQR)	P-value
Groups				
G1 Control group	2 (1) B b	2 (1) B ab	4 (1) B a	0.050*
G2 Mebo group	3 (1) AB ab	3 (1) AB a	1 (1) A b	0.050*
G3 Endoform group	4 (1) A a	4 (0) A a	0 (1) A b	0.050*
P-value	0.050*	0.050*	0.048*	

Table 3: Histopathological Scores of the Angiogenesis (newly blood vessels)

Scores of angiogenesis	7 th D (N=3) Median (IQR)	14 th D. (N=3) Median (IQR)	21 st D (N=3) Median (IQR)	P-value
Groups				
G1 Control group	1 (1) B b	2 (1) B ab	4 (1) A a	0.025*
G2 Mebo group	4 (1) A a	4 (1) A ab	1 (1) AB a	0.050*
G3 Endoform group	3 (1) A ab	4 (1) A a	1 (1) B a	0.050*
P-value	0.050*	0.050*	0.025*	

Table 4: Histopathological Scores of the of Inflammation

Scores of inflammations	7 th D (N=3) Median (IQR)	14 th D (N=3) Median (IQR)	21 st D (N=3) Median (IQR)	P-value
Groups				
G1 Control group	4 (1) B a	3 (2) B ab	2 (1) B b	0.050*
G2 Mebo group	1 (1) AB ab	1 (1) AB a	0 (1) A b	0.296
G3 Endoform group	1 (0) A a	1 (1) A a	0 (0) A b	0.139
P-value	0.034*	0.050*	0.034*	

Table 5: Histopathological Scores of the Re-epithelialization RE

Scores of re-epithelialization	7 th .D (N=3) Median (IQR)	14 th D (N=3) Median (IQR)	21 st D (N=3) Median (IQR)	P-value
Groups				
G1 Control group	0 (0) B b	2 (1) A ab	3 (1) B a	0.045*
G2 Mebo group	2 (1) AB a	3 (2) A ab	4 (0) A a	0.050*
G3 Endoform group	2 (2) A ab	3 (1) A ab	4 (0) A a	0.050*
P-value	0.050*	0.100	0.021*	

Data expressed as Median & IQR ((Inter-Quartile-Range) (N= 3 animals) (Kruskal-Wallis test)
 The different capital letters indicate presence of significant differences among groups at $P \leq 0.05$.
 The different small letters indicate presence of significant differences among periods at $P \leq 0.05$.

DISCUSSION

The essential requirements for any successful healing process especially for that of full-thickness wounds necessitate the partnership and harmonization of three elementary foundations including the extracellular matrix (ECM), growth factors and cells (Rodrigues *et al.*, 2019; Nikahval *et al.*, 2020; Al-Mutheffer *et al.*, 2024). Several strategies had been adapted to deal with full-thickness cutaneous wounds, some based on using of various kinds of traditional therapeutic preparations or procedures that can promote wound healing (Guanghuai *et al.*, 2022; Shihab *et al.*, 2023; Madyan *et al.*, 2024). While others tried using many synthetic and biologic dermal and epidermal regenerative alternative options (Capella *et al.*, 2020; Raizman *et al.*, 2020; El-Shaer *et al.*, 2023). In this research we applied two different approaches to deal with full-thickness wound in dogs, one of them designed to benefit from the accelerating, anti-inflammatory, anti-infection properties of mebo patches (Jiang *et al.*, 2021; Guanghuai *et al.*, 2022). While the second approach used the Endoform patches to profit from its dimensional architecture and its richness with more than one thousand various protein molecules that govern tissue homeostasis and maintenance (Raizman *et al.*, 2020).

The gross observation following using of mebo and endoform showed existence of a good adhesion between these two patches and wound beds since the 1st week following surgery that agreed with other results (Magin *et al.*, 2016; Lucich *et al.*, 2018). On the other hands, the healing process within both treated groups started earlier since day four after induction and was more evident after seven days. Moreover, the progress and speed of that healing events were much better in both treated groups especially Endoform group as comparable to control group during the whole period of study. These amazing

observed results of Endoform could be due to the presence of a good extracellular matrix construction that provided the full-thickness wounds with the needed structural support that supplied the host cell with the required scaffold to invade and grow because it holds abundant ratio of intact native collagen that considered as the perfect requirement for any ideal progress of wound healing (Rosenbaum *et al.*, 2018). Besides, its extracellular matrix structure facilitated that swift healing through its open porous architecture that permitted the swift attachment of cell (Sizeland *et al.*, 2017). Furthermore, these remarkable gross data of Endoform scaffold could be highly associated with its ability to supply the wounded area with many essential factors like collagens, laminin, fibronectin, glycoproteins, signaling particles, and growth factors that are responsible for the essential regenerative cellular events like growth, migration, and differentiation (Hynes, 2009; Schultz & Wysocki, 2009; Raizman *et al.*, 2020). Furthermore, the ECM itself is the significant controller of the inflammatory response because of its components that modify tissue proteases associated with wound healing (Raizman *et al.*, 2020). All these possible explanations could clarify the speedy early beginning and amazing progress of healing events associated with Endoform group that was also observed with other researchers work who noticed the beneficial impact of using Endoform forestomach matrix in shortening the time needed for wound closer during their surgical management of various kinds of soft-tissue wounds (Capella *et al.*, 2020; Bosque *et al.*, 2023; Melnychuk *et al.*, 2023). On the other hands, the Mebo groups healing was also better and faster than control group, this could be to its included ingredients especially its important linoleic fatty acid, which is essential for building of cellular membrane (Zhan *et al.*, 2021; Najim *et al.*, 2022). Furthermore, the oily formula of Mebo patches retained moisture and softened the bed area that speed up the wound healing process, while berberine

displayed analgesic and antimicrobial properties that furtherly supported the beneficial advantages of Mebo and in turn encouraged and upgraded the quality of tissue repair events without less or no complications (Tan *et al.*, 2019; Mabvuure *et al.*, 2020; Najim *et al.*, 2022). Although most uses of mebo focused on burned wounds (Mabvuure *et al.*, 2020; Guanghuai *et al.*, 2022) but its accelerating impact on wound healing were in a great coincides with other researcher's data like (Melnychuk *et al.*, 2023) who documented its accelerating healing effects on diabetes-related wounds or even (Alshehabat *et al.*, 2020) who noticed the significant enhancement and acceleration of healing process within Mebo group comparable to honey in their comparative study on wound healing using healthy and immunocompromised dogs. While (Tang *et al.*, 2014) mentioned that Mebo significantly promoted and reduced the duration of cutaneous excisional wound repairing in rats. All these series of events associated with the progress of wound healing following application of both Endoform and Mebo patches participated significantly in the obvious lessening in the wound size area that was noticed in both treated groups especially Endoform group comparable with control groups on days 14 and 21 as the lessening in wound extent is a distinct clue for the good progress of wound healing process that agreed with others researchers outcomes (Coerper *et al.*, 2009) who mentioned that more than 50% reduction in wound size is greatly correlated with progress of diabetic wound healing, or even (Vu *et al.*, 2020) who stated that the rate of wound size reduction could be considered as a trusted valuable indicator for wound healing activities. Furthermore, there was perfect dissolution and full biosorption of these two dressing patches into the wound beds within the 1st week following dressing that even need the re-application of these patches as a result of the oily melting texture of Mebo patch (Moustafa, 2016) or the biodegradable

nature of endoform (Irvine *et al.*, 2011) that was in the same line of (Melnychuk *et al.*, 2023) results who reapplied Endoform patch every 5 to 7 days. The other parameter that was used to evaluate the efficiency of using Endoform and Mebo patches was the histopathological examination to study the progress and phenomena of healing process. The obtained microscopic outcomes exhibited presence of significant difference in the most observed healing features among the three experimental groups especially the severity of inflammatory reaction which was less in both treated groups particularly Endoform group comparable to control group, this observation was in the same line of (Magin *et al.*, 2016) who noticed the less inflammatory response associated with using Endoform as a dressing for rat full-thickness wounds, besides, (Capella *et al.*, 2020) who compered Endoform with other biomaterial for wound healing and noticed its less inflammatory reaction. These obtained data of less inflammatory response could be to the fact that endoform is slightly treated, handled and decellularized to detached its layers of tissues and to keep its essential tissue repair elements (Agostinis *et al.*, 2021) which in turn participated in that low level of inflammatory reaction toward it. In addition to the fact that ECM of the Endoform patch itself is a chief controller of the inflammatory response that made Endoform group showed the less inflammatory reaction due to its contents of growth factors and signaling molecules (Raizman *et al.*, 2020) that modify tissue proteases associated with wound healing (Negron *et al.*, 2014). Furthermore, the intact structure of endoform ECM was closely identical in composition and structure to skin histology (Raizman *et al.*, 2020). On the other hands, the less inflammatory reaction that was associated with Mebo group was in the same line of other researchers work who studied the influence of Mebo on wound healing (Magin *et al.*, 2016; Guanghuai *et al.*, 2022) and noticed the less existence of

inflammatory cells due to the anti-inflammatory and anti-infection belongings of Mebo because of its beta-sitosterol and berberine contents that hold these anti-inflammatory and antimicrobial properties (Tan *et al.*, 2019; Mabvuure *et al.*, 2020; Najim *et al.*, 2022). These beneficial ingredients reduced both the level and intensity of the inflammatory reaction and promoting wound healing. Furthermore, the new blood vessels formation and angiogenesis was also compared histopathologically and statistically. These observed comparisons exhibited presence of significant difference among the three experimental groups since the 1st week. It was noticed that the angiogenesis was better and even started earlier in both treated groups especially Endoform groups comparable to control group. Besides, this revascularization was developing more and more with the progress of the healing process which is considered as the foundation for the ideal healing process as formation of new capillaries during angiogenesis is one of the main participating issues in wound repair process due to its vital role in sending of essential vital elements and oxygen to the quickly multiplying restored cells at wound spot (Guerra *et al.*, 2018; Veith *et al.*, 2019). Within wound healing events, new blood vessels are formed into the wounded area in a regular percentage, causing the formation of a rich system of recently blood vessels that even reaches near to 10 times denser than of the usual tissue (Dipietro, 2016). These observations could be due to the fact that Endoform reveals more angiogenic properties (Lucich *et al.*, 2018) because it holds more than 150 signaling molecules and growth factors that inspires the progress of vasculature (Coerper *et al.*, 2009; Raizman *et al.*, 2020) besides its structure upholds remaining angiogenic channels to funding the creation of new blood vessels (Agostinis *et al.*, 2021; Melnychuk *et al.*, 2023). Furthermore, Endoform could also impact positively on the wound beds microenvironment that even shift and

promote its transition into the perfect healing phase (Lucich *et al.*, 2018). On the other hand, the good angiogenesis of Mebo group could be due to the positive ability of Mebo on vascular endothelial growth factor (VEGF) expression that is crucial in the perfect initiation of angiogenesis as VEGF is the greatest significant moderator of angiogenesis within wound, and its presence encourages new capillary growth (Guerra *et al.*, 2018; Veith *et al.*, 2019). Besides that, the start of new blood vessels formation is totally governed by many growth factors particularly VEGF which is the greatest controlling proangiogenic element within healing wounded area by behaving as a commanding proangiogenic mediator (Dipietro, 2016). These data agreed with (Tang *et al.*, 2014) who noticed the high expression of VEGF that hastened the abundant neovascularization within granulation tissue since day 8 during his work with rats to study the beneficial impact of Mebo on cutaneous excisional wound healing. Another microscopic criterion that was also compared statistically and histopathologically among experimental groups was re-epithelialization, because it is an essential step during wound healing that could be considered as a crucial parameter for the successful wound closure (Pastar *et al.*, 2014) that enables the skin to restore its normal barrier role (Bornes *et al.*, 2021). The obtained data from these comparisons exhibited presence of significant difference in the degree and time of beginning of that re-epithelialization which was very clear and even started earlier since the 1st week in both treated groups especially Endoform groups comparable to control group, this could be due to the helpful impact of Endoform on healing events because of its three dimensional structure that enabled the swift rebuilding of the epidermis, in addition to the presence of signaling molecules, and growth factors that stimulates the good re-epithelialization that was also noticed with other researches (Capella *et al.*, 2020; Raizman *et al.*, 2020;

Sharma *et al.*, 2023). Moreover, the good re-epithelization of Mebo group was also mentioned within other works especially (Alshehabat *et al.*, 2020) who studied wound healing in immunocompromised and non-immunocompromised dogs and discovered that MEBO-treated wounds exhibited improved epithelialization area, compared with other treatments and (Gong *et al.*, 2023) who founded that MEBO promoted the re-epithelialization of diabetic wound. These gained data of Mebo patch could be due to the ingredients of Mebo preserves moisture within the wound environment, which in turn enhanced the epithelialization process through facilitating the migration of epidermal cells (Tan & Dosan, 2019). Finally, The existence of abundant and well developed granulation tissue was also compared microscopically and statistically among the three experimental groups. The outcomes of these comparison clearly exhibited presence of a significant variations toward both treated patches groups especially for Endoform in comparison to control group which was evident since day 7 post dressing indicating that both Mebo and Endoform patches incorporated very well into the tissue as the granulation tissue which is branded microscopically by the existence and propagation of fibroblast cells and recent delicat-walled capillaries, is greatly addressed the presence of the well development of regenerative process within the wounded site (Hakkinen *et al.*, 2011; Jiang & Scharffetter, 2020; Fadilah *et al.*, 2023). These obtained data were in great agreement with other researchers data who mentioned the presence of good granulation tissue that in turn support re-epithelization associated with application of Endoform patches (Ingegneri & Romanelli, 2020; Agostinis *et al.*, 2021). Besides (Magin *et al.*, 2016; Bosque *et al.*, 2023) ho stated that Endoform enhanced granulation tissue development on day 7 or even earlier, as with (Davis *et al.*, 2022), who noticed that the granulation tissue development experienced initial improvement since day 4 for

those wounds treated with using Endoform native extracellular matrix. On the other hand, several studies noticed that MEBO application stimulate granulation tissue growth within wound (Zheng *et al.*, 2020). Furthermore, granulation tissue was also detected with other work (Tang *et al.*, 2014) who notice existence of granulation tissue with MEBO since day eight that significantly promoted the healing process when compared with other therapeutic options. The existence of all these good histopathological indicators since the early first week following dressing helped the well performance of both Endoform and Mebo patches comparable with control group.

CONCLUSIONS

The macroscopic and the microscopic outcomes of this study exhibited clearly that full-thickness cutaneous wounds dressing in dogs can be done successfully and perfectly using either Endoform or Mebo biodegradable patches with superiority of Endoform.

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Conflict of Interests

All authors declare that they have no conflict of interest associated with this article.

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مقارنة بين تأثير رقعة الإندوفورم والمييو على إصلاح الجروح الجلدية في الكلاب

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تم التخطيط لهذا العمل لمقارنة قدرة لاصقات المييو وإندوفورم على إصلاح جروح الجلد لدى الكلاب. وقد استخدم في الدراسة سبعة وعشرون كلبًا بالغًا من السلالات المحلية. تم استحداث جرح جلدي مربع كامل السماكة بإبعاد 2×2 سم على الجانب الجانبي لمنطقة مرفق اليد في جميع الحيوانات ثم تم تقسيم الحيوانات إلى ثلاث مجموعات متساوية. في المجموعة الأولى (مجموعة السيطرة)، تُركت هذه الجروح المستحدثة مفتوحة للشفاء تلقائيًا دون استخدام أي نوع من العلاج، بينما بالنسبة للمجموعتين الثانية والثالثة، تم وضع لاصقات المييو وإندوفورم بنفس حجم الجرح على تلك الجروح وتم تضميدها بإحكام. تم تقييم النتائج استنادًا إلى العلامات العيانية والنسجية المرضية في اليوم السابع والرابع عشر والحادي والعشرين بعد الجراحة. أظهرت النتائج الإجمالية أن سرعة عملية الشفاء كانت أفضل بكثير في كل من مجموعتي المييو والإندوفورم منذ الأسبوع الأول مقارنة بمجموعة السيطرة، ومع ذلك، كانت مجموعة الحيوانات الإندوفورم هي الأفضل، إلى جانب أن كلتا المجموعتين المعالجتين أظهرتا انخفاضًا تدريجيًا واضحًا في حجم الجرح مقارنة بمجموعة السيطرة، على الرغم من أن حجم الجرح أصبح أصغر بكثير في الإندوفورم من مجموعة المييو. علاوة على ذلك، أظهرت التقييمات النسيجية المرضية تكوينًا مفرطًا وأكثر نضجًا للأنسجة الحبيبية مع ارتشاحات خلوية التهابية قليلة وتكوين أوعية دموية جيدة وإعادة تكوين الظهارة وتكوين بصيلات الشعر وملحقات الجلد الأخرى في كل من مجموعتي العلاج مقارنة بمجموعة السيطرة. في الختام، يمكن استخدام كل من رقعة المييو والإندوفورم لتسريع الشفاء التام للجروح الجلدية ذات السماكة الكاملة في الكلاب على الرغم من أن الإندوفورم يُصنف على أنه الخيار الأول الأفضل.