Treatment of Full-Thickness Cutaneous Wounds Using a Novel Fish Skin Acellular Dermal Powder as Hydrogel with Gene Expression of Some Growth Factors Genes

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Wound Healing, Hydrogel, Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF).

ABSTRACT
The purpose of this study is to evaluate how well fish skin acellular dermal powder hydrogel (FADPH) promotes the healing of cutaneous wounds in Iraqi bucks. 48 of full-thickness cutaneous wounds (3X3 cm) created on the dorsal back of twelve bucks, each two wounds on both sides. Then, divided into two groups according to treatment method (6 bucks /group). The wounds in control group (A), were left without treatment and the wounds in treatment group (B) were treated by topical application of (FADPH). The outcomes were assessed clinically, morphometrically scoring at (7, 14, 35, and 42) days post treatment, and gene expression by RT-PCR of two genes including fibroblast growth factor (FGF) and epidermal growth factor (EGF) at three times periods (0,7 and 14) days and histopathological assessment at (7,14, 35 and 42) days post treatment. The scoring results of group B showed that the percentages of scoring results were significantly higher than that of control wounds at the level of ($P<0.001$) from the first 7 days until the end of the study. Also, results of gene expression of FGF and EGF was considerably higher in the treated group than control group at the level of ($p<0.001$). The histopathological results in group B showed faster re-epithelialization of the epidermis, proliferation of fibrin and regenerated epithelia, dense collagen fibers, and finally complete regeneration of the epidermal layer at the end of study in compared with group A which was showed slowly development in wound healing proses and highly inflammatory reaction. In conclusion, FADM hydrogel enhanced and accelerated the healing of skin wounds in male goats.

1. Introduction

Wounds are defined as damage or disruption to the normal anatomical structure and function of tissues (Khalaf & Salih, 2018). Full-thickness wounds are characterized by the whole destruction of epithelial regenerative tissues and refer to extensive scarring due to lack of epithelialization and can't be healed by standard healing process require replacements of missing tissue to save the wound free of contamination (Mohammed & Salih, 2022). Cutaneous wound healing is an important pathophysiological phenomenon called wound healing to restore the skin barrier after trauma. This process is naturally divided into three stages; inflammation, proliferation, and remodeling phase (Ghaima, 2013; Wilkinson and Hardman, 2020; Atiyah and Al-Falahi, 2021). To avoid the potential harmful effects of synthetic non-absorbable materials, surgeons are developing biological materials and using them to treat wounds, these biomaterials are derived from a variety of tissues, including the pericardium, small intestine submucosa, and dermis of humans, fishes and pigs, these materials allow for neovascularization and regeneration, as well as the ability to change into tissue and resist infection, (Mahdi and AL-Bayati 2019).

Bioscaffolds are essential for tissue engineering, facilitating cell adhesion, proliferation, migration, and differentiation, they allow cellular invasion, proliferation, and secretion of extra cellular matrix (ECM), causing regrowth of natural tissue (AL-Bayati *et al.*, 2016). Fish skin scaffold speeds up wound healing process by stimulating cell proliferation, division, and adhesion, so that fish collagen is used effectively for scaffolds fabrication as a natural polymer (Zedan, 2023). Acellular fish skin has recently used in variables of clinical requirements because of its physicochemical properties and amino acid composition (Gumaa and Al-Bayati, 2021). Decellularized fish skin as biological materials is the good supply of collagens type I and II which is regarded to be able to breakdown, safe scaffold, and pure natural ECM (Bassam, 2021). ECM consists of proteins and polysaccharides, providing biological and mechanical support to adjacent cells and controlling their regeneration process (Al-Falahi *et al.*, 2018).

The main growth factors (GFs) involved in the wound healing process include FGF, EGF, PDGF, VEGF and keratinocyte growth factor (KGF) (Grazul-Bilska *et al.,* 2003). FGFs are heparin-binding GFs that are produced in most tissues in the body and play an essential role in the earliest stages of embryonic development, proliferation, organogenesis, and in healing process of several organs of body (Barrientos *et al.,* 2008). FGF secreted by inflammatory cells, vascular endothelial cells, fibroblasts, and keratinocytes. They act as a part of re-epithelialization, angiogenesis, granulation tissue formation and regulate ECM production, also crucial for tissue repair and reconstruction, promoting cell division and proliferation. It increases during acute wounds, aiding angiogenesis, granulation tissue formation, re-epithelialization, and tissue remodeling (Al-Ebadi, 2018; Al Qaseer *et al.,* 2021).

EGF is a growth factor that increases cell development, proliferation, and differentiation by attaching to the Epidermal Growth Factor Receptor (EGFR) (Oda *et al.,* 2005). In acute wounds, EGF is mainly secreted by platelets, macrophages and fibroblasts and is upregulated within a short period of time following injury (Shiraha *et al.,* 1999). EGF is up-regulated after acute injury significantly accelerating re-epithelialization and increasing tensile strength in wounds (Barrientos *et al*. 2008). EFG supports wound closing by boosting fibroblast proliferation and migration and dermal maturation by connecting with the EGFR in the cells at the wound location, and accelerate keratinocyte migration, which promotes re-epithelialization (Richard, 2013).

2. Material and Methods:

2.1 Experimental Animals:

The current study was conducted in the animal house of the College of Veterinary Medicine /University of Baghdad, throughout the duration of the study 3 months. After obtaining an official approval from the ethical committee of the college (number 1163 on 2024/0609). Twelve healthy mature local breed bucks weighing (15-20) kg and (1.5-2) years old were recruited for this study. The animals were housed in the farm animal house of the College of Veterinary Medicine, University of Baghdad. All animals were evaluated clinically by a physical examination before initiation of the experiments. The animals were left for four weeks to adapt to the experimental condition and received free access to water and food during the period of the experiment. After obtaining an official approval from the ethical committee of the college of Veterinary Medicine/University of Baghdad (number 1163 on 2024/0609)

2.2 Surgical Operation:

The dorsal surface of thoracic regions was clipped free hair and prepared aseptically for the wounding. Four Full-thickness cutaneous square wounds (3X3) centimeters were created on the dorsal back of 12 male bucks (2 wounds on each side one cranial and one caudal), with 10 cm between each wound (Figure 1). To induce full thickness cutaneous wounds on the dorsal area of each animal, all the animals were injected intramuscularly (I.M) with 10,000 IU and 10mg/kg body weight of (penicillin-streptomycin/Kepro-Holand)12 hours before surgery respectively, and submitted to surgical operation under sedation by IM injection of 2% Xylazine hydrochloride at dose of (0.2 mg/kg) and local anesthesia using inverted L local infiltration of lidocaine hydrochloride 2% at dose 10mg/kg B.W in wound borders (**Sarkar *et al.*, 2016)** (24 wounds/group) then the animals will be divided into the following groups:

A. **Control group (n=6):** the area of the wound will be left without treatment only dressing with sterile gauze.

B. **Treatment group (n=6):** the area of the wound will be treated by local application of FADPH and dressed in sterile gauze.

2.3 post-operative care:

All wounds were covered with sterile gauze fixed with medical tape and changed every day

(**Elbialy *et al*., 2020**), and the animals were injected with penicillin/streptomycin (10mg/kg IM) post-wounding for five days.The wound healing processes in control and treatment groups were evaluated clinically, morphometrically, gene expression by rt-PCR and histopathologically during four periods of study, as follows:

2.4 Evaluations:

A complete clinical examination was performed daily on all animals throughout the period of the study, then the wounds areas were shaved carefully, and digital photograph was taken. By using saline, the scab of each wound was gently removed for better vision of areas of epithelialization and granulation tissue. The percentages of re-epithelialization, wound contraction and total wound healing were calculated for each wound, depending on the parameters (Bohling *et al.*, 2004) (Figure 2). Gene expression of FGF & EGF genes by rt-PCR at (0, 7 and 14) days post wounding using primers in (Table 1). The primers were used in quantification of gene expression by using RT-qPCR techniques based BRYT Green DNA binding dye (Promega, USA). The histopathological evaluation was performed post-treatment with control and treatment groups (four wounds/period). Full-thickness incisional biopsy samples were obtained (1 cm^2) they involved approximately (3-4) mm of unwounded skin on both sides of the wound which were fixed in (10%) neutral formalin solution, then exposed to routine tissue handling and sectioned at (5-6 micron) using rotary microtome and staining with Hematoxylin-Eosin (H&E) (Luna, 1992).

Table 1: showing primers which are used in rt-PCR.

Primer Name	Sequence 5`-3`	Annealing Temp. (ºC)
EGF	F-(TCCCAGGTTCTCTTAAGTGCCT) R- (AACAGCCGCTTATCAAGCACATCC) **(Frota, *et al.*, 2010)**	58
FGF2	F-(AGTGTGTGCAAACCGTTACCTTGC) R- (ATACTGCCCAGTTCGTTTCAGTGC) **(Almeida, *et al.*, 2012)**	65
GAPDH	F-(TGTTTGTGATGGGCGTGAACCA) R-(ATGGCGTGGACAGTGGTCATAA) **(Almeida, *et al.*, 2012; Kate, 2019)**	65

Fig. 1: Shows the shapes and sites of four full-thickness wounds on lateral thoracic areas.

Fig. 2: Shows the changes in wound size at four periods in control (C) and treatment (T) groups according to the time.

2.5 Preparation of Fish ADM:

According to the method described by **Mahdi (2021)** fish skin was collected by skinning and cleaning, then washing it with tap water and PBS. 10x10 cm of skin was preserved in cold PBS with 0.1 Amikacin. De-epithelization was done by putting the skin in a hypertonic solution containing tris base, sodium chloride, and ethylene diamine tetra acetic acid (EDTA) for 2 hours. After de-epithelization, the skin pieces were decellularized using 1% sodium deoxycholate for 12 hours. The fish ADM was soaked in sterile PBS and saved in a PBS solution with amikacin and sodium azide. The skin parts were shaken on an orbital shaker for de-epithelization and decellularization routes. Macroscopic and microscopic assessments have proven these processes. After decellularization, the frozen decellularized skin was melted at room temperature, then divided to pieces of 3 × 3 cm^2 and freeze-dried (lyophilized) using (vacuum freezer drier, China) at (-60 C˚) for 24 h. The freeze-dried pieces were grounded in a cryomill. The prepared powder was stored in (4) until use **(Vijay *et al.*, 2017).**

2.6 Preparation of Hydrogel:

The powder was enzymatically thawed by adding 1mg /ml pepsins solution with 0.02M (HCl) and distilled water, the prepared material concentration 50 mg/ml and pH (2.2-2.4) then it was liquified for 24 hrs. with constant stirring on a magnetic stirrer at room temperature **(Cai *et al.*, 2021)**. The resulting liquid was cooled on ice while the pH raising to 8 to inhibiting the pepsin enzyme by using (NaOH), and then pH was dropped to (7.0-7.4), the concentration of the salt was fixed by using 10x PBS to reach an isotonic solution, then the hydrogel kept at 4ºC in the freezer

2.7 Statistical analysis:

The mean and standard errors of continuous variables were reported, and significant differences were tested using the analysis of variance (ANOVA) test for days or independent samples T-test for groups, followed by the least significant difference (LSD) test. Statistical significance was defined as a probability value ($p \leq 0.05$) and ($p \leq 0.001$) **(Snedecor & Cochran,1973)**.

3.Results and Discussions:

3.1 Clinical evaluations:

Skin wounds in both groups were healed without any problems; throughout the follow-up days until

the end of study, no visible symptoms of bleeding, contamination, or pus were seen, and all animals showed normal appetite and normal physiological parameters. This might be attributed to the use of extremely effective antibacterial and suitable post-operative care and the properties of fish skin hydrogel; these results were agreed with research by **Azari *et al*. (2008)** which show there is no infection and secondary complication in wounds after 28 days follow up in goats**.** Fish skin is a useful resource for wound healing as studies have demonstrated its antiviral, antibacterial, and faster healing rates, so that acellular fish skin has antimicrobial, anti-inflammatory and analgesic effects, have excellent wound healing properties and to be an effective skin replacement material (**Magnusson, *et al*., 2017)**. Fish skin contains extracellular matrix (ECM) components including collagen and unsaturated fatty acids omega-3 fatty acids, it can function as an absorbable scaffold to fend off bacterial invasions, speed up regeneration and boost signaling during both tissue remodeling and the inflammatory stage of wound healing. **(Magnusson *et al*., 2018).**

3.2 Morphometric Results:

3.2.1 Percentage of wound re-epithelialization:

The epithelialization development in treatment wounds has a significant difference ($p<0.05$) than that of control group initiated from **14** days post wounding which was **(25.91±1.45)** in control group and **(29.17±1.25)** in treatment group. Then, the percentage of epithelialization in control group was **(82.50±0.80)** and in treatment group was **(95.50±2.13)** at **35** days post-treatment (see **Table 2**).

Table 2: Show's the means and standard error of re-epithelialization percentage in control and treatment groups.

Periods **Groups**	**7 days** **Mean ±SE.**	**14 Day** **Mean ±SE.**	**35 days** **Mean ±SE.**	**42 days** **Mean ±SE.**
Control Group	14.25±1.63	25.91±1.45	82.50±0.80	98.50±1.02
Treatment Group	15.58±1.61	29.17±1.25	95.50±2.13	100.00±0.00
p-value **LSD**	**0.000*** **5.35**			

*High Significant differences at probability value ($p\leq0.001$).

3.2.2 Percentage of wound contraction:

The percentage of wound contraction showed significant differences ($p\leq0.001$) in treatment group as compared with control group started from **7** days (**20.00±1.84**) in control group and (**25.17±2.09**) in treatment group, then reach **(88.75±1.29)** in control group and **(95.67±1.74)** in treatment group at 35 days post-treatment (see Table **3**).

Table 3 Show's the means and standard error of wound contraction percentage in control and treatment groups.

Periods **Groups**	**7 days** **Mean±SE.**	**14 days** **Mean ±SE.**	**35 days** **Mean ±SE.**	**42 days** **Mean ±SE.**
Control Group	20.00±1.84	35.67±2.01	88.75±1.29	99.20±1.05
Treatment Group	25.17±2.09	41.50±2.28	95.67±1.74	100.00±0.00

p-value	0.000*
LSD	4.50

*High Significant differences at probability value (p≤0.001).

3.2.3 Percentage of wound healing:

The percentage of wound healing showed significant differences (p≤0.001) in the treatment group as compared with control group which started from **7** days (**26.17±1.62**) in control group and (**44.33±1.84**) in treatment group and at the end of study (**89.83±1.35)** in control group and **(100.00±0.00)** in treated group at 35 days post-treatment (see Table **4**).

Table 4: Show's the means and standard error of wound healing percentage in control and treatment groups.

Groups	7 days Mean±SE.	14 days Mean±SE.	35 days Mean ±SE.	42 days Mean ±SE.
Control Group	26.17±1.62	40.33±1.71	89.83±1.35	99.10±0.03
Treatment Group	44.33±1.84	66.17±1.99	100.00±0.00	100.00±0.00
p-value	0.000*			
LSD	4.48			

*High Significant differences at probability value (p≤0.001).

According to results of our study, the treated group wounds had high percentages of re-epithelialize (P<0.001) than control group. FADPH promotes the growth of epithelial cells and forming thick collagen, which in turn reduces the size of the wounds and the distance between the edges of the wound (Biazar *et al.,* 2022). Shortly after wounding, epithelial keratinocytes become activated through the combined effects of the exposure to pro-migratory matrix molecules within the wound site, growth factors and cytokines that are released by inflammatory and other wound cells and from the blood clot and surrounding extracellular matrix, these factors necessary for re-epithelialization (Beaudry *et al.,* 2010). Wounds start to re-epithelialize a few hours after the damage. Damaged stroma and clots of blood are rapidly removed from the wound area by epidermal cells found in skin appendages like hair follicles (AL-Bayati *et al*., 2013). The FDAPH contain growth factors including TGFα EGF and FGF-2, which show a crucial part in re-epithelialization by promoting vascularization, cell migration, proliferation, and differentiation, eventually leading to ECM synthesis (Chicharro-Alcántara, *et al*., 2018).

As the healing progressed, the wound area decreased significantly at different times intervals in both groups with significant differences between them. Closure of second-intention wounds is accomplished by epithelialization and contraction (Rivera and Spencer, 2007). Because wound contraction happens more quickly than epithelialization, it has a larger role in accelerating healing. The initial wound edges moving centripetal is known as wound contraction. The contraction of myofibroblasts in granulation tissue is the cause of this process (AL-Bayati *et al.,* 2013). Myofibroblasts are necessary for the contraction and repair of wounds. Their characteristics include the existence of tension fibers with the α-actin isoform, which is expressed in smooth muscle, and their differentiation from fibroblast (Diller & Tabor, 2022).

The current study found that FADPH promotes quicker wound healing by protecting growth factors from enzymes and controlling their release in damaged areas (Park, *et al*., 2017).

The wound healing ratio showed significand differences (p≤0.001) between treatment and control groups. These results indicating that hydrogel enhanced the wound healing process, and this agree with many studies (Tang and Saito, 2015) which declare that stratified squamous epithelium with wide layers of collagen was discovered through histological investigations employing fish skin as a scaffold to repair wounds. One of the most significant ECM constituents in the skin of fish is collagen type Ⅰ promotes three crucial cytokines for wound healing: fibroblast growth factor (FGF), epidermal growth factors (EGF) and keratinocyte growth factor (KGF). Additionally, using skin dressings maintains tissue hydration, which is essential for accelerating the process of re-epithelialization and healing of wounds (Chen, *et al.*, 2019).

3.3 Molecular Results:

RT-PCR results of the genes levels in current study indicate presence of significant differences (P>0.001) in the means values of FGF &EGF genes in control and treatment groups in different periods of study. It was quiet enough that there were no significant differences at **0** times, then there was elevation in means values of these genes at **7** and **14** days post-wounding in the wounds of treatment group than control group with significant differences (p≤0.001). The FGF gene reaches (**3.15±0.29**) in control group and (**4.01±0.02**) in treatment group at **7** days post wounding and (**4.12±0.05**) in control and (**5.32±0.34**) at **14** days post wounding (**Table 6**).

The results of EGF gene reaches **(2.57±0.03**) in control group and **(4.02±0.03**) in treated group at **7** days post wounding and (**2.99±0.00**) in control group and (**5.92±0.14**) in treated group at **14** days post wounding (**Table 7**).

Table 6: Show's, the means ±SE values of b-FGF quantity in control and treatment groups

Groups	**0 day Mean ±SE.**	**7 days Mean ±SE.**	**14 days Mean ±SE.**
Control Group	0.81±0.10	3.15±0.29	4.12±0.05
Treatment Group	0.70±0.04	4.01±0.02	5.32±0.34
p-value **LSD**	0.001* 0.51		

*High Significant differences at probability value (p≤0.001).

Table 7: Show's, the means ±SE values of EGF quantity in control and treatment groups

Groups	**0 day Mean ±SE.**	**7 days Mean ±SE.**	**14 days Mean ±SE.**
Control Group	0.79±0.01	2.57±0.03	2.99±0.00
Treatment Group	0.94±0.03	4.02±0.03	5.92±0.14
p-value **LSD**	0.001* 0.20		

*High Significant differences at probability value (p≤0.001).

The current study showed that the increased levels of b-FGF gene in the tissues of wound sites at seven days post-treatment in both groups, but treatment group showing superiority, due to inflammatory response and development of the healing mechanism post-treatment, which is marked by inflammatory cell infiltration in accordance with type of hydrogel effect on the host tissue and inflammatory cell-released b-FGF (Al-Ebadi, 2018; Kate, 2019). These results agree with many studies indicating that every biologic substance stimulates an acute host response, which is represented by a strong MNC invasion (Badylak and Gilbert, 2008), while Londono and Badylack, (2015) mention that biomaterials can induce a transition from an inflammatory process to a constructive remodeling and functional tissue restoration process by enhancing growth factors production, hence modulating the various phases of the healing response. Other studies indicated that the composition of the biomaterial may influence the level and profile of the inflammatory reaction (Wooley *et al*., 2002). The increasing level of b-FGF gene in treated group indicating of more fibroblast's proliferation that led to more of collagen formation (Areeg and Ahmed 2022). The b-FGF is elevated in the acute wound because it is essential in granulation tissue formation, re-epithelialization, and tissue remodeling (Barrientos *et al*. 2008). While Werner *et al*. (2003) referred that FGFs have been found at the wound site and in the wound fluid particularly in the early phases following damage, suggesting that endogenous proteins are also regulators of wound healing. The fast early angiogenesis and repair of surgical wounds, as noted by (Gonzalez *et al*. 2016 and Al-Ebadi 2018), is partially mediated by b-FGF, which can be optionally released by cellular injury. Tissues and platelets also store b-FGF and act to deliver it to the site of injury to aid in the initiation of wound repair. Hammoodi, (2019) mention that, the using of biological materials in wound healing led to an earlier increase in the level of b-FGF at the wound site because of the biological materials' ability to attract inflammatory cells, which in turn secrete growth factors at the site of injury. Also, the progressive rise in the level of b-FGF in the healing from 5 to 30 days post-wounding was because of the existence of inflammatory cells at this time of healing.

Blood components are introduced to the site of the wound to start the clotting cascade. The clot that results from this also leads to hemostasis and facilitates an influx of inflammatory cells. Platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and transforming growth factor-beta (TGF-b) are among the growth factors produced by alpha granules, which are released when platelets degranulate (Young and McNaught, 2011). Acellular fish skin hydrogel maintains its collagen and natural structure, providing a significant benefit, these characteristics improve the structure's mechanical strength and create an ideal environment for tissue repair and regeneration (Dorweiler *et al*., 2017). GFs are kept in the biomaterials and will be released during post-application breakdown gradually and by infiltration of inflammatory cells that bind to ECM proteins, according to several studies explaining the association between GF levels and biomaterials (Badylak, 2007). Furthermore, other authors state that GFs will release and carry out their physiological effects until the implant has been destroyed once they are activated and dissociate from their binding proteins (London and Badylak, 2015). According to Hantash *et al.*, (2008) proinflammatory cytokines facilitate the recruitment of neutrophils to eradicate infected microorganisms. Macrophages also cause the production of granulation tissue by releasing proinflammatory cytokines and growth factors (FGF, EGF, TGF-b, and PDGF). The level of GFs in biomaterials can vary based on factors such as species, tissue source, manufacturing method, decellularization efficiency, and post-processing modification (London and Badylak, 2015). Fish skin grafts have been shown to increase FGF, EGF, and cytokine production in the epithelium and connective tissue during neovaginoplasty (Dias, *et al*., 2020).

3.4 Histopathological results:

The histopathological sections of the skin biopsies taken from control and treatment groups show significant differences between them. at **7** days control group was show hemorrhage, necrotic tissue, and neutrophils infiltration (**figure 2**) while treated group revealed mild hemorrhage, severe infiltration of neutrophils in the dermal layer, with proliferation of fibroblasts and fibrocytes with formation of newly blood vessels (**figure 3**), at the same period. On **14** days post wounding control

group shows focal aggregation of inflammatory cells consist of polymorph nuclear cells (PMNCs) and mononuclear cells (MNCs) surrounded by irregular collagenous tissue (**figure 4**), while treated groups shows regeneration of epidermal epithelia which characterized by elongation and vacuolation of the cytoplasm with severe congestion of blood vessels under the epithelial layer and formation of immature granulation tissue, the dermis showed fibrous encapsulation (**figure 5**). On **35** days post-wounding the control group shows dense fibrous tissue and thick regenerated epithelia with hyperactive basal layer (**figure 6**), while treated group revealed regeneration of epithelial cells of the dermal layer with proliferation of fibrous tissue in the dermis, perivascular cuffing consists of inflammatory cells mainly MNCS with proliferation of mature collagenous and fibrous tissue (**figure 7**). Finally at **42** days post wounding both groups show complete regeneration of the epidermal layer, the dermis shows proliferation of dense fibrous tissue, vacuolation of the cytoplasm of the epidermal epithelia with keratin formation (**figure 8,9**).

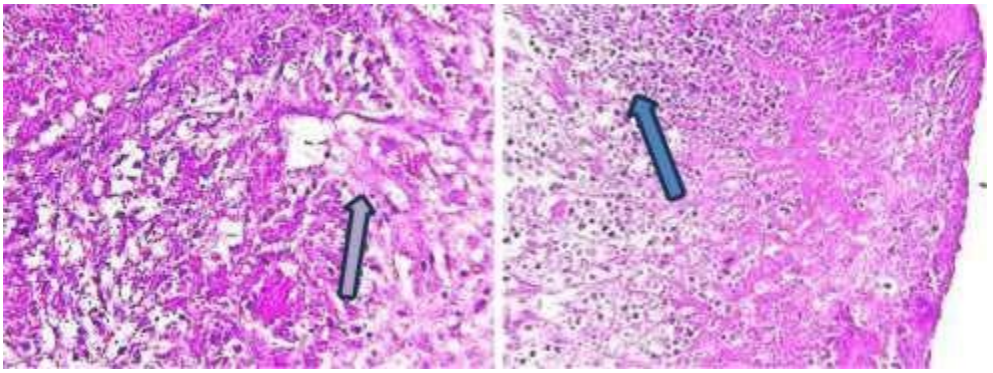

Fig 2: Histopathological section of skin wound from goat in control group at **7** days post wounding, shows hemorrhage, necrotic tissue (yellow arrow) and neutrophils infiltration (blue arrow) (H & E stain 400x, 200x).

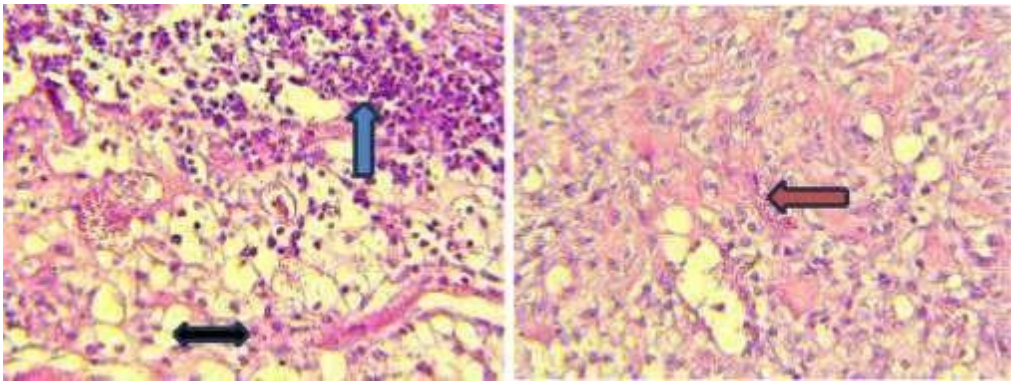

Fig 3: Histopathological section of skin wound from goat in treated group at **7** days post wounding show's severe infiltration of neutrophils in the dermal layer (blue arrow), with proliferation of fibroblasts and fibrocytes (black arrow) with formation of newly blood vessels (orange arrow) (40x).

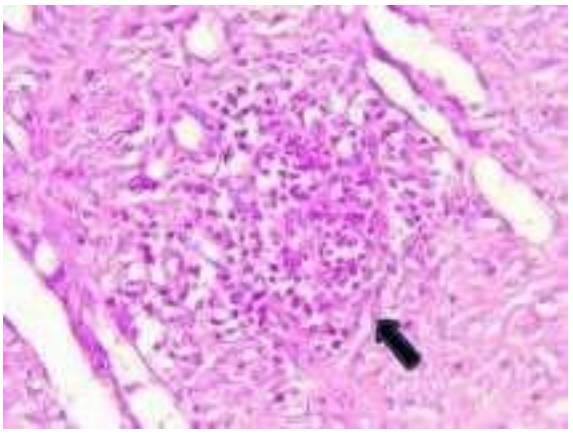

Fig. 4: Histopathological section of skin wound from goat in control group at **14** days post wounding, shows focal aggregation of inflammatory cells consist of PMNCs and MNCs surrounded by irregular collagenous fiber (black arrow) (H & E stain 100×).

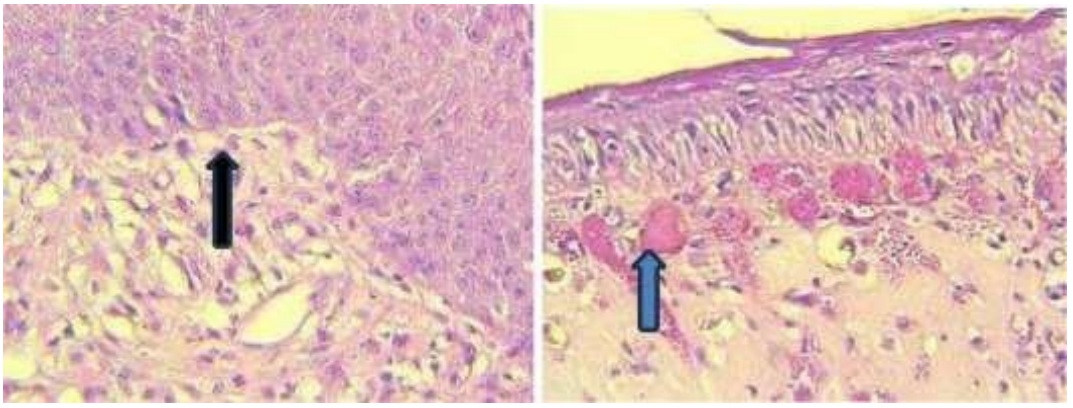

Fig 5: Histopathological section of skin wound from goat in treated group at **14** days post wounding show's regeneration of epidermal epithelia which characterized by elongation and vacuolation of the cytoplasm (black arrow) with severe congestion of blood vessels under the epithelial layer (blue arrow) and formation of immature granulation tissue (40x H&E).

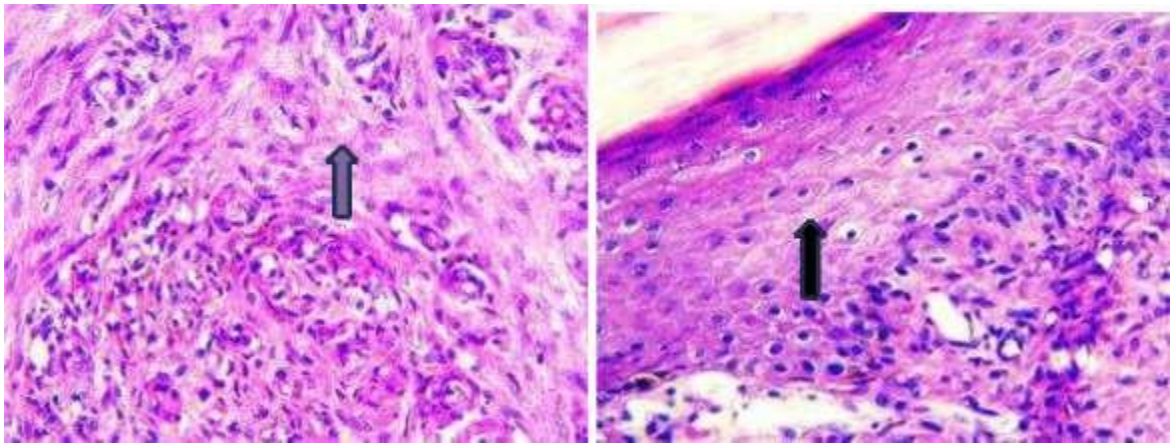

Fig 6: Histopathological section of skin wound from goat in control group at **35** days post wounding show's dense fibrous tissue (yellow arrow) and thick regenerated epithelia with hyperactive basal layer (black arrow) (40x H&E).

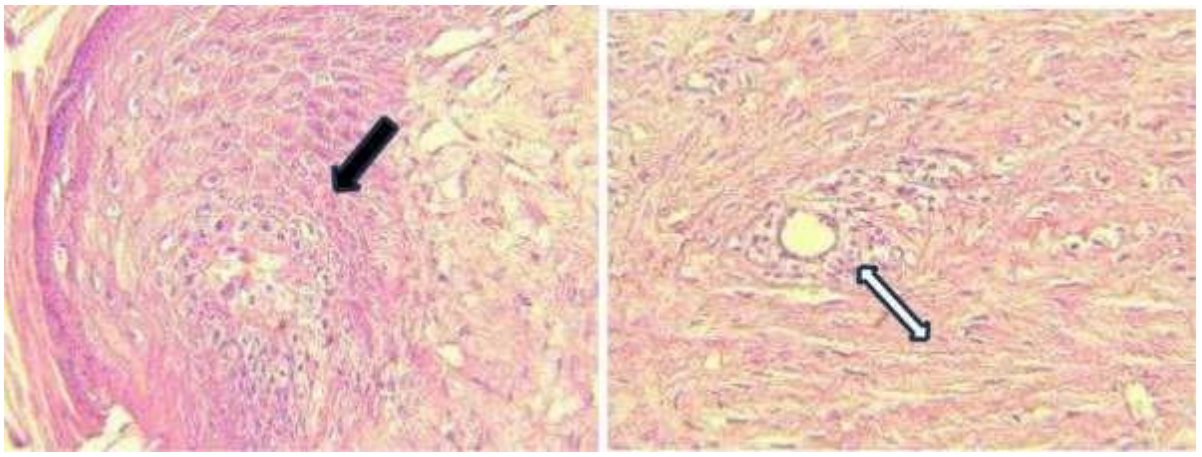

Fig 7: Histopathological section of skin wound from goat in treated group at **35** days post wounding show's regeneration of epithelial cells of the dermal layer with proliferation of fibrous tissue in the dermis (black arrow), perivascular cuffing consists of inflammatory cells mainly MNCs with proliferation of mature collagenous and fibrous tissue (white double head arrow) (40x H&E).

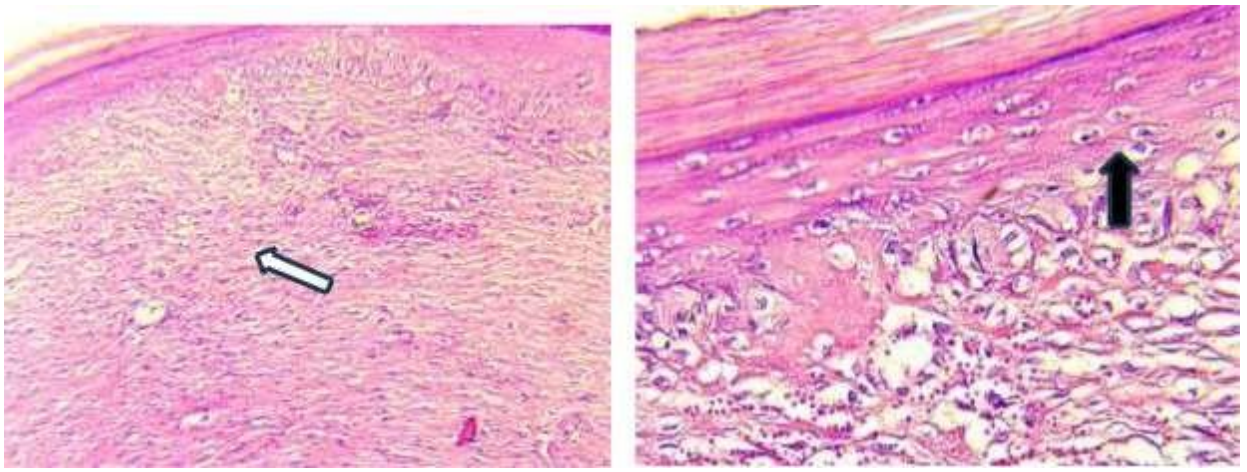

Fig 8: Histopathological section of skin wound from goat in control group at **42** days post wounding shows complete regeneration of the epidermal layer (black arrow), while the dermis shows proliferation of dense fibrous tissue, vacuolation of the cytoplasm of the epidermal epithelia with keratin formation (white arrow) (20x &40x H&E).

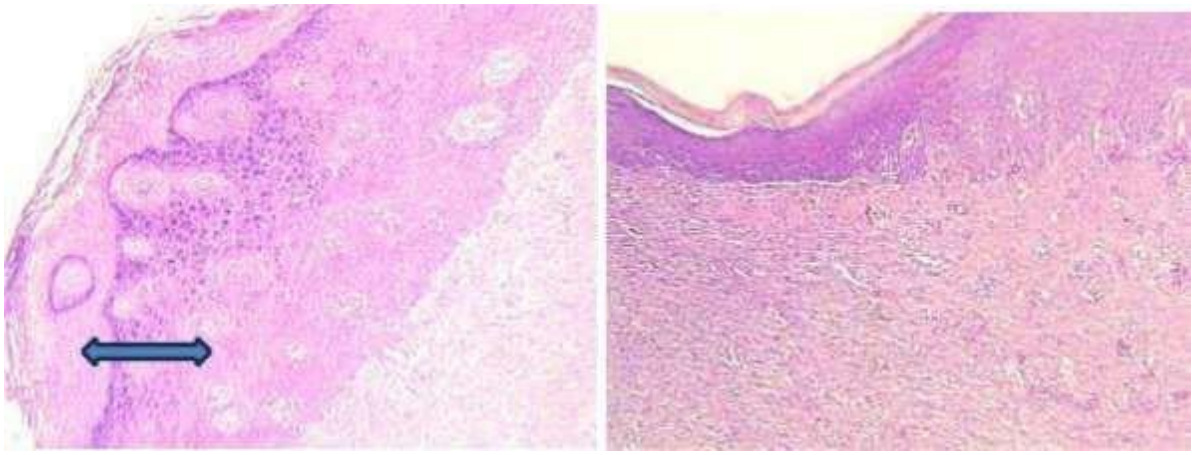

Fig 9: Histopathological section of skin wound from goat in treated group at **42** days post wounding shows complete regeneration of the epithelial layer of the epidermis tissue with dense fibrous tissue of the dermis layer with keratin formation (blue double head arrow) (20x H&E).

Histopathological results of current study found that the healing process of treated group was

faster than the control group, with early signs of healing such as reactive fibroblasts, enlarged fibroblasts, collagenous stroma, fibrous encapsulation and collagen formation. This could be due to differences in the quantity and quality of cells triggering healing, the composition of biomaterials, which promotes early inflammatory cell infiltration, and the variance in healing progression levels between the treatment and control groups (Hammoodi, 2019). Sections of the histopathology show bleeding and within the first seven days of treatment, development of granulation tissue, inflammatory cell infiltration was present in both groups, showing fast response to injury. However, the treated group had a severe response to wound healing compared to the control group, this agrees with Azari *et al*. (2008) who observed that in goats, the newly formed stroma, or granulation tissue invades the wound area around four days after trauma. This granular stroma is created by new capillaries, at the same time fibroblasts and macrophages enter the wound area. Fibroblasts create extracellular matrix for cell development, macrophages supply growth factors for fibroplasia and angiogenesis, and blood vessels transport nutrients and oxygen for cell metabolism. According to Werner and Grose (2003), growth factors along with the extracellular matrix (ECM) encourage fibroblasts in the surrounding tissue to multiply and create collagen fibers. Fibroblasts are also in charge of synthesizing, depositing, and remodeling the extracellular matrix. For the freshly produced granulation tissue to survive, new blood vessels must develop.

The sections at 14 days post wounding in control group show's focal aggregation of PMNC and MNCs with irregular collagen fibers while treatment group show's regeneration of epidermal epithelia with formation of granulation tissues, FDAPH contain growth factors which play a crucial role in epithelialization of epidermis by promoting cell migration, proliferation, differentiation and angiogenesis, eventually leading to extracellular matrix synthesis and granulation tissue formation (Chicharro-Alcántara, *et al*., 2018). The acellular fish dermal matrix is wealthy origin of collagen and amino acids such as proline and alanine so that it can promote the proliferation of fibroblasts, formation of the granulation tissue, and produce of collagen in the wound site (Biazar, *et al*., 2022). Angiogenesis is triggered by many angiogenic factors, including bFGF and VEGF, and is an essential mechanism for the preservation of granulation tissue and accelerating wound healing (Li, *et al*., 2019). The outcomes of our research indicate that the wound tissue from the treated group had much higher levels of bFGF gene expression than the control group. This suggests that the collagen obtained from fish skin has a strong capacity to stimulate the synthesis of these angiogenic agents (Chen *et al.,* 2021), the superior expression of this factor is markedly involved in promoting the wound healing process by regulating the inflammatory response and promote angiogenesis and collagen deposition. The complex progression of angiogenesis depends on the ECM present in the injury bed, endothelial cell migration, and mitogenic activation (Wu *et al*., 2007). Eming, (2007) study discovered that inflammatory response is crucial for supplying growth factor and cytokine signals, which are necessary for cell and tissue movements in mammalians.

On 35 days post wounding in control group figures show's dense fibrous tissue and thick regenerated epithelia with hyperactive basal layer, while treated group exposed complete renewal of epithelial cells of the dermal layer with proliferation of fibrous tissue in the dermis, perivascular cuffing contains of inflammatory cells mostly MNCS with propagation of mature collagenous and fibrous tissue. Fibroblasts provide extracellular matrix, while macrophages provide growth factors for angiogenesis and fibroplasia (Sunderkötter *et al*., 1994). Previous research, including Baldursson *et al*., (2015) study found that fish ADM lipids and proteins facilitate faster wound healing in full-thickness wounds. Alam and Jeffery (2019) study found that fish skin xenografts had a shorter median healing period for burn injuries, when Fish ADM was used for skin grafting, resulting in a half-healing period. Scarring appeared on day 35 after incision, and skin gradually healed from wound edges (Badois *et al*., 2019). The new epidermis showed potential changes, with a higher proliferative index at the wound center compared to the wound periphery, where the neoepidermis was more differentiated and the dermo-epidermal junction was regenerating (AL-Bayati *et al*., 2013).

2. Conclusion:

In summary, our research has shown that the use of FADPH aided in the recovery of full thickness skin wounds in bucks, as seen by better injury close during the proliferative stage of the healing process and increasing of GFs gene expressions. FADPH offers the perfect environment to promote the growth of dermal tissue, which leads to favorable healing results. It also has strong biocompatibility and may speed up the healing of wounds. The model of acute and sterile wound repair applied in this work is important since it does not suggest a different mechanism for poor healing in chronic and contaminated wounds.

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Authors' Contribution: Research is extracted from a doctoral Desertion of the Ph. D for the Student Yasir Salah, University of Baghdad/ Baghdad/ Iraq. Asst. Proof Dr Alawadi (adviser) and Dr. prof. Albayati (secondary adviser) designed all the study experiments. Yasir Salah performed all the experiments, collected all samples of fish and goat skin and experiment animals and conduct the experiment, and wrote the draft of the research under Dr Alawadi and Albayati supervision contributed to check the analyses of the data to the finalize the manuscript for journal submission. All authors approved the final version of the current manuscript for publishing in the respected journal.

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