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Accelerating γ-irradiated Skin Wound Healing in Rats by Human Amniotic Membrane and/or Chitosan

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SKIN wounds usually a reason of physical pain for patients, also are an economic load for them. It is an essential to find out an efficient approach to stimulate skin healing. Seventy-two male rats were equally divided into 8 groups; rats were submitted to a surgical damage induction except group1 as follow: Group1; control. Group2; gentamycin. Group3; γ-rays (8Gy). Group4; human amniotic membrane. Group5; Chitosan. Group6; γ-rays+ human amniotic membrane. Group7; γ-rays+ Chitosan. Group8; γ-rays+ human amniotic membrane + Chitosan. Three rats from each group were decapitated on the 3rd, 7th and 21st day after wound made. Histopathological study, immuno-histochemical staining for epidermal growth factor receptor (EGFR) and biochemical parameters; tissues tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β) and interleukin-6 (IL-6) in wound area of skin samples were estimated. γ-irradiation of the rats' skin wound delayed the healing process with less re-epithelization and prominent fatty tissues with severe rise in the level of the proinflammatory markers. Treatment of rats' irradiated skin wounds with amniotic membrane and/or Chitosan improved the healing process, as indicated by pathological, biochemical and EGFR stain results. The repairing in the wound area was the best in the group4 compared to other rats' groups. Also, group6 at all the experimental times was significantly better than all the other groups treated with γ-rays. **Conclusions:** Treatment of irradiated rats' skin wounds by amniotic membrane and/or Chitosan enhanced the healing of wounds. However, group4 was the best at all times of the experiment compared to the other groups.

Keywords: *skin, wound, human amniotic membrane, Chitosan, γ-rays, rats.*

Introduction

The skin wound was global circumstances obtained by surgery, grazing, scolding, chronic ulcers, radiation or other trauma. Skin wound repairing is a physiological process exaggerated by several reasons, which may be enhanced by accurate wound treatment with real wound maintenance supplies. Best wound repair materials should be static, flexible, decomposable, and commonly appropriate, with the capability to save wounds rest bleeding, moist and adsorb exudate (Abd El-Hack et al., 2020).

Radiation is used beside chemotherapy after or before surgery dealing with malignancy solid-tumors. Although effective in treating several malignances, radiation of covering normal tissues may cause severe complications. high-cell turnover tissues, as well as the integument are most commonly affected (Yu et al., 2021). Acute skin damage after radiation may persist for days or weeks, characterized by temporary erythema and painfulness, dehydrated and wet desquamation hyper-pigmentation, and initial ulceration (Bolton, 2020).

The villous placenta, (Jauniaux et al., 2022; Zhao et al., 2022) fetal membranes (Sandora et al., 2022; Doudi et al., 2022) and amniotic fluid have vital roles in healing (Nyman et al., 2022). The results of extensive studies discovered that the amniotic epithelial cells share several of the features of pluripotent embryonic stem cells

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and multi-potent mesenchymal stem cells and differentiate into multiple cell lineages in vitro (Orietti et al., 2021). Amniotic epithelial cells may produce several elements which could enhance healing of tissues (Fathi & miki, 2022).

Chitosan, an innate polysaccharide used frequently as wound healing material that has antibacterial and hemostatic assets as well as the ability to help granulation tissues progress. Compared to other forms, hydrogels have the benefits of tissues union and water-retaining activities, with the capability to make physical barriers for wound and suitable cooling effect that relieves pain (Feng et al., 2021). In order to improve the effects of chitosan-based hydrogel in the fate wound regeneration and repair, recent endeavors or investigations have been achieved or conducted in new year's (Feng et al., 2021; Wei et al., 2022).

This work aims to study the efficiency of human amniotic membrane and Chitosan in boosting the healing of skin wounds exposed to γ-rays.

Material and methods

Experimental animals

Seventy-two adults male *Wistar* rats were used (weighting 250-300g). Animals were obtained from the animal house that belongs to the NCRRT, Cairo, Egypt. Animals were maintained under standard ventilation conditions and had free access to water and normal concentrated pellet and were adapted in standard designed cages 9 rats per cage in a 12/12-h light-dark cycle and under standard pressure and temperature conditions.

All animal treatments were carried out according to the Ethics Committee of the National Research Centre standards and guidelines and in accordance with the recommendations for the proper care and use of laboratory animals published by the National Institutes of Health (HIN publication No. 85–23, revised 1985). The study was approved by the Central Scientific Publishing Committee, Egyptian Atomic Energy Authority. Rf. (203) - 10/3/2021.

Radiation facility

Rats were whole-body gamma-irradiated with a single dose of 8Gy using an indoor shielded AECL/Cesium 137 Gamma cell-40 biological irradiator (Atomic Energy of Canada Ltd, Ottawa, Ontario, Canada), installed at the "National Centre for Radiation Research and Technology (NCRRT)", Atomic Energy Authority, Cairo, Egypt. The dose rate was 0.37 Gy/minute at the time of experimentation.

Experimental surgical procedures

The animals were anesthetized with xylazine hydrochloride (2%, 0.01 ml/kg) and ketamine hydrochloride (10%, 0.005 ml/kg) intramuscularly after weighing. Next, a 6cm^2 area on the abdominal side of the animal was shaved with an electric clipper, and local antisepsis was done using 0.5% topical chlorhexidine alcohol. The surgical wounds were done on all groups except group1, determining 3 cm in diameter and 1 mm in depth, were made by a standardized fashion with the help of a circular metal instrument with a metal blade (nº4) removing the whole thickness of the skin. Immediately after surgical excision, the lesions of the animals in all groups received gentamycin ointment locally on wounds for seven successive days, after completing the surgical procedures, the rats in all groups except group1 were kept in individual cages.

Chemical reagents

Patent amniotic membranes were used; REGE pro, purified biological membrane, sterilized by gamma radiation, made in Egypt at NCRRT.

REGE-pro is a natural collagen rich matrix that promotes re-epithelialization and granulation progression in wounds, burns, and ulcers. In addition, REGE-pro reduced scar microbial infection, inflammation and pain. The REGE-pro membrane is applied in direct contact with the wound.

Preparation of REGE-pro:

The amniotic membrane was dissected from placenta obtained from healthy women after caesarean delivery. Donors were tested for syphilis, hepatitis-B virus, hepatitis-C virus, and human immunodeficiency virus. The amniotic membrane processing was performed by washing out blood and debris by sterile isotonic solutions; 0.9% sodium chloride. Cleaned amniotic membrane was dried and packaged into polyamide bags and finalized by sterilization into 60Co gamma cell, dose 25 kGy; the recommended dose stated by the International Atomic Energy Agency (IAEA) for tissue allograft sterilization (Singh et al., 2016).

Chitosan was purchased from Aladdin, Shanghai, China. The moisture content was not greater than 5%, the degree of deacetylation greater than 80%, and ashes no more than 1%.

Experimental design

Seventy-two male rats were divided into eight equal groups (n=9) as follows: Group1 (Normal intact skin; animals were shaved only). Group2 (wound treated with gentamycin ointment; gentamycin ointment topically was applied on the wound for 7 successive days). Group3 (γ-rays exposure; gentamycin ointment topically applied on the wound, and one hour after wound was made, rats were exposed to a single dose of 8Gy γ-rays). Group 4 (human amniotic membrane; gentamycin ointment topically applied on the wound, and one hour after wound was made, amniotic membrane was applied daily on the wound for 7 days). Group5 (Chitosan; gentamycin ointment topically was applied on the wound, and one hour after wound was made, Chitosan was applied daily on the wound for 7 days). Group6 (γ-rays+ human amniotic membrane; gentamycin ointment topically applied on the wound and one hour after wound was made, rats were irradiated with a single dose of 8 Gy γ-rays then amniotic membrane was applied daily on the wound for 7 days). Group7 (γ-rays+ Chitosan; Gentamycin ointment topically applied on the wound and one hour after wound was made, rats were irradiated with a single dose of 8 Gy γ-rays, then Chitosan was applied daily on the wound for 7 days). Group8 (γ-rays+ human amniotic membrane+ Chitosan; gentamycin ointment topically applied on the wound and one hour after wound was made, rats were irradiated with a single dose of 8Gy γ-rays, then amniotic membrane and Chitosan were simultaneously applied daily on the wound for 7 days).

Sample collection and preparation

After an overnight fast, three animals from each group were decapitated on the 3rd, 7th and 21st day from the beginning of the experiment (after wound was made). Skin samples were collected and divided into two parts. The first part was washed, and a homogenate (10% weight/volume w/v) was prepared in phosphate-buffered saline (0.02M sodium phosphate buffer with 0.15 M sodium chloride, pH7.4) using Teflon-glass homogenizer (Glass-Col, Terre Haute, Ind., USA) and centrifuged at 1000xg for 15min in a cooling refrigerated centrifuge (K3 Centurion Scientific Ltd, London, UK). The supernatant was obtained for the further biochemical analysis. The second part was put in 10% buffered formalin for histopathological study.

Biochemical analysis

Assessment of TNF-α, IL-1β and IL-6 was performed using ELISA method (Bio Source International, Camarillo, CA, USA) according to the manufacturer's instructions (Thermo Scientific Multiskan MK3, USA).

Immunohistochemical staining

Immunohistochemical staining for epidermal growth factor receptor (EGFR) was performed on the tissue microarray slides using the EGFR pharm Dx kit (Dako Cytomation, Carpentino, CA, USA) according to the manufacturer's instructions. The stains were scored as no stain when there was no specific membrane staining within the tissues, and positive when there was any staining of wound cells membrane above background level. The positive cases were further classified into nil, few, mild, moderate to intense based on the staining intensity (Shia et al., 2005).

Histopathological study

The skin sections were fixed in 10% neutral formalin solution. After 5days, skin was dehydrated through a series of graded alcohol, embedded in paraffin, and cut into 4-micron sections and stained with hematoxylin and eosin (H & E) according to Bancroft et al. (2008) and examined with a light microscope (Olympus, Japan).

Statistical analysis

All data are presented as means+ SE. Statistical analyses of the results were calculated using ANOVA followed by LSD as Post Hoc-test. Acceptable significance was recorded when the P-values were less than 0.05 (Mishra et al., 2019).

Results

Inflammatory markers

The inflammatory markers; TNF-α, IL-1β, IL-6 (Tables 1-3) in Group1 showed no significant changes during all the experimental period from the 3rd to the 21st day.

The inflammatory markers on the 3rd day: In groups2-8, the level of TNF-α, IL-1β and IL-6, exhibited a significant rise (P<0.05), related to group1. The highest increase was recorded in the group3 followed by group2. While the lowest increase was documented in the group4 (Tables 1-3).

The inflammatory markers on the 7th day: In groups2-8, the level of TNF-α, IL-1β and IL-6, was lower than the 3rd day, but still higher than the corresponding level in group1 (Tables 1-3).

The inflammatory markers on the 21st day: The level of TNF-α, IL-1β and IL-6 reached the normal value in group4 and was approximately normal in group5. While the highest level of TNF-α, IL-1β and IL-6 was recorded in group3, followed by group2, compared to its level in group1 (Tables 1-3).

TABLE 1. The level of skin tissues Tumor Necrosis Factor alpha (TNF-α) (pg/ mg tissue) in the different rat groups at different times.

Animal groups	3 days	7 days	21 days
Normal Skin	12.4± 0.97 ^{aA}	12.4± 0.73 ^{bA}	12.4± 0.88 ^{bA}
Control (Wounded)	56.1± 2.77 ^{bA}	22.4± 1.56 ^{bB}	23.1± 2.02 ^{bB}
γ-rays	65.2± 1.90 ^{cA}	38.4± 2.11 ^{cB}	35.1± 1.44 ^{cC}
Human amniotic membrane	28.7± 3.00 ^{dA}	25.6± 2.07 ^{bA}	13.6± 1.80 ^{aC}
Chitosan	39.8± 0.89 ^{eA}	34.2± 3.00 ^{eA}	15.4± 2.31 ^{aC}
γ-rays + Human amniotic membrane	35.7± 1.99 ^{fA}	30.6± 0.97 ^{fA}	23.2± 1.55 ^{bC}
γ-rays + Chitosan	45.1± 2.02 ^{gA}	39.2± 1.40 ^{eA}	20.5± 2.11 ^{aC}
γ-rays+ Human amniotic membrane+ Chitosan	42.1± 1.77 ^{gA}	26.1± 2.00 ^{bB}	19.7± 1.11 ^{aC}

- The superscripts A, B, C in the same row indicate the significance of changes at different times in the same group. Similar letters signify non- significantly different. Different letters mean significantly different at P<0.05.
- The superscripts a, b, c, d, e, f, g in the same column, indicate the significance of changes at different times between the different groups. Similar letters signify non- significantly different. Different letters mean significantly different at P<0.05.
- a Significance vs normal; b Significance vs control; c Significance vs γ- rays. d Significance vs Human amniotic membrane group. e Significance vs chitosan; f Significance vs γ- rays + Human amniotic membrane +; g Significance vs chitosan.

TABLE 2. The level of skin tissues Interleukin 1 Beta (IL-1β) (pg/ mg tissue) in the different rat groups at different times.

Animal groups	3 days	7 days	21 days
Normal Skin	14.6±1.29 ^{aA}	14.6±1.22 ^{aA}	14.6±1.18 ^{aA}
Control (Wounded)	61.2±1.29 ^{bA}	24.1±2.89 ^{bB}	24.4±1.90 ^{bB}
γ-rays	69.1±3.21 ^{cA}	40.3±3.20	30.2±2.88 ^{cC}
Human amniotic membrane	36.5±1.99 ^{dA}	27.3±1.88 ^{bB}	15.5±1.22 ^{aC}
Chitosan	47.3±2.00 ^{eA}	37.2±1.80 ^{eB}	16.2±1.78 ^{aC}
γ-rays + Human amniotic membrane	41.1±3.76 ^{fA}	30.3±2.99 ^{fB}	20.5±1.29 ^{fC}
γ-rays + Chitosan	50.9±1.75 ^{gA}	42.3±2.20 ^{eB}	22.0±2.40 ^{gC}
γ-rays+ Human amniotic membrane+ Chitosan	49.0±1.98 ^{gA}	27.2±2.50 ^{bB}	20.6±1.87 ^{fC}

- The superscripts A, B, C in the same row indicate the significance of changes at different times in the same group. Similar letters signify non- significantly different. Different letters mean significantly different at P<0.05.
- The superscripts a, b, c, d, e, f, g in the same column, indicate the significance of changes at different times between the different groups. Similar letters signify non- significantly different. Different letters mean significantly different at P<0.05.
- a Significance vs normal; b Significance vs control; c Significance vs γ- rays. d Significance vs Human amniotic membrane group. e Significance vs chitosan; f Significance vs γ- rays + Human amniotic membrane +; g Significance vs chitosan.

TABLE 3. The level of skin tissues Interleukin 6 (IL-6) (pg/ mg tissue) in the different rat groups at different times

Animal groups	3 days	7 days	21 days
Normal Skin	10.4± 1.55 ^{aA}	10.4± 1.42 ^{aA}	10.4± 1.53 ^{aA}
Control (Wounded)	52.1± 3.25 ^{bA}	20.3± 3.66 ^{bB}	19.3± 3.85 ^{bB}
γ-rays	53.7± 4.25 ^{bA}	35.4± 4.15 ^{cB}	25.8± 3.77 ^{cC}
Human amniotic membrane	25.4± 3.00 ^{dA}	22.9± 3.11 ^{bA}	11.0± 3.20 ^{aC}
Chitosan	35.9± 3.99 ^{eA}	31.2± 4.00 ^{eA}	13.4± 3.29 ^{aC}
γ-rays + Human amniotic membrane	29.8± 2.25 ^{fA}	25.8±2.88 ^{fA}	16.0± 3.11 ^{dC}
□-rays + Chitosan	40.8± 2.16 ^{gA}	35.3± 1.54 ^{eA}	18.7± 1.25 ^{eC}
γ-rays+ Human amniotic membrane+ Chitosan	39.7± 4.63 ^{gA}	23.4± 2.25 ^{fB}	15.6± 1.66 ^{dC}

Legends as for Table 1

Histopathological findings

In group1: The skin is arranged in three principal layers; the epidermis, the dermis, and the hypodermis (hypodermal fat). The emphasis of this topic is on the epidermal and dermal layers of skin. Skin appendages such as sweat glands, hair follicles and sebaceous glands are located in dermis layer (Fig. 1).

Rat skin wound on the 3rd day: Group2; the wound is containing necrotic debris, leukocytes, and proliferation of granulation tissues within wound defect and surrounding tissues (Fig. 2A) and numerous leukocytes (++) with less granulation tissues in the wound gap are detect (Fig. 2a). While in group 3 there are extra-vascular hemorrhage and little fibrous tissues containing massive leukocytes (+++), and little young fibrous tissues are shows in the wound gap (Fig. 2B & b). But in group6, maturation of fibrous tissues which filling the majorities of the gap and beginning of re-epithelialization of the epidermis above the wound gap beside less vascularization and containing few leukocytes (+) are recorded in the wound gap (Fig. 2C & c). Wound defected in group7 is devoid from blood and permanent fibrous tissues containing little collagen fibers with proliferation capillaries sprouts, few leukocytes (+) scatter with young fibrous tissues in wound gap (Fig. 2D & d). In group 8, the reduction of wound gap with maturation of fibrous tissues and permanent granulation tissues containing numerous capillaries within immature fibrous tissues are reveal (Fig. 2E & e).

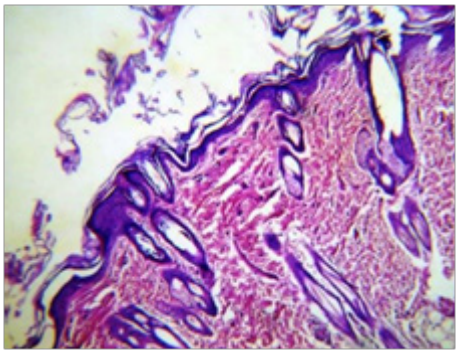

Fig. 1. Normal intact skin rat: normal epidermis, dermis and skin adnexa (H&E 100x).

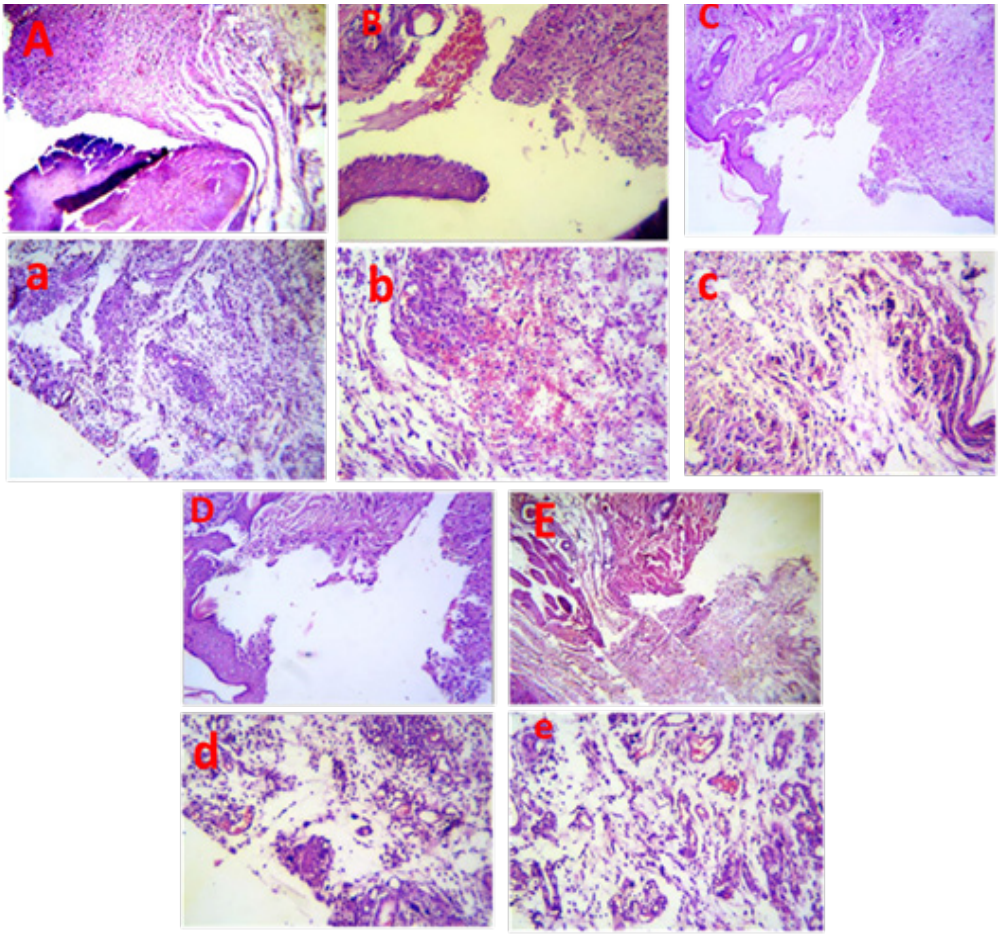

Fig. 2. photomicrograph of histopathological examination of rat's skin wound in the different groups on the 3rd day [H&E; A, B, C, D. E (200 x) a, b, c, d, e (100x)], A)

The skin wound on the 7th day: in group2, permanent granulation tissue's containing moderate leukocytes (++) are shows in the wound gap (Fig. 3A). While in group3 persistent necrotic tissues in the center of the wound encircled by edematous granulation tissues are detect (Fig. 3B). In group6 reduction of the wound gap with little crust and re-epithelialization of the wound are notice (Fig. 3C). Likewise permanent mature granulation tissues and little crust on the surfaces are present at wound area in group7 (Fig. 3D). In group8, complete filling wound gap by granulation tissues are pronounce with proliferative skins adnexa, re-epithelialization of wound border and few inflammatory cells (+), Fig. 3 E.

The skin wound on the 21st day: Group2 shows less re-epithelization of wound with complete filling of the wound by fibrous tissues and fat (Fig. 4 A). In group3 the wound defect shows maturation of fibrous tissues, less re-epithelization with prominent fatty tissues and lack inflammatory cells (Fig. 4 B) while in group6 the epidermis and skin adnexa are full the majority of the wound gap (Fig. 4 C). Furthermore, in group7 wound gap is shows incompletely covered by epidermis and contain collagen fibrous and few fat tissues (Fig. 4 D). In group8 the wound gap are contains collagen deposits, covered by epidermis and fat in vicinity of the wound (Fig. 4 E).

In groups (4 & 5): Skin wound healing is considered better than the other groups at all experimental times.

Immunohistochemistry

The EGF stain on the 3rd day from wound made, revealed few, nil, intense, mild to moderate stains in groups (2-8), Fig. 5 A, B, C, D, E. There are no stains in rat groups on the 7th and 21st day from wound made.

Discussion

The reliability of fit skin plays an essential role in conserving physiological homeostasis of the human and animal body. The integument is the basic structure tissue of all creature's bodies. It plays a crucial role in the defense against infections

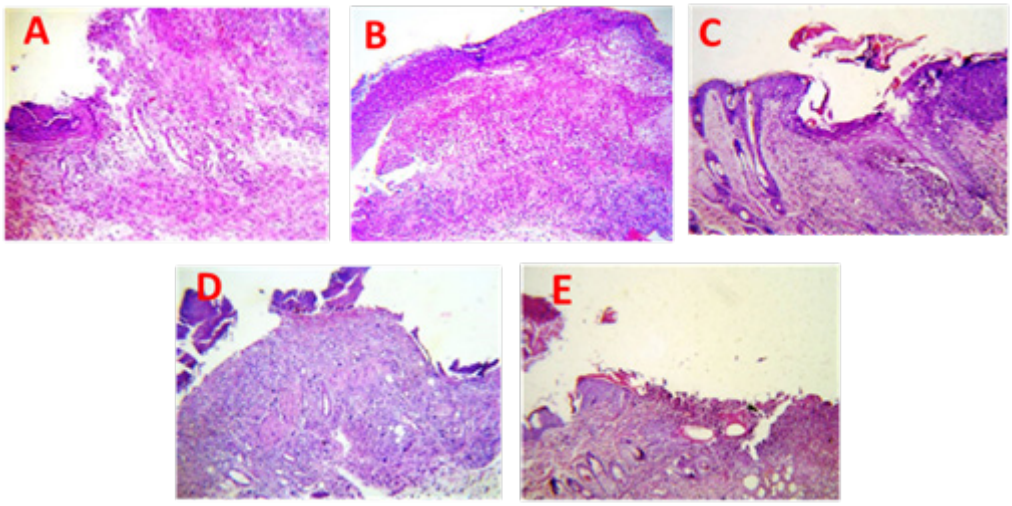

Fig. 3. photomicrograph of histopathological examination of rat's skin wound in the different groups on the 7th day (H&E 100x). A) Control: permanent granuolation tisues contaning numerous leukocytes in the wound gap B) γ-rays: presistant necrotic tissues in the center of the wound encircled by edematous granulation tissues. C) γ-rays+ Human amniotic membrane: reduction of the wound gap and little crust and re-epithelialization of the wound. D) γ-rays+ chitosan: permanent mature granulation tissues and little crust on the surfaces. E) γ-rays+ Human amniotic membrane+ chitosan: complete filling wound gap by granulation tissues with proliferative skins adnexa and re-epithelialization of the border.

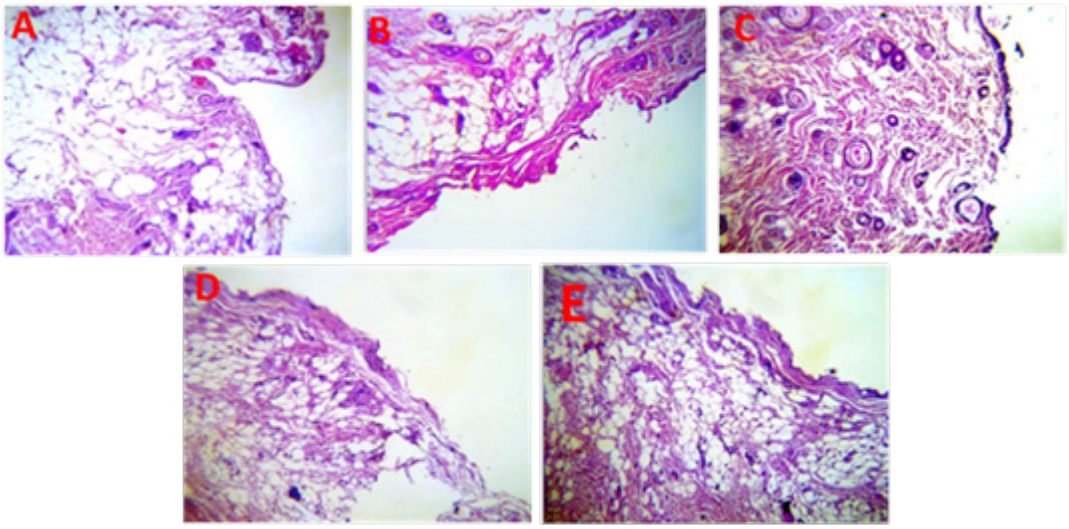

Fig. 4. Histopathological of histopathological examination of rat's skin wound in the different groups on the 21st day (H&E 100x). A) Control: less re-epithelization of wound with complete filling of the wound by fibrous tissues and fat. B) γ-rays: maturation of fibrous tissues, less re-epithelization with prominent fatty tissues. C) γ-rays+ Human amniotic membrane: the epidermis and skin adnexa filled the majority of the wound gap. D) γ-rays+ chitosan: wound gap not completely covered by epidermis but contain collagen fibrous and fat tissues. E) γ-rays+ Human amniotic membrane+ chitosan: wound gap contains collagen deposits and partially covered by epidermis and fat in vicinity of the wound.

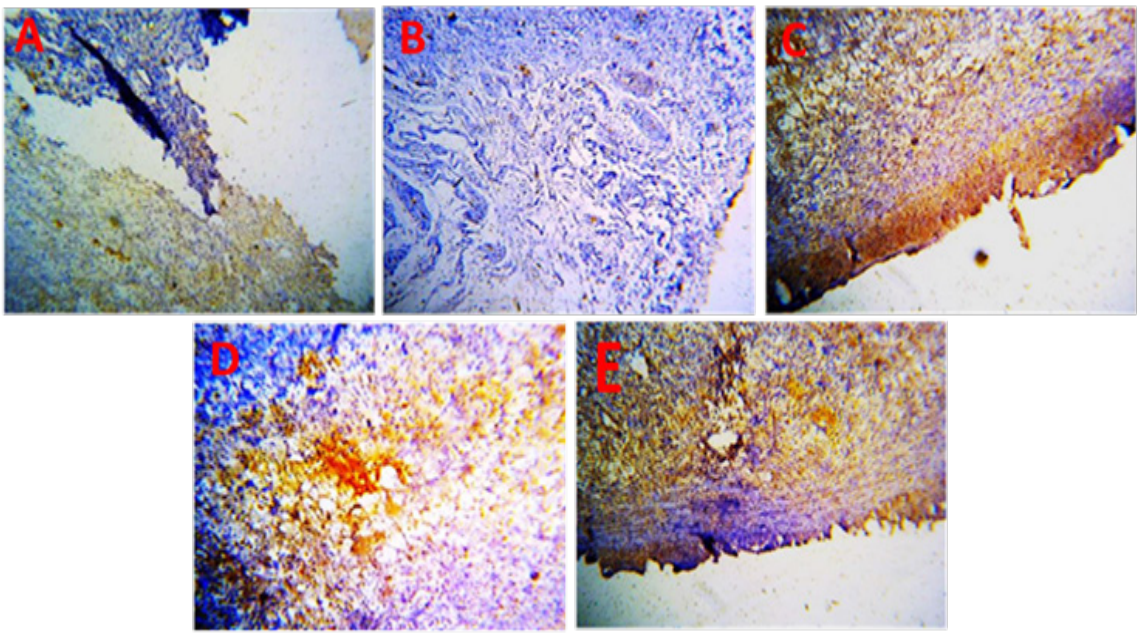

Fig. 5. photomicrograph of histopathological examination of Immunohistochemical staining (EGFs 100x) for epidermal growth factor receptor (EGFR) in the different groups on the 3rd day. A) Control: low immunohistochemical deposits of vascular epidermal growth factors. B) γ-rays: no stain. C) γ-rays+ Human amniotic membrane: intense immunohistochemical deposits of vascular epidermal growth factors. D) γ-rays+ chitosan: mild immunohisto-chemical deposits of vascular epidermal growth factors. E) γ-rays+ Human amniotic membrane+ chitosan: moderate immunohistochemical deposits of vascular epidermal growth factors.

and mechanical forces, thermal dysregulation and fluid imbalance. Several occurrences lead to insufficient wound repair which demands therapeutic interference. Prolonged circumstances such as diabetes mellitus, radiation or peripheral vascular sickness can lead to weakened wound healing. The damage of skin could follow an acute trauma distress such as significant thermal wounds utility rendering the organism susceptible to infections, thermal dysregulation, and fluid loss (Sorg et al. 2017). In wound healing, the inflammatory phase is essential to be a preliminary process for the creation of new tissues. A monocyte-derived cytokine TNF-α is a highly preserved fragment that may be played a main role in the pathogenesis of gram (-ve) injury. Also, TNF-α could play either a useful or injurious role in wound remedial (Chen et al., 2024).

The current study reveals a significant increase in the level of skin tissues inflammatory factors which was recorded in all wounded groups, compared to group 1, especially on the 3rd day. This was followed by a progressive decrease on the 7th day with a tendency towards normal level on the 21st day. The highest increase of TNF-α, IL-1β, and IL-6 levels occurred in group 3 at all the experimental times, then the group 2; and the lowest increase was noted in group 4.

The results verify the conclusions of Zhang et al. (2021) that radiation induced increase of TNF-α and IL-6 as a result of radiation-induced H_2O_2.

Also, Yang et al. (2023) reported that the lower increase recorded in the group 5 is attributed to the role of chitosan in the down regulation of TNF-α, IL- 1β and IL-6 in skin lesions.

The lowest increase recorded in group 4 are in agreement with finding of Azimi-Bahnamiri et al. (2024) that human amniotic epithelial cell transplantation through subcutaneous addition significantly increase wound healing in rats, and this effect may be linked with the down regulation of the inflammatory cytokine TNF-α. Their results suggest that the application of human amniotic membrane or chitosan, to rat skin wound has the ability to significantly attenuate this increase.

The results are in agreement with the previous finding of Satyamitra et al. (2023) that radiation induced fibrosis is arbitrated by

inflammation, which starts directly after radiation. In this context, Wu (2024) added that the initial inflammatory reaction to radiation is primarily produced by pro-inflammatory cytokines; IL-1, IL-6, TNF-α and adhesions molecules. These issues can produce a limited inflammatory response of leukocytes, chief to self-perpetuating tissues injury and loss of defensive barriers. Sharma et al. (2024) determined that IL-1β had a significant reason in the advance of radiation-induced skin reactions. In addition, Noh et al. (2023) described that Fibroblasts enhanced by human amniotic membrane to secrete IL-1β and IL-6 that contribute to regulating and improving wound repair processes.

Elhabal et al. (2024) writes that chitosan induces a decrease in the levels of the three proinflammatory cytokines, TNF-α, IL-1β, and IL-6, while increases the anti-inflammatory IL-10. The IL-10 inhibits the manufacture of numerous soluble mediators, including IL-6, TNF-α and IL-1β (Li et al. 2023). The delayed wound healing associated with the highest increase TNF-α, IL-1β, and IL-6 in the group3 are in agreement with Rübe et al. (2024) who reported increased induction of proinflammatory cytokines in irradiated skin.

Jabbar et al. (2023) concluded that, in adding to their role in delayed the inflammatory stage of repair, proinflammatory cytokines also avoid collagen deposition. In the long term, upregulated proinflammatory cytokines stimulate macrophages and stromal cells to create TGF-β1, resulting in fibrosis (Qian et al., 2024).

According to the immune-histochemical results obtained on the 3rd day for all groups there were intense immunohistochemical deposits of vascular EGF in group 6 compared with group 7 and group 8. In the group3, EGH stain was absent, which may be due to impairments in wound blood supply. The results agree s with Elkady et al. (2019) who suggested that radiation induced fibrinoid necrosis in blood supply considered by circumferential bright pink area of necrosis with depositions of protein in endothelium of blood vessels. Yin et al. (2024) describes, EGF as a good wound healing mediator because its healing function exciting skin cell growth, propagation and differentiation. However, the trans-dermal supply of EGF poses a main trial due to its rapid half-life and lack of effective formulation.

The results obtained from pathological studies revealed that skin wound healing in γ-rays group is delayed than other groups on the 7th day and showed extra-vascular hemorrhage and little fibrous tissues containing huge inflammatory cells in wound gap, and on the 21st day revealed maturation of fibrous tissues, less re-epithelization with noticeable fatty. The severe wound reaction compared to the other groups is substantiated by the highest increase in proinflammatory cytokines which, stop collagen deposition and contribute to reduced wound strength. Kato et al. (2022) described that pathogenesis of improper wound repair and fibrosis next radiotherapy is a complex, dependent procedure concerning cellular reduction, extra-cellular matrix variations, capillary injury, and changed pro-inflammatory mediators. Irradiation also has a significant effect on skin cell function, mediating altered collagen manifestation in fibroblasts, diminished contractility of myo-fibroblasts and the stimulation of proinflammatory cytokines (Johnson et al. 2019). Kato et al. (2022) decided that cells in the granulation and remolding phases of the cell series are greatly liable to ionizing radiation, in the skin, this targets the epidermal stratum basale where cells are quickly dividing (Wang et al., 2006). Moreover, radiation-induced apoptosis partially accounts for delayed wound healing. Simões et al. (2020) reported delayed epithelial hyperplasia and dermal infilling in an irradiated rat model. Supplementary studies have confirmed the upregulation of apoptosis, concurring with the timing of granulation tissue creation (DeLeo et al., 2021). Apoptosis of dermal stem cells has been suggested as a mechanism for long-term injuries (Lin et al., 2023).

In the current study, treatment of γ-rays wound with Human amniotic membrane or chitosan as in group6, group7, and group8 has accelerated the healing process, which was substantiated by the decrease in the level of proinflammatory mediators. The pathological results revealed also that the best healing of wound was recorded in group6 than either group 7 or group 8 manifested by maturation of fibrous tissues filling the majorities of the gap, little leukocytes, less vascularization and beginning of re-epithelization and the epidermis and skin adnexa filled the majority of the wound gap on the 21st day. Absorption of wound tissue for treatment in group 6 (γ radiology + human amniotic membrane) appears to be better and faster than absorption of treatments in groups 7 and group 8. It appears that multiple treatments hindered its absorption by wound tissue and that the succession of treatments within two hours was

not sufficient to produce a more successful effect than individual treatment in group 6 (Authors).

The amniotic membrane is a placental biomaterial with many biological and mechanical properties important for tissue regenerative medicine. The amniotic membrane is non-immunogenic and acts as a tissue barrier not permeable for microbial cells as well as a substrate for wound cellular migration and re-epithelialization (Kate et al., 2023). In addition, the amniotic membrane secretes many wound healing and protecting factors that prevent microbial colonization such as antimicrobial protein, growth factors (TGF-β) that induce re-epithelialization and granulation, and anti-inflammatory cytokines that reduce wound inflammation and pain (Fénelon et al., 2021; Radwan & Nemr, 2020; Tacktill et al., 2022).

Aghayan et al. (2022) reported that amniotic membrane is an attractive technique of grafting for wounds as it has distinctive properties, including anti-inflammatory properties, bacteriostatic, wound defense, reduced scarring, and pain reduction properties, as well as epithelialization initialization capacities. Furthermore, amniotic membrane is widely available and less costly than other bioengineered skin substitutes. El-Heneidy et al. (2016) revealed amniotic membrane bud can be used, as placental tissues have a large amount of TGF-β. Also, amniotic membrane down-regulates transforming TGF-β and its receptor look by fibroblasts and reduces the risk of fibrosis. Therefore, an amniotic membrane scaffold can regulate the healing of a wound by exciting tissue reconstruction sensibly than stimulating scar tissue formation. Peng et al. (2023) recorded that treating amniotic membrane with adhesive hydrogels (chitosan) to increase its mechanical characteristics will further promote its application as a tissue repairing material. On the other hand, Yang et al. (2024) reported that Chitosan can be used due to its antimicrobial properties for infected wounds, as well as, it can stimulate TGF-β to increase wound healing (Ribeiro et al. 2024).

To our awareness, this is the first investigation exploring the usage of gamma-rays, amniotic membrane and Chitosan in the handling of wound healing specially irradiated one.

Conclusion

Amniotic membrane and as well as Chitosan implant can be used to enhance the healing of irradiated wound. In addition, Amniotic membrane is a good scaffold, it has exclusive biological assets that are significant for tissues healing, including anti-inflammatory, antimicrobial, anti-fibrosis and anti-scarring, as well as a realistic price and little immunogenicity.

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