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Effect of Nano Fat Graft on the Healing of Donor Site of Split Thickness Skin Graft

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ABSTRACT

Background: Split-thickness grafts (STSGs) harvesting is a common technique to reconstruct absent skin and to re-establish the skin barrier in burn and skin defects. Donor site morbidity is not uncommon. Various materials and dressings had been used to improve healing of donor site. Fat grafting or its components had been used in promotion of healing in chronic and irradiated wounds indicating the possibility of improving healing.

Objective: To evaluate the effect of adding nano fat grafts to donor sites of STSGs.

Patients and Methods: Twenty (20) adult patients were included in this comparative self-controlled clinical trial from March 2020 to April 2021. These patients had raw areas of skin needing STSG. STSG thickness was 0.12.5 inch leaving an area measuring 7x15cm ($105cm^2$) at the donor site. The donor site area in the lower limb was used as a test area where it was divided into two equal areas, one area was covered by nano fat graft and Vaseline gauze (group A), the other acting as a control group was covered by Vaseline gauze only (group B). Group A was compared to group B as regard healing time from 10^{th} day onward and quality of healing after one month using Vancouver scale (VSS). Tissue biopsy was taken at day 21 from both groups. Any Donor site complications were noted.

Results: Comparing The rate of donor site healing in group A with group B, group A showed faster healing with a mean of (13.30±2.61) versus (16.05±2.43) days from the date of harvesting of STSG. Histologically the mean thickness of neo epithelium in group A was more than that of group B with a mean value of 255.04±15.27μm and 161.15±28.75μm consecutively. Group A showed better vascularity and pliability while no difference was detected as regard pigmentation and height. Infection occurred in donor site of one patient.

Conclusions: Using topical nano fat graft and Vaseline gauze on the donor site of STSG improves healing time as well as vascularity and pliability in comparison to Vaseline gauze alone.

Key Words: *Donor site – Split thickness skin graft – Nano fat graft – Vancouver scar scale.*

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Ethical Committee: Institutional Review Board (IRB) ethical approval and patient consents were obtained in Ain Shams University, Faculty of Medicine.

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INTRODUCTION

The difficult issue of donor-site morbidity after split-thickness skin grafting (STSG) is often treated as a simple matter. STSG donor sites sometimes lead to more postoperative morbidity for patients than the wound covered with the graft. The presence of an ideal dressing for STSG donor site is still occupying a lot of ongoing research. There had been a lot of dressings that had been described for utilization in donor site wounds but none of them can be considered ideal **[1-10]**. Patient comfort, reduction of wound pain and avoiding leakage of exudate, the speed of epithelialization and treatment costs are the main criteria for an ideal dressing **[11]**. Nano fat grafting is a relatively new liquid form of fat that have a large spectrum of usage in plastic surgery in recent years. Various techniques have been designed to obtain a fat graft as rich as possible of adipose-derived stem cells (ASCs) whether mechanically or enzymatically. The use of mechanical method had recently proven that it may have more ASCs. It showed that nano fat contains abundant stromal vascular fraction cells and adipose-derived stem cells that helps tissue regeneration **[12]**. Adipose derived stem cells have a huge proliferative capacity and the ability to differentiate into mesoderm, ectoderm, and endoderm lineages **[13]**. Better quality and faster rate of new epithelium formation in donor site is a possibility with the usage of nano fat graft.

Aim of work:

To evaluate the effect of adding nano fat grafts to donor sites of STSGs.

PATIENTS AND METHODS

Patients:

Institutional Review Board (IRB) ethical approval and patient consents were obtained in Ain Shams University, Faculty of Medicine. Twenty (20) adult patients were included in this randomized self-controlled clinical trial from March 2020 to May 2021. All patients had raw areas that necessitate skin reconstruction by STSG. An area of 7X15 cm (one sheet of STSG) at donor site was used as a test area where it was divided into equal half where one was covered by with nano fat and Vaseline gauze (group A) while the other by Vaseline gauze, control group (group B).

Inclusion criteria:

1- Patients with Full Skin loss of an area at least 7x15cm.

Exclusion criteria:

1- Patients suffering from diabetes.

2- Patients having Hgb less than 10mg%.

3- Patients on systemic corticosteroids.

4- Patients with well-known liver or kidney disease.

5- Smokers.

6- Patients having any previous scar or injury in Donor area.

Methods:

All included patients had:

• History taking:

A- Personal history.

B- Medical history.

C- Surgical history.

D- Special habits (e.g., smoking).

• Clinical examination:

A- General examination.

B- Vital signs.

C- Local examination of the affected raw area, to exclude local sign of infection, donor site (thigh or leg) is free from current or previous injury.

• Investigations: Laboratory Investigations (e.g., CBC, Coagulation profile, Renal functions, liver functions, Hb A1c level).

• Operative procedure:

After signing an informed consent and under general anaesthesia:

All included patients had the following:

A- *Nano fat graft preparation:* The infraumbilical abdominal area was infiltrated with tumescent fluid about 20 cc with concentration of 1/200000 adrenaline. Liposuction was done to harvest fat using a 3mm cannula. The resultant fatty tissue, 20cc was washed with 20cc of saline solution then fluid was removed. The fat tissue aspirated was transferred mechanically 30 times through 2.4-mm and 1.2-mm Luer-to-Luer connector Leading to disaggregated emulsion. This Mechanically deconjugated fat was passed through the Nano Transfer device producing 0.6- to 0.4-mm fragments, resulting in nano fat as an end product.

B- *Harvesting STSG:* One sheet of STSG was taken used electric dermatome. The thickness of graft is 0.0125-inch thickness. This resulted in an area measuring 7x15cm at donor site which was used as a test area. It was divided into equal half, one was covered by nano fat (3cc) topically applied and Vaseline gauze (group A) while the other was covered by Vaseline gauze only, control group (group B) (Fig. 1). A biopsy was taken using 3mm punch biopsy instrument. Closure of donor site by bandage was done followed by fixation of STSG in recipient area and bandage application.

• Follow-up.

Group A was compared to group B up to one month for:

A- Timing of macroscopic complete healing by the ability of removing adherent layer (Vaseline gauze) from 10^{th} day onward in both groups.

B- Tissue biopsies were taken one intraoperatively and two at day 21 postoperative from both group in 10 cases, for the light microscopic histological study, punch biopsies taken from donor site test area, were fixed in 10% formalin, then processed to obtain paraffin blocks. Five µm thick serial sections were cut and stained with Haematoxylin and Eosin stain (H&E) 14. Morphometric study and statistical analysis: Total thickness of the epidermis was measured in H&E-stained sections from five different fields in five serial stained sections of each biopsy (at magnification 100 X). This was done using the image analyser Leica Q win V.3 program at the Histology Department, Armed Forces College of Medicine, Cairo, Egypt. The computer was connected to a Leica DM2500 microscope (Wetzlar, Germany).

C- Clinical evaluation of quality of healing at one month postoperatively using Vancouver scale.

D- Any complication at donor area.

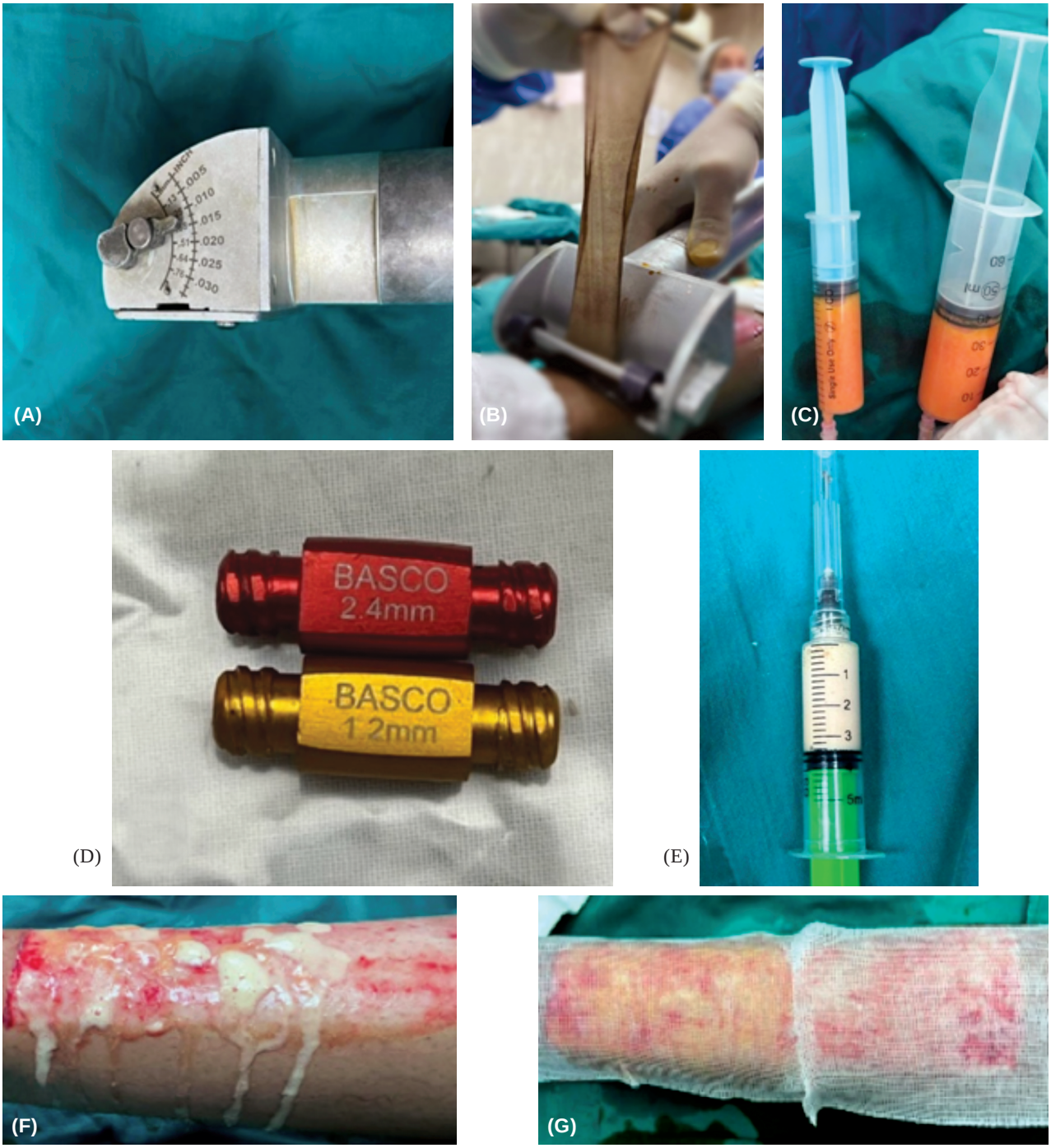

Fig. (1): Operative procedure harvesting of STSG and nano fat application. (A): Electric dermatome showing thickness of STSG, (B): STSG, (C): Fat harvested from abdomen, (D): Two connector used to obtain nano fat graft. (E): Nano fat emulsion obtained after 30 times in each connector, (F): Application of nano fat on half of test area (group A), (G): Application of Vaseline gauze separately on both areas (group A and group B=Control area).

Statistical analysis:

Systematic randomization was done where in odd patients (1,3,5,..up to 19) nano fat was applied in upper part of test area where in even patients (2,4,6…20) nano fat was applied in lower part of test area. Data were analysed using the Statistical Package for Social Sciences (SPSS version 27). Descriptive analyses were done to obtain the means, and deviations for quantitative data, and Numbers and frequencies for qualitative data. Different types of graphs were used according to the type and distribution of data (pie, and error bars), Bivariate analyses were performed using the Wilcoxon signed test, and Marginal test of homogeneity. *p*-value <0.05 was considered significant.

RESULTS

Twenty (20 patients), (20 donor area) were included in this study. Twenty 20 donor sites had half of test area covered with nano fat and Vaseline gauze (group A) and other half covered by Vaseline gauze only (group B) acting as a control group. There were 13 (65%) males, and 7 (35%) females. Their ages ranged from 25 to 45 years, with a mean age of 33 years ±6 (SD). The cause of skin loss was burn in 19 cases (95%) and venous ulcer in one case (5%). (Fig. 2). There were no complications in 19 donor site (95%) and one complication (5%) in the form of infection. (Table 1).

Regarding the timing of healing in group A versus group B, the mean time of healing of group A was 13.30±2.61 versus 16.05±2.43 day which was statistically significant showing faster time of healing in group A. (Table 2) (Fig. 3).

Table (1): Demographics and clinical data.

Age Mean ± SD	33±6
Gender:	
Female N (%)	13 (65%)
Male N (%)	7 (35%)
Cause:	
Burn N (%)	19 (95%)
Venous ulcer N (%)	1 (5%)
Complications:	
Negative N (%)	19 (95%)
Positive N (%)	1 (5%)*

* Complication was a case of infection in donor site.

Table (2): Difference between healing time, total scores, and histology between the two groups (N=20).

	Mean ± SD	Wilcoxon Signed Ranks Test	*p*-value
Fat (A) Healing from 10th days onward	13.30±2.61	3.57	<0.001*
Non (B) Healing from 10th days onward	16.05±2.43		
Fat total score	3.75±1.86	3.65	<0.001*
Non total score	5.40±1.5		
Histology fat group (skin thickness)	255.04±15.27	2.80	0.005*
Histology non-fat group (skin thickness)	161.15±28.75		

*Significant *p*-value.

Histological results:

Light microscopic examination of the specimen taken from test area at the same time of STSG harvesting showed a denuded skin with preserved dermal architecture, showing intact hair follicles and organized collagen bundles (Figs. 4-A,B).

The biopsies taken at day 21: In group A, reepithelization was evident, where an epidermis made of a complete stratified squamous epithelium was noted, with a mean thickness of (255.04±15.27) μm. The newly formed epidermis showed a layer of basal columnar cells with oval nuclei of stratum basalis, many layers of polyhedral cells with rounded vesicular nuclei in stratum spinosum, and layers of flat cells containing intensely basophilic granules in the stratum granulosum. However, the epithelium was of the immature para keratinized type showing the stratum corneum as thin layer of flat elongated acidophilic cells, retaining their flat nuclei. Subepidermal hemorrhage was seen with separation of the epidermis in some areas. Areas of granulation tissue was seen in the papillary dermis consisting of fine collagen fibers intermingled with extravasated red blood cells, numerous fibroblasts, mononuclear inflammatory cells and small blood vessels (Figs. 4-C,D).

The histological examination in group B revealed nearly the same findings with a mean thickness of (161.15±28.75) μm. In addition, areas of acanthosis were seen as focal thickening of the epidermis dipping into the underlying dermis. Heavy subepidermal inflammatory cell infiltrate was seen in the granulation tissue underneath the newly formed epidermis, and few areas of minimal subepidermal hemorrhage were also observed (Figs. 4-E,F,5).

The histological results of the patient that developed infection are described. Improper epithelization was noticed in group A. The surface was almost entirely covered with a thick layer of granulation tissue, except for a tiny area showing a thin, disorganized epithelium, two to three cell-thick, with rounded nuclei. This small part of incomplete epidermis was significantly decreased in thickness as compared to other patients (either group A or B), measuring (3.44μm±1.01) Nevertheless, parts of hair follicles were seen formed of properly organized stratified epithelium (Figs. 4-G,H). Regarding group B in this patient, lack of proper epithelization was also evident. The surface was seen highly disorganized and covered by the moderately thick irregular granulation tissue, heavily infiltrated by mononuclear cells. Simple squamous epithelium was noticed covering focal parts of the specimen, especially in its periphery, measuring (2.92μm±0.85), that was significantly decreased in thickness as compared to other patients

whether group A or B as well as group A of the same patient (Figs. 4-I,J).

When comparing group A with group B as regard the quality of healing using Vancouver scar scale, group A showed significant change in vascularity than group B with a 90% pink in group A vs 95% red in group B. As regard pliability, group A showed more significant pliability with a (90%) yielding versus (85%) firm. There was no statistical significance between group A and B as regard height and pigmentation. The mean of overall total Vancouver score was 3.75±1.86 in group A and 5.40±1.5 in group B which is statistically significant. (Table 3) (Fig. 6-A,B).

Table (3): Differences between group A and group B regarding Vancouver score.

	Fat group		Non group		Marginal Homogeneity Test	*p*-value
	N	%	N	%		
Vascularity:						
Normal	0	0.0	0	0.0	4.12	<0.001*
Pink	18	90.0	1	5.0		
Red	2	10.0	19	95.0		
Purple	0	0.0	0	0.0		
Pigmentation:						
Normal	17	85.0	18	90.0	1	0.317
Hypo-pigmented	0	0.0	0	0		
Hyper-pigmented	3	15.0	2	10.0		
Pliability:						
Normal	0	0.0	2	10.0	3.8	<0.001*
Supple	0	0.0	0	0.0		
Yielding	18	90.0	0	0.0		
Firm	0	0.0	17	85		
Ropes	2	10.0	3	15		
Contracture	0	0.0	0	0.0		
Height:						
Flat	17	85.0	0	0.0	1	0.317
<2mm	1	5.0	16	80.0		
2-5mm	2	10.0	0	0.0		
>5mm	0	0.0	4	20.0		

* Significant *p*-value.

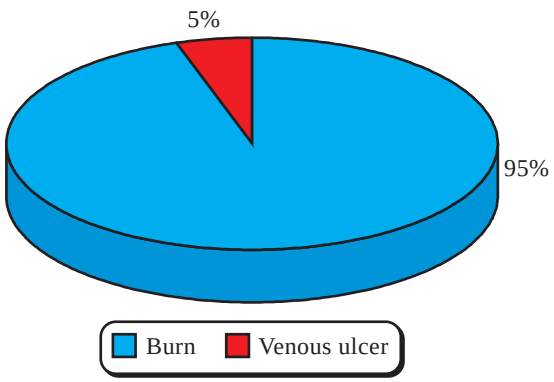

Fig. (2): Causes of skin injury.

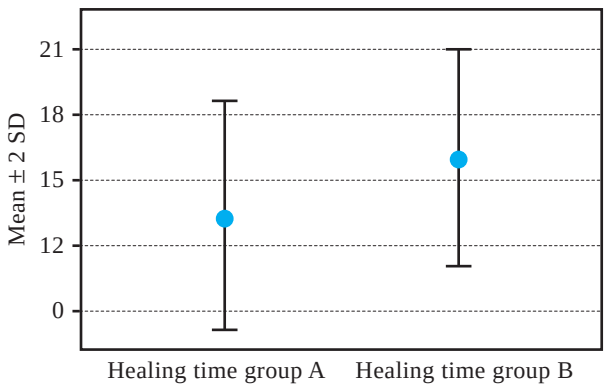

Fig. (3): Error bars show the mean ± 2 SD healing time of the two groups (Group A: Fat, Group B: Non).

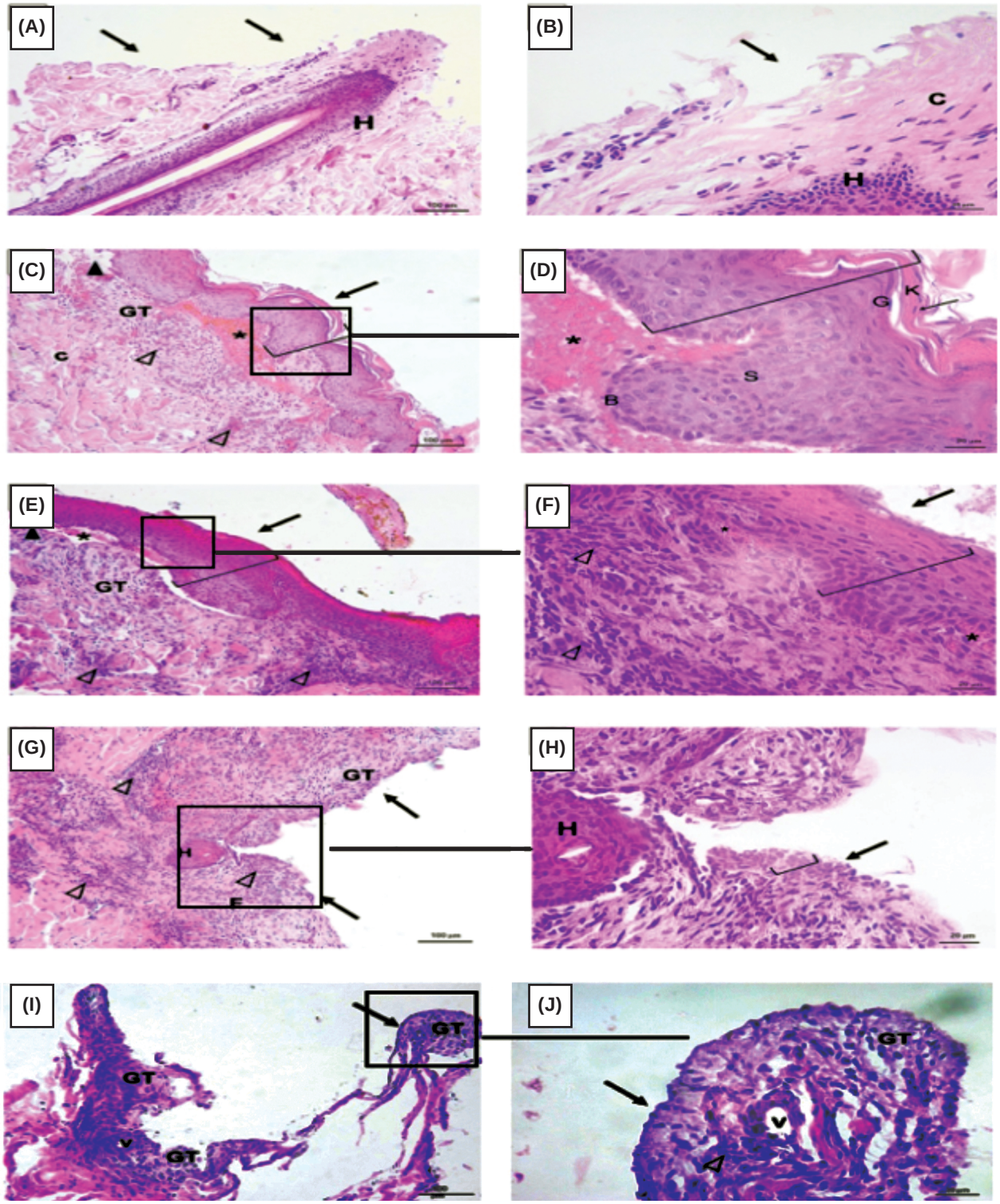

Fig. (4): Punch biopsies, H&E-stained sections: (a, c, e, g, i x100, scale bar = 100 µm) and (b, d, f, h, j x 400, scale bar = 20µm). a, b = Histological result of punch biopsy taken at same time of STSG harvesting c,d = Group A (after fat grafting) e, f = Group B (without fat graft) g, h = Complicated patient (after fat grafting) i, j = Complicated patient: (without fat graft). ↑ = Surface of the specimen, either denuded (A, B), covered by stratified squamous epithelium (Fig. C, D, E, F), covered by granulation tissue (Fig. G, H), or simple squamous epithelium (Fig. I, J). H = Hair follicle; C = Collagen bundles;] = Thickness of stratified squamous epithelium; * = Hemorrhage; B = Stratum basalis; S = Stratum spinosum; G = Stratum granulosum; K = Keratin of stratum corneum; GT = Granulation tissue; Δ = Mononuclear cellular infiltrate; ▲= Subepidermal space; V = Blood vessel.

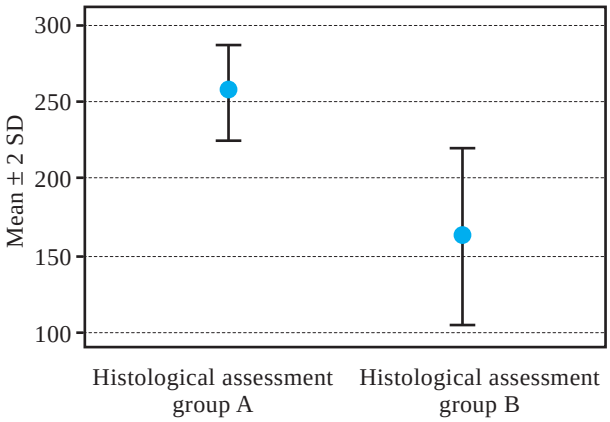

Fig. (5): Error bars show the mean ± 2 SD histological assessment of the two groups (Group A: Fat, Group B: Non).

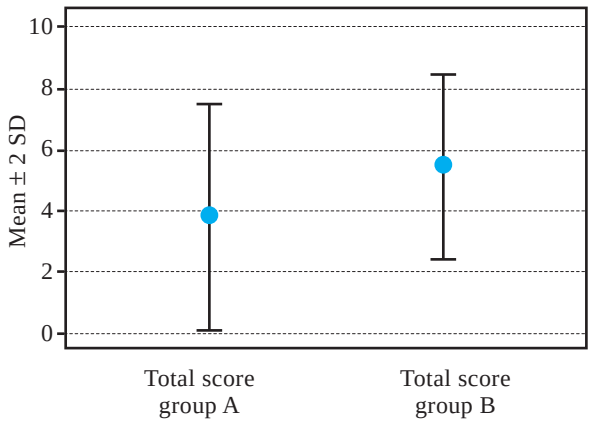

Fig. (6-A): Error bars show the mean ± 2 SD total score of the two groups (Group A: Fat, Group B: Non).

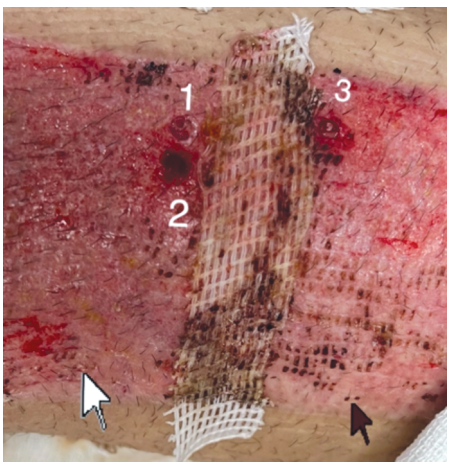

Fig. (6-B): Healed Donor area in group A and group B showing biopsy sites (1,2,3), group A (black arrow) showing pink vascularity and group B (white arrow) showing red vascularity.

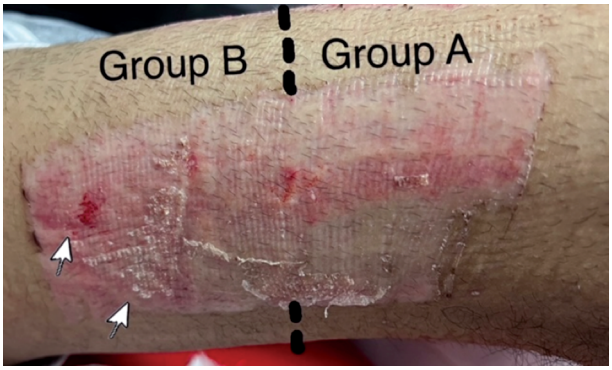

Fig. (7): Group Institutional Review Board (IRB) ethical approval and patient consents were obtained in Ain-shams University Faculty of Medicine B shows signs of immature healing (white arrows).

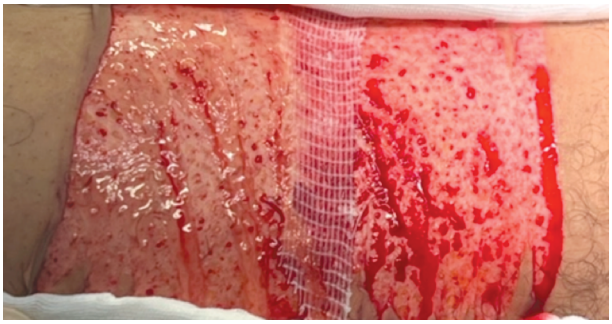

Fig. (8): Nano fat application with possible hemostatic effect on dermal bleeding.

DISCUSSION

STSG has become one of most used technique to treat skin loss due to various reasons. Burns are considered one of major reasons **[15]**. In this study the majority (95%) of cases were due to burns that resulted in full skin loss and necessitate coverage by STSG. The take of split-thickness skin graft (STSG) results in a wound with residual dermal element that needs wound care **[16]**. There are multiple different ways for wound care in donor site between institutions. The most important objectives in donor site dressing or adding a substance to the dressing are to promote rapid healing, reduce pain, decrease hospital stay and end up in a scar of good quality without occurrence of complications. The use of products that result in moist wound healing are better than non-moist products as it decreases pain and promote healing **[17,18]**. Acute wound pain result in decreased quality of life as well as delayed wound healing. Unfortu-

nately, in this study pain recording was not feasible due to proximity of two test area. STSGs can be classified into thin (0.2 to 0.3mm), medium (0.4mm), and thick grafts (0.5 to 0.6mm) **[19]**. In this study the STSG thickness was 0.0125 inch (0. 375mm). This thickness was chosen as the deeper the donor area the slower the healing at donor site especially if the graft is more than 0.5mm it was taken by electric dermatome to ensure same depth of donor area in group A and group B. In 2013, Tonnard et al., **[20]** succeeded in isolating an injectable by-product of fat tissue called "nano fat", which was the result of emulsification and filtration of the aspirated adipose tissue. Nano fat is an ultrapurified adipose tissue-derived product that doesn't contain mature adipocytes but contains CD34+ rich ASCs, microvascular fragments, growth factors and cytokines **[21]**. There has been a lot of terminology describing adipose derived extracts that contains mechanically stromal cells as Super microfat **[22]**, Total stromal-cell transfer **[23]**. The presence of adipose tissue in abundant quantity in human body and easiness of harvesting makes it a potential donor for mesenchymal stromal cells (MSCs). In this study we use the nano fat mechanically prepared by tulip nano transfer system that provides adequate number of stromal cells. This confines with the fact that the mechanical methods have a lot of privilege over enzymatic methods, the most important benefit is that no dissolving chemical is used, such as an enzyme, so the structure and existence of stromal cells are maximized, just like ECM. This ensures that the stromal cells are not destructed and found. The regenerative potential of autologous fat relates to the presence of adipose-derived stem cells (ASCs) within the stromal vascular fraction (SVF). ASCs can differentiate into fibroblasts and keratinocytes, as well as secreting soluble mediators with angiogenic and anti-inflammatory properties.

Chung et al., **[24]** suggested that STSGs donor site wounds would usually heal within 7 to 21 days whatever the dressing type. However, a slow donor site wound healing, even by days, could increase patient uneasiness and increase the burden on health care resources with excess costs. In this study in group A the healing time was statistically significant less than group B (control group) which was 13.30±2.61 versus 16.05±2.43 days. This can be attributed to the rich ASCs and other growth factors and cytokines present in nano fat that promote epithelization. The decreased time for donor healing would allow the patient to return to work earlier and resume his normal life which defiantly have a good impact on his psychological aspect and quality of life. In one survey patients reported an average of 15.6 up to 33.8 days they could not go to work due to the donor-site wound.15 The using of nano fat graft was not only beneficial in decreasing healing time of donor area but it was responsible for a better epithelial thickness (Fig. 7). Histologically group A showed a statistically significant increased thickness more than group B. This can be explained by the fact that ASCs can differentiate into different cell types including keratinocytes and fibroblast that share in healing process greatly, in addition it promotes revascularization by the paracrine secretions of bioactive molecule **[25]**.

The quality of scar formed at the donor site is of utmost concern to patients it affect his quality of life. Scare scores had been used for such type of assessment. Variable evaluation methods were used, including the Vancouver Scar Scale (VSS), the Patient and Observer Scar Assessment Scale (POSAS), and other scores (adapted POSAS and VSS, Bates-Jensen wound assessment tool) **[26]**. In this study For scarring, the VSS was used which uses a grading scale from 0- to 13 to assess pigmentation, vascularity, pliability, and scar height, with higher scores suggesting the probability of hypertrophic scar. VSS score is one of most reliable score for scaring specially in burns **[27]** in this study Group B shows a significant increase in VSS score than group A with a mean of 5.40±1.5 versus 3.75±1.86 consequently. This indicates that there is a higher incidence in group B to develop hypertrophic scar than group A and that the presence of nano fat resulted in better quality of scar. This can be attributed to the fact that at donor site the adipose derived stromal cells initiate a site-specific reparative response comprised of remodelling of extracellular matrix (ECM), enhanced and sustained angiogenesis, immune system modulation, and cellular turnover **[28]**. There were specific changes in the VSS score apart from total score where group A showed significant better results than group B as regard vascularity and pliability while no significant difference in pigmentation and height. (Table 3).

The donor site can be complicated by infection, dyspigmentation, and hypertrophic scarring **[29]** in this study one case (5%) was recorded as a complication where there was infection. The infected area was present in group A and group B in same patient constitutes around 50% of test area. Frequent dressing was done until healing occurred at day **[30]**. Histological studies showed improper epithelization in both groups with focal epithelium. The mean thickness of this epithelium was less in group b than group A with a mean of (3.44μm±1.01)

versus (2.92μm±0.85) which was statistically significant. This result indicates the presence of role of nano fat graft even in infection, one postulation is the fact that Nano fat (ASCs) showed different immunomodulatory effects on host immune cells in both wound healing and transplant biology, releasing immunosuppressive factors, downregulate the inflammatory response and move the wound past the state of persistent inflammation **[30]**. This point needs further in-depth histological studies with isolation of ASCs alone from nano fat and checking its role histologically on microscopic level. We observe in group A that while Application of nano fat grafting dermal bleeding stops while was still persistent in group B (Fig. 8), this was not quantified or measured to test possible hemostatic effect of nano fat graft and further studies is recommended to prove or disprove that observation.

Conclusion:

In donor site of STSG, the use of nano fat graft decreases the time needed for wound healing, increased epithelium thickness, and resulted in a better vascularity and pliability as regard healing quality.

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