MJMR, Vol. 36, No. 1, 2025, pages (75-83).

Open Access
ISSN:2682-4558

Research Article

Effect of Human Amniotic Membrane Application on Microskin Graft

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DOI: 10.21608/MJMR.2024.297542.1727

Abstract
Background; Severe full-thickness burns and significant injuries that cause extensive skin damage hinder natural regeneration and jeopardize patient survival. Immediate coverage is essential to restore normal skin function. When a donor site is available, using an autograft is always the preferred and most effective option. **Aim and objectives**; To assess the impact of applying human amniotic membrane at the recipient site of micro-autogenous split-thickness skin grafts for covering post-burn raw areas, this study focuses on several factors: the graft take percentage, infection rates, time required for complete wound healing, the expansion ratio of the recipient site to the graft donor site, and the scar evaluation of the graft. **Subjects and methods**; This prospective controlled clinical study was conducted on 20 patients at the Plastic Surgery Department of Minia University Hospitals. The study involved applying human amniotic membrane to the recipient site of micro-autogenous split-thickness skin grafts on only one half of the raw area, with the other half serving as a control. **Results;** The mean time for complete healing in study side which is covered by amniotic membrane is (25.5±4.2), and it is significantly lower than the control side which is not covered by amniotic membrane which is (30.8±5). No need for another session of grafting at both sides.

Keywords; Microskin graft (MSN), Split Thickness Skin graft (STSG), Amniotic Membrane (AM).

Introduction
Severe full-thickness burns or major injuries that severely damage the skin can impede natural regeneration and threaten patient survival. Prompt intervention is crucial to restore normal skin function. When donor skin is available, an autograft is widely regarded as the most effective solution [1].

Skin grafts are primarily classified into three basic types: full-thickness skin grafts (FTSG), split-thickness skin grafts (STSG), and composite grafts. FTSGs include both the epidermis and the entire thickness of the dermis. In contrast, STSGs comprise the entire thickness of the epidermis and only a portion of the dermis, and they can be further subdivided into thin, medium, and thick grafts [2].

The healing process of skin grafts typically involves three stages: anchorage, inosculation, and maturation. While split-thickness autografts are preferred, the limitation of available donor sites has driven the search for alternative solutions [3].

Converting both split and full-thickness grafts into micrografts transforms them into individual units that promote regeneration and release growth factors to support the healing process [4].

Microskin grafts (MSG) are thin split-thickness grafts that come in different sizes and thicknesses. Smaller grafts tend to heal faster because they have more active edges, which accelerate regeneration [5].

Cells in the basal layer of microskin form epithelial islands that expand outward, connecting to the wound surface's epidermis and creating an epidermal cyst structure. Autologous micrografts are rich in progenitor cells, growth factors, and extracellular matrix particles derived from the patient's own tissue [6].

The amniotic membrane (AM), which is the innermost part of the placenta, has been used in therapeutic applications for the past century. Its thickness ranges from 0.02 mm to 0.5 mm and it is composed of three primary histological layers: the epithelial layer, the thick basement membrane, and the avascular mesenchymal tissue [7].

Applying an amniotic membrane in burn treatment promotes rapid healing and re-epithelialization by mitigating several harmful factors. Covering microskin grafts with a human amniotic membrane accelerates wound [8].

The amniotic membrane significantly shortens the duration for complete graft take, making it especially suitable for children or burns on the extremities. The graft is applied to the wound bed, and the amniotic membrane is wrapped around the grafted extremity, followed by dressing. The membrane adheres to itself when wrapped around the extremity. Using an amniotic membrane as a graft fixator is associated with accelerated re-epithelialization and recovery [9].

Aim of the work

To assess the impact of applying human amniotic membrane at the recipient site of micro-autogenous split-thickness skin grafts for covering post-burn raw areas, as regard to:

1. The graft take percentage.
2. Infection rates.
3. Time required for complete wound healing.
4. The expansion ratio of the recipient site to the graft donor site.
5. Scar evaluation of the graft.

Patient and Methods

A. Technical Design

This prospective controlled clinical study was conducted on 20 patients at the Plastic Surgery Department of Minia University Hospitals. The study involved the application of human amniotic membrane at the recipient site of micro-autogenous split-thickness skin grafts.

Inclusion Criteria:

- Age: pediatrics & adults (2: 40 years old).
- Both sexes
- Post burn raw areas.

Exclusion Criteria:

- Exposed bone, tendon, nerve, cartilage.
- Clinically infected recipient area.
- Uncontrolled DM
- Immune-compromised patients
- Uncooperative patients.
- Drug abuse

B. Operative Design:

All patients will be subjected to:

- **An informed consent was taken from all patients or their guardians**
- **Complete history taking:**
  - Personal history
  - Any complaint
  - Past medical history and past surgical history
  - Family history
- **Complete physical examination**
- **General examination**
  - Vital signs (Blood pressure, Temperature, Heart rate, Respiratory rate)
- **Microskin grafts are applied over the whole raw area, then the raw area was divided into two parts, and amniotic membrane is applied on only on part of it.**
- **Preparation of the amniotic membrane:**
  - The preparation of amniotic membrane (AM) involves collecting the membrane from volunteer mothers who have been screened for HIV-1, HIV-2, hepatitis B, hepatitis C, and syphilis.
  - The placenta is decontaminated to eliminate potential surface pathogens, followed by rinsing with sterile saline and antibiotic solutions. An antibiotic-antimycotic cocktail containing Ampicillin, Ceftriaxone, and Fluconazole is used to combat both Gram-positive and Gram-negative bacteria, as well as fungi. Large sections of the amniotic membrane (AM) are then removed and thoroughly rinsed to clear any blood or blood clots. It was then transferred to a container filled with 50% glycerol and stored at -28 °C (household

freezer)[9]. Just before use, the human amniotic membrane (HAM) is defrosted and soaked in sterile saline for two hours to remove glycerol. It is then placed in a sterile stainless steel surgical bowl

- **Preparation of the micro-autogenous split-thickness skin grafts:**

- During preparation of raw area, thin autogenous split-thickness skin grafts is also harvested by the Zimmer® dermatome adjusted at 25mm split thickness graft setting with a ratio of 1:8 which is expansion ratio of the graft donor site to the recipient site measured by sterile steel surgical ruler.
- Preparation of micro-autogenous split-thickness skin grafts by cutting harvested skin grafts with blade 24 into small pieces on sterile wooden board.

- **Application of microskin grafts on raw area covered by amniotic membrane:**

- A graft split technique is used by dividing raw area into two parts by imaginary line separates the raw area.
- Microskin grafts are spread over whole of post-burn raw area using forceps and the prepared amniotic membrane is applied on top of one half of the raw area and fixated by disposable skin stapler (study group) while the other half isn't covered by amniotic membrane (control group).
- Vaseline gauzes are applied on both parts of raw area.

- **Follow up started after 5 days then every 2 days for 6 months with documentation of progression at both compared halves as regard the graft take percentage, infection rates, time required for complete wound healing, the expansion ratio of the recipient site to the graft donor site, and the scar evaluation of the graft.**
- **Three assessors were used to assess the results to reduce bias**

C. Administrative Design:

- The protocol was submitted for approval of Research Ethics Committee
- Informed consent was obtained from the patients before enrollment of the study
- All data was kept confidential
- All participants had the right to withdraw from the study without affecting their management Department

Results

Table (1): baseline data of studied cases

Baseline data	Descriptive statistics (n=20)
Age	
Mean ±SD	21.4±12.4
Range	4-40
Median (IQR)	18(9.25-32)
Sex	
Male	12(60%)
Female	8(40%)
Occupation	
Students	9(45%)
Housewife	2(10%)
Clerk	2(10%)
Farmer	4(20%)
Children	3(15%)
Medical history	
Controlled diabetes	2(10%)
Controlled hypertension	3(15%)
No comorbidities	15(75%)
Special habits	
None	17(85%)
Smoker	2(10%)
Alcohol	1(5%)
Cause of burn	
Scald	6(30%)
Flame	11(55%)
Electric	2(10%)
Contact	1(5%)

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- As shown in previous table, total number of studied cases were 20 cases with mean age 21.4±12.4, ranged from 4 to 40 years with 60% males and 40% females.
- Regarding occupation of studied cases, the majority were students with percentage of 45%, then 20% were farmers, 15% were children.
- As regard comorbidities and special habits, the majority had no comorbidities and special habits of medical importance with percentage of 75% and 85% respectively while only 2 cases had controlled diabetes and 3 cases were controlled hypertensive.
- As regard Cause of burn, more than one half had flame burn (55%) then 30% had scald while only 10% had electric burn and 1 case had contact burn

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Table (2): Frequency and percentage of Site of raw area among studied cases

Sites of raw area	Frequency (n=20)	Percentage (100%)
Abdomen	**1**	(5%)
Back	**1**	(5%)
Buttocks	**2**	(10%)
Upper limbs	**6**	(30%)
Lower limbs	**10**	(50%)

Regarding Site of raw area among studied cases, one half of cases had burn in lower limb (50%), then 30% had burn in upper limb, then 10% had burn in buttocks while only 2 cases had burn in abdomen and back separately.

Table (3): comparison between study side and control side regarding healing time in days

Healing time	Study group	Control group	P value
Healing time (days)			
Mean ±SD	25.5±4.2	30.8±5	0.001*
Range	20-31	23-42	
Median (IQR)	25.5(21-30)	30.5(27-34.5)	

* significant at p value <0.05

By comparing between study side and control side regarding healing time in days, the result was highly statistically significant (p value <0.001) as mean healing time in study side was significantly lower in study side (25.5±4.2) than in control side (30.8±5).

Table (4): comparison between study group and control group regarding graft take

Graft take	Study group	Control group	P value
Excellent (100%)	7(35%)	2(10%)	0.008*
Good (>75%)	12(60%)	9(45%)	
Poor (<75%)	1(5%)	9(45%)	

- Regarding the comparison of graft take between study side and control side, the result was statistically significant (p value <0.05).
- As the percentage of excellent graft take was higher in study side (35%) than in control side (10%), also 60% of study side had good graft take compared to 45% while only 5% of study side had poor take compared to 45% of control side

Figure (1): Photograph shows amniotic membrane application over microskin graft in a post burn raw area

Figure (2): Photograph shows complete healing of the graft after 26 days post-operatively

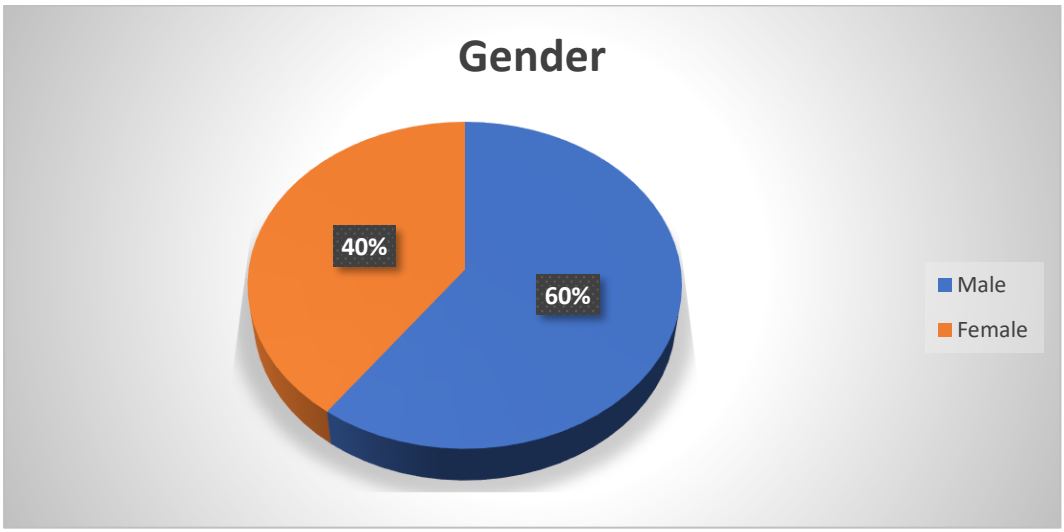

Figure (3): Illustration shows a pie chart represent distribution of gender among studied cases

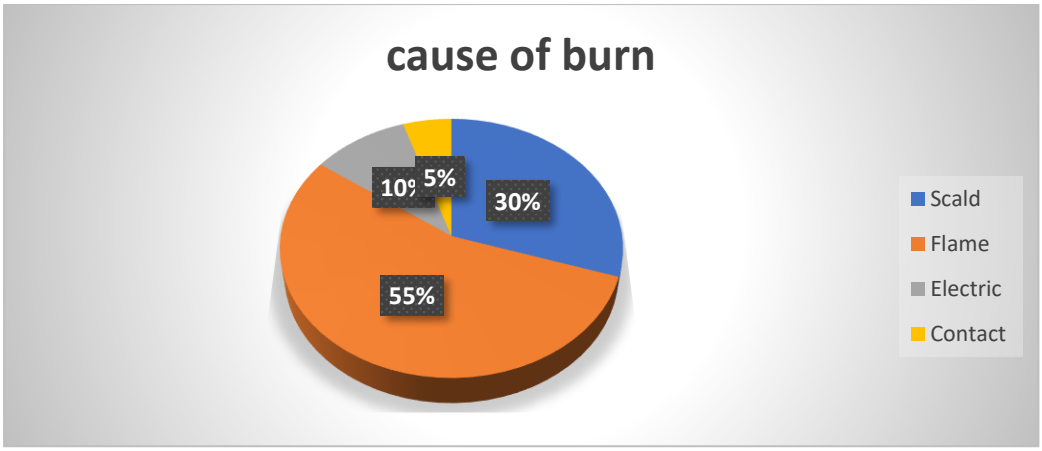

Figure (4): Illustration shows a pie chart represent distribution of cause of burn among studied cases

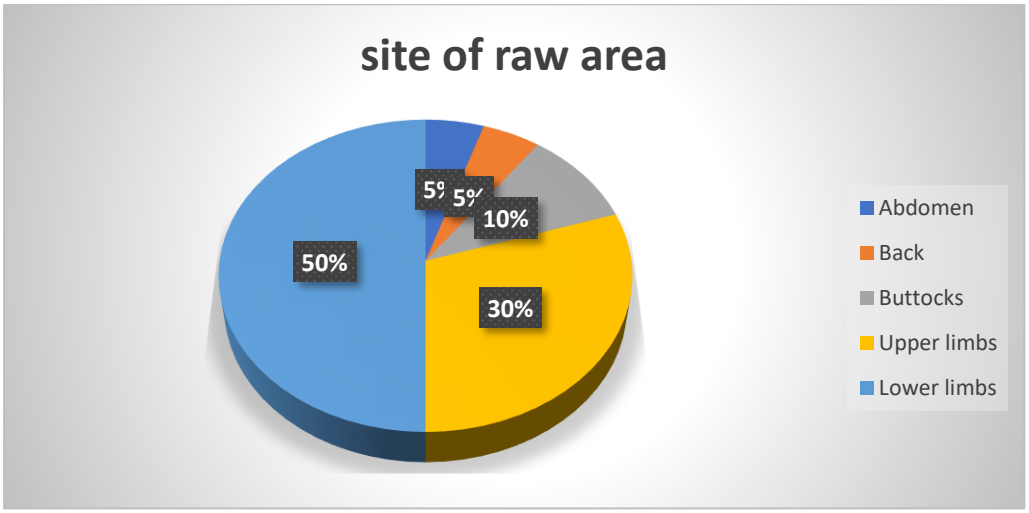

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Figure (5): Illustration shows a pie chart represent distribution of site of raw area among studied cases

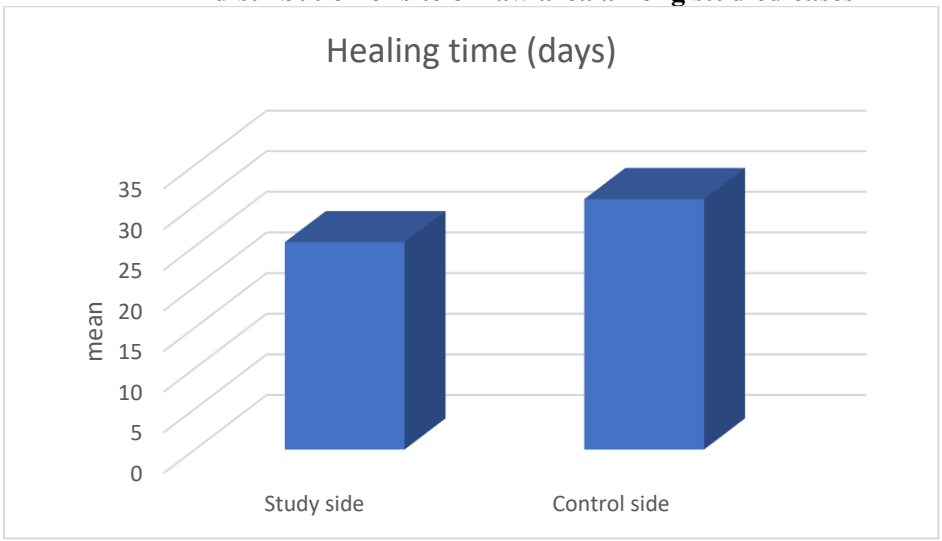

Figure (6): Illustration shows a bar chart represent comparison between study side and control side regarding healing time

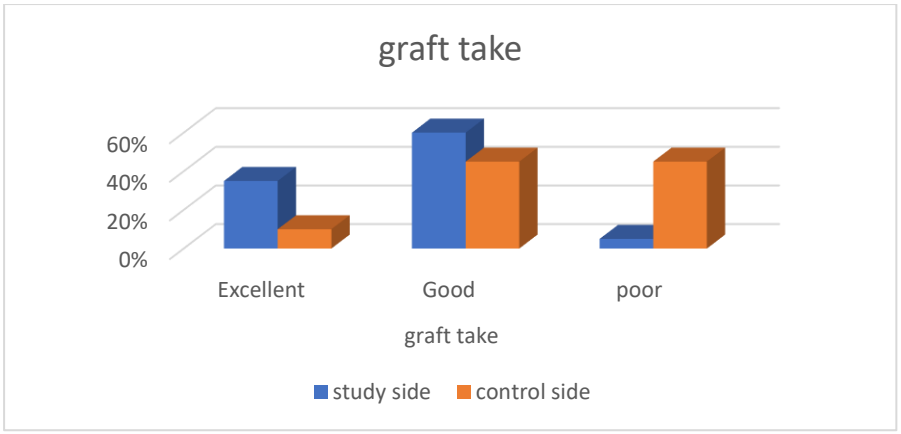

Figure (7): Illustration shows a bar chart represent comparison between study side and control side regarding graft take

Discussion

In the current thesis, regarding the demographic characteristics among the 20 studied cases, the mean age of the patients was 21.4±12.4 years ranged from 4 to 40 years, 12 males and 8 females.

In a previous study by Agarwal et al., in 2021 aimed to use the microskin autografting for post burn raw areas, but without a cover for the microskin graft, the demographic data among 25 studies were mean age of the patient was 27.52 years ranging from 18 to 54 years, 15 males and 5 females.

As regard the causes and site distribution of the studied patients we found that, the most common cause was flame burn in (55%) of the patients, followed by scald burn (30%). The most common site was lower limbs (50%), followed by upper limbs (30%).

In agreement with these findings, X. L. Chen et al., in 2011 showed that the most common cause was thermal injury was flame (63.5%), followed by explosion (19.1%), scald (12.7%). *The present study showed that,* the mean healing time in study side is (25.5±4.2) which is significantly lower than the control side which is (30.8±5) and no need for another session of grafting at both sides.

X. L. Chen et al., in 2011 clarified that the wound healing time was determined by evaluating the appearance or signs of epithelialization in the grafted areas and the wounds healing time of these 63 major burn patients was between 35 and 55 days.

Conclusions

On conclusion, we found that autologous microskin grafts covered by human amniotic membrane are superior to non-covered microskin grafts for wound coverage of post burn raw areas. It has more graft take, less duration of complete wound healing, less donor site morbidity and a more desirable aesthetic result.

On the other hand, autologous microskin grafts covered by human amniotic membrane are more liable to infection. Amniotic membrane has no effect in decreasing graft contractures.

Recommendations

Further studies with larger sample size are needed to confirm the current results.
Further studies will be needed to confirm that application of amniotic membrane on grafts increase graft take and decrease time needed for complete wound healing of post burn raw areas.
Further studies will be needed to assess the occurrence of infection with the application of amniotic membrane on skin grafts.

Further studies will be needed to determine the effect of the application of amniotic membrane over microskin grafts regarding graft contracture.

We recommended the use of microskin grafts covered by amniotic membrane for extensive post burn raw areas with limited donor sites for grafting.

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