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Healing Effects of Dried and Acellular Human Amniotic Membrane and Mepitelas for Coverage of Skin Graft Donor Areas; A Randomized Clinical Trial

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ABSTRACT

Objective: To compare the healing effects of dried and acellular human amniotic membrane and Mepitel for coverage of split-thickness graft donor site (STGDS).
Methods: Twenty patients who underwent STGDS regeneration surgery in identical anatomic regions were enrolled in this randomized controlled clinical trial conducted in Hazrate Fatemeh hospital (Iran). Patients were randomly assigned in 3 groups of wound dressing; group A by Mepitel, group B AmiCare (Dried amniotic membrane) and group C OcuReg-A (Acellular amniotic membrane). Re-epithelization rate (healing time), pain sensation, scar formation and infection rate were assessed till complete healing was achieved.
Results: Our results showed no significant difference between Amicare, OcuReg-A and Mepitel in the features analyzed by us including: Re-epithelization rate (healing time) p=0.573, Pain sensation p=day 4 th: 0.131, day8 th: 0.93 and day 12 th: 0.365, Scar formation p>0.05 and Infection rate.
Conclusion: Our findings confirmed the safety and efficacy of AmiCare (dried amniotic membrane) and OcuReg-A (Acellular amniotic membrane) in treatment of split-thickness donor site in comparison with Mepitel as a standard wound dressing.
Trial registration number: IRCT201511118177N12

Keywords: Split-thickness donor site; Dried amniotic membrane; Acellular amniotic membrane; Mepitel; Re-epithelization.

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Introduction

Split-thickness graft donor site (STGDS) is a common procedure in reconstructive surgery and burned patients. The donor area will be healed spontaneously after a while, mostly in 10-21 days, depends on the thickness of harvesting graft. Pain, healing speed, prevention of infection, permanent scar and pigmentation changes in the donor site are the main concerns of this technique. Different types of dresses were recommended for temporary covering of the wound with variable results. The dressing should be cheap with simple storage and easy to apply, promote healing, reduce pain, and prevent scar development.

Amniotic membrane(AM) has been used as a versatile skin alternative in dermatology for more than one century [1]. The structure of AM and its growth factor contents make it suitable as an ideal biological dressing material. Moreover, it shows important features such as promoting re-epithelization, anti-microbial, anti-inflammatory properties and inhibiting of fibrosis and scar formation [2, 3]. Most of these characteristics are based on contents including collagen types I, II, III, IV, laminin-1, laminin-5 and fibronectin in the basement membrane. EGF, TGF-a, -b1, -b2 and -b3, keratinocyte growth factor (KGF), KGFR, HGF, HGFR, bFGF, VEGF, PDGF [4] are well-known growth factors which are released by epithelial cells at amniotic membrane. Amniotic membrane has recently been used as a scaffold for transferring cells to the wounds [5]. It is vastly used in different surgeries, as a carrier for cell transfer from bench to bed, tissue engineering and regenerative medicine [3]. Patient comfort and cost effectivenessmak this an ideal skin substitute for wound healing [6]. Furthermore there is no ethical concern regarding the utilization of human amniotic membrane.

Over the past few decades different amniotic membrane products have been developed specifically for cornea and wound coverage .It works as a mechanical barrier which regulates the humidity of the wound environment and prevents tissue desiccation of [7]. Human amniotic membrane was used in different preservation types: fresh, air-dried, freeze dried cryopreserved, acellular and extract forms. In comparison between different types of amnion preservation, dried type was better than cryopreserved [8, 9]. This study aimed to compare the effectiveness of two forms of amniotic membrane products; dried amnion (AmiCare, Royan Institute, Iran) and Acellular amnion (OcuReg-A, Royan Institute, Iran) in comparison with Mepitel (Mölnlycke Health Care Company, Sweden) for coverage of skin graft donor site.

Materials and Methods

Institutional and Ethical Approval

This study was approved by institutional review board (IRB) and ethical committee of Royan Institute as well as Iran University of Medical Sciences (IR.ACER.ROYAN.REC.1394.81). We have aslo registered the study protocol with the Iranian clinical trial registry (IRCT01511118177N12; www.irct.ir). All the patients provided their informed written consent before inclusion in the study.

Amniotic Membrane Preparation

Fresh amniotic membranes were obtained from placenta of healthy mothers who underwent elective caesarean section with negative serological test for HIV, HCV, HBs, cytomegalovirus, Syphilis. Also they had negative molecular tests for HIV I, II, HBV, HCV and were negative in microbial test. The criteria for AM's selection was in accordance to Royan Cord Blood Bank regulations and all AMs procedures were based on Royan amniotic membrane bank protocols. To produce dried amniotic membrane, fresh amnion was processed by isolation from placenta; washing three times with normal saline (containing penicillin and streptomycin), laying overnight on a sterile gauze in the biosafety cabinet for air dry. Dried products were irradiated with Gama waves (25 kGy). A AmiCare can be stored for one year at Room temperature. To produce Acellular amniotic membrane also known as OcuReg-A epithelial cell were separated from amnion surfaces by incubated processed AM in EDTA (0.025%) for 1 hour at 37 ^{0}C. Then cells were slowly removed using a cell scraper and the membrane was washed three times with normal saline. This product can be stored for one week in the refrigerator. To prevent any contamination all steps were performed in sterile conditions.

Patients and Clinical Observation

All adult patients between the age of 18 to 65 years who needed partial thickness skin graft and had not any systemic disease or comorbidity were included in the study. A split-skin graft taken from an anterior thigh donor site for all patients and the technique was explained to the patients. All skin grafts were harvested by a single surgeon (MJF) by using a free hand large Humby-type knives and all effort was made that the thickness of the graft be the same in different parts of the donor area. The patients were then randomly divided into three groups. We used block randomization. In the first group (A) the wound covered with Mepitel (ready to use), in the second group (B) with AmiCare and in the third group(C) with OcuReg-A. The amnions in group B and C were covered with sterile gauze. In all three groups circumferential bandage was used for fixation of dressing. Dressing was changed with 4-day intervals till complete healing was achieved; but if the amnion was adhered to the wound, it left untouched. Digital photography and evaluation of healing by an independent observer were made during 12 days after harvesting the skin graft. The re-epithelialization was

scored as excellent, good, fair and poor (Table 1). Also pain score (Visual Analogue Scale) measurements and evaluation for infection (with any signs such as discharge, unusual pain or peripheral cellulites, a swab culture was obtained from the wound) were made during dressing change. The residual scar was evaluated in 3 and 6 months in patients using Vancouver Scar Scale). The four characteristics of the scar, vascularity (0-3), pigmentation (0-3), pliability (0-5) and height (0-3) were evaluated in a blind manner by an independent plastic surgeon and the final score (0-14) was used for statistical analysis.

Table 1. Numeric score of Re-epithelialization

Defined numeric unite	Rate of Re-epithelization
Excellent=4	More than 90%
Good=3	70% to 90%
Fair=2	30% to 70%
Poor=1	Less than 30%

Statistical Analysis

We used Cohen table based on power 0.8 and α error 0.05 and β error 0.2. At the beginning the calculated sample size was 75. However, during the study only 20 patients were convinced to be followed for the rest of study. The data were analyzed by statistical package for social sciences (SPSS Inc. Chicago, Illinois, USA), Version 19.0. Data are presented as mean ± SD and proportion as appropriate. Data was analyzed with use of Kruskal Wallis and one-way analysis of variance (ANOVA) tests with LSD as the post hoc test. Proportions were compared using the chi-square test. A 2-sided p-value of less than 0.05 was considered statistically significant.

Results

Twenty patients including 16 men and 4 females were incuded in this study. The age range was 28.92±10.65. The donor areas were covered with Mepitel in 8 patients, dried amnion(AmiCare) in 7 patients and Acellular amnion(OcuReg-A) in 5 patients.

Re-epithelialization

The result of Re-epithelialization is shown in Table 2. Although poor results were observed in dried and acellular amnion, but the differences between them and Mepitel were not statically significant (Figure 1). (Dried Amnion versus Mepitel and Acellular, p=0.573).

Pain sensation

As shown in Table 3 and Figure 2, Pain sensation during dressing changes was not significantly different between mepitel and AM groups (Mepitel compared with Acellular and Dried Amnion. p=day 4 th: 0.131, day8 th: 0.93 and day 12 th:0.365).

Scar Formation

There was no significant difference of Vancouver Scar Scale (VSS) between groups in 3 and 6 months post operation (p=0.70 and 1.00 respectively) (Figure 3).

Table 2. Score of Re-epithelization rate in 12th day

Groups	Patients no.	Excellent	Good	Fair	Poor
Mepitel[a]	8	1	3	3	1
Dried Amnion(AmiCare)[a]	7	0	3	1	3
Acellular Amnion(OcuReg-A)[a]	5	1	1	0	3

[a]Differences between groups were not significant (p=0.573).

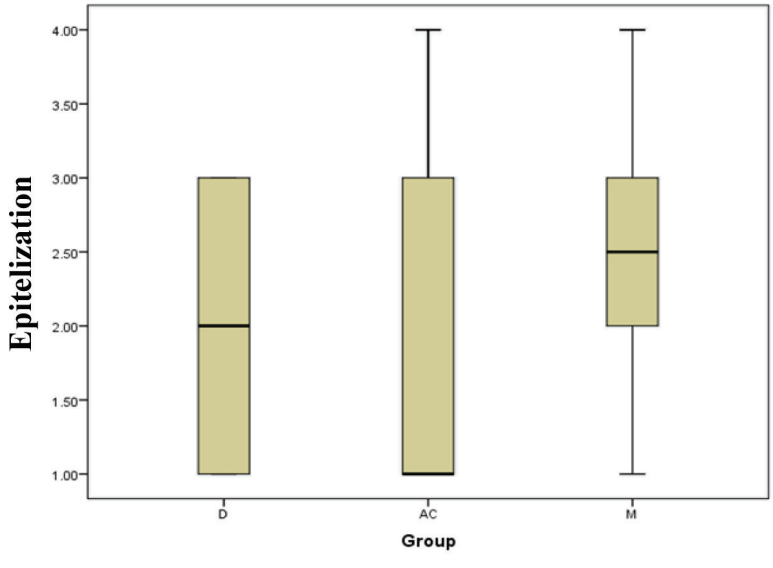

Fig. 1. The comparison of Re-epithelization rate on 12th day between group D (Dried Amnion) versus AC (Acellular Amniotic membrane) and group M (Mepitel). (P=0.573)

Table 3. Pain sensation during dressing

Groups	Patients NO.	Average of pain		
		4th day	8th day	12th day
Mepitel	8	4±3.28	2.1±1.8	0.4±0.8
Dried Amnion(AmiCare)	7	5.6±2.3	5.2±2.4	1.6±1.8
Acellular Amnion (OcuRage)	5	8±2.1	2.5±1.7	1.5±1.6
***p* value**		0.131	0.93	0.365
Statistical test		ANOVA	Kruskal Wallis Test	Kruskal Wallis Test

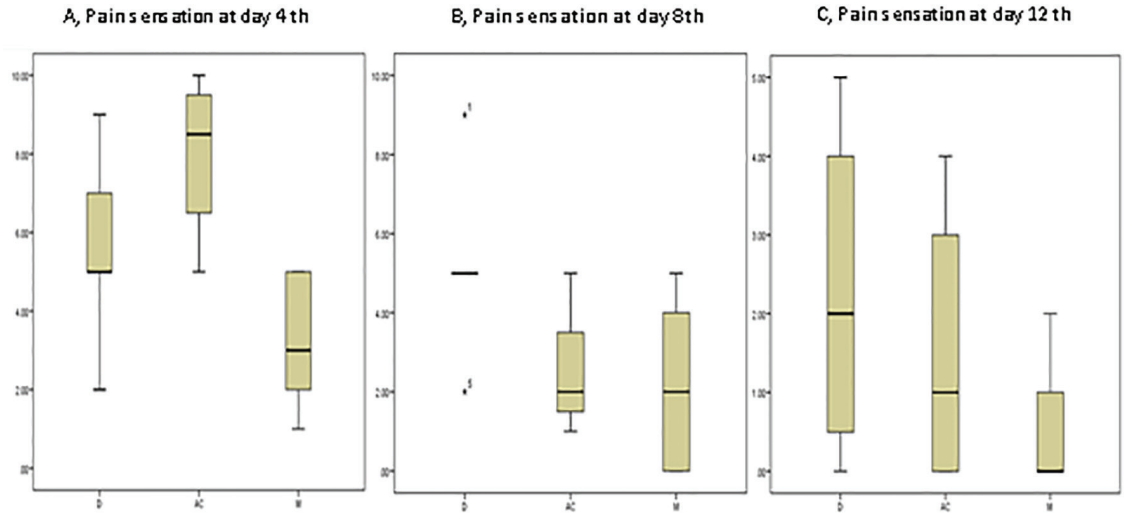

Fig. 2. The results of pain sensation in different dressing group. D (Dried Amnion) versus AC (Acellular Amniotic membrane) and M (Mepitel).P value day 4 th 0.131, day 8 th093 and day 12 th .365

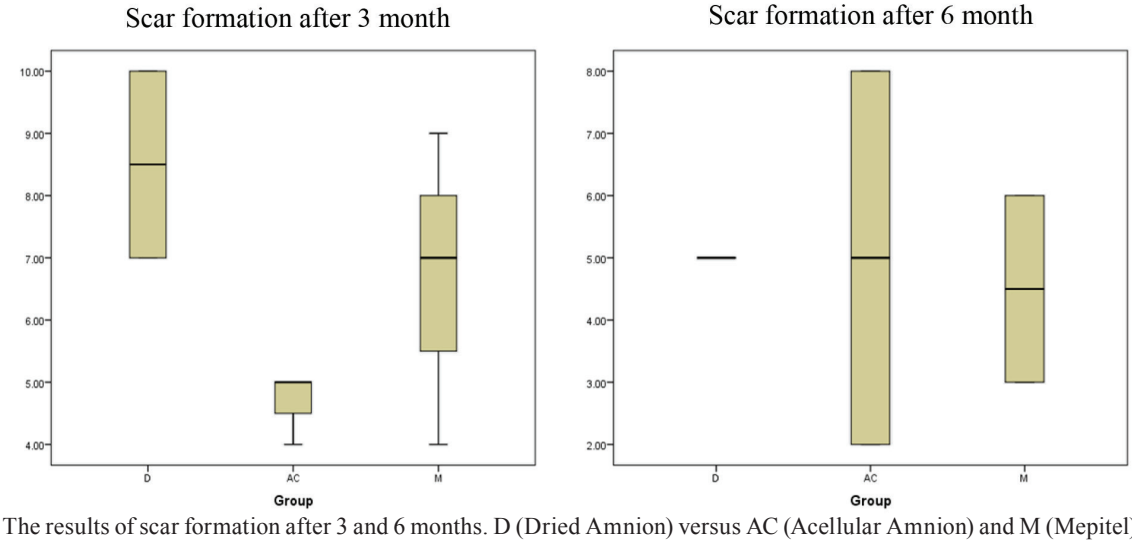

Fig. 3. The results of scar formation after 3 and 6 months. D (Dried Amnion) versus AC (Acellular Amnion) and M (Mepitel). P=070 and 1 respectively.

Discussion

Split thickness graft is mostly used in skin graft which is a common procedure in reconstructive surgery. The wound in donor area will gradually heal but taking care of it is a challenging problem [10]. Different dressing materials and also coverage methods are recommended for donor areas with a wide range of results. These include Gauze, Films, Hydrogels, Foams, Alginates, Composites, Hydrocolloids and Interactive Dressings [11]. An ideal wound dressing should have certain properties like accelerating re-epithelization, preventing infection, pain reductiob and scar formation accompanied by cost-effectiveness especially in developing countries [4].

In our study the difference between dried and acellular amniotic membrane with respect to pain, healing time and scar formation on a donor wound was negligible. In multiple studies as in ours, dried irradiated amnion could significantly reduce pain during and between dressing change, promote healing and decrease hospital stay [12-16]. In Mostaque AK et al study, Study on 102 burn patients, dried amnion in comparison with silver sulfadiazine could significantly

decrease hospital stay, number of dressing change, pain during and between application and healing time [12]. In Bujang-Safawi et al study, these results were repeated in 33 patients with superficial burn of the face and they recommended this cheap ideal dressing for facial burns [14]. In a study on donor site of the graft where the patient was used as control, freeze-dried amnion only reduced pain but the healing time and prevention of infection were not different with antibiotic impregnated gauze group [16].

It is clear that the dried amnion can preserve its characteristics as an ideal wound dressing material during long time of room temperature stocking [13]. There is some controversy about the effect of amnion on post healing scar and hyper pigmentation [16]. In most studies the acellular amnion was evaluated as a carrier for cell transport [5, 17]. Although, amnion could promote healing and neo-vascularization without cells [18-20] the extraction of cells eliminates immunological rejection and seeding, attachment and proliferation of fibroblast, keratinocytes or stem cells with better delivery than with a cellular amnion [8, 21, 22].

AmiCare and OcuReg-A are new products from amniotic membrane and Mepitel is a Primary wound contact dressing [23].Our results showed that the rate of epithelialization is not different between two types of amnion and specialized expensive wound dressing material. In our study the effect of dried (AmiCare) and acellular(OcuRege) amnion on healing and pain were not significantly different, although dried amnion was better. The preparation, preservation and transport of Acellular amnion are more complex than dried amnion. We conclude that for simple coverage of the wounds, dried amnion is more practical and cheap, but when the plan is to transfer the autologous or allogeneic cells into the wound, then acellular amnion is the material of choice. There are some limitations with using amniotic membrane as a wound dressing material. Currently, there are no data of communicable diseases transmission and rejection of amniotic membrane transplant in clinical use, but to avoid of any possible risk, strict screening of donor has to be performed and the GTP and GMP requirements have to be considered. Despite this widespread use, Procurement, preparation and preservation methods have effect on the quality of amniotic membrane products including Dried and Acellular. Thus, optimization and standardization of handling procedures have to be done. Therefore, quality control tests should be defined, which lead to monitoring all of products and releasing the best.

In conclusion, this clinical investigation showed safety and efficacy of AmiCare and OcuRage as well as other clinical features including: Re-epithelization rate (healing time), Pain sensation, Scar formation for treatment of split-thickness donor site with no significant differences in comparison of Mepitel as a standard wound dressing. It recommends AmiCare and OcuRage as a point for STDS wound treatment.

Conflicts of Interest: None declared.

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