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BURNS

Article

ANALYZING THE EFFICACY AND OUTCOME OF AMNIOTIC MEMBRANE IN BURN CARE: A SYSTEMATIC REVIEW AND META-ANALYSIS

Nandita Melati Putri[1], Aditya Wardhana[1], Normalina Sandora[2], Akhmad Noviandi Syarif[1], Nadya Farhana[1], & Sheila Oklia[1]

1. Plastic Reconstructive and Aesthetic Division, Department of Surgery, Cipto Mangunkusumo Hospital, Universitas Indonesia, Jakarta, Indonesia
2. Indonesia Medical Education and Research Institute (IMERI), Universitas Indonesia, Jakarta, Indonesia

ABSTRACT

Introduction: Managing burn injuries is a significant clinical challenge in promoting wound healing and minimizing complications. Advances like amniotic membrane as a biological dressing have been introduced to improve wound healing. This study analyzes the efficacy of amniotic membrane in burn care and its impact on wound healing outcomes.
Methods: We conducted a systematic review in Pubmed, Cochrane, and ScienceDirect, using "Amnion", "Burns", and "Wound healing" as keywords. The inclusion criteria are studies assessing the application of amniotic membrane on burn wounds. The outcome measures were mean healing time, wound healing rate, incidence of wound infection, dressing renewal frequency, pain score, and LOS.
Results: We identified eleven trials (n=971) ranging from the year 1989 to 2023, containing eight RCTs, and three NRCTs. The pooled RR showed statistically significant differences between amniotic membrane group and control group in mean healing time (RR -4.52 [95% CI; -6.93, -2.11]; p=0.0002), wound healing rate (RR 1.60 [95% CI; 1.09, 2.33]; p=0.02), incidence of wound infection (RR 0.48 [95% CI; 0.30, 0.77]; p=0.002), and dressing renewal frequency (RR -1.64 [95% CI; -2.48, -0.79]; p=0.0002).
Conclusion: This meta-analysis indicates that amniotic membrane is advantageous as a biological dressing for burn patients.

Keywords: *Amniotic Membrane; Burns; Dressing Renewal Frequency; Incidence of Wound Infection; Length of Hospital Stay; Mean Healing Time; Pain Score; Wound Healing Rate*

Latar Belakang: Penanganan luka bakar merupakan tantangan klinis yang signifikan dalam upaya mempercepat penyembuhan luka dan meminimalkan komplikasi. Perkembangan terbaru, seperti penggunaan membran amnion sebagai balutan biologis, telah diperkenalkan untuk meningkatkan penyembuhan luka. Studi ini menganalisis efektivitas membran amnion dalam perawatan luka bakar serta dampaknya terhadap luaran penyembuhan luka.
Metode: Kami melakukan tinjauan sistematis pada basis data PubMed, Cochrane, dan ScienceDirect dengan menggunakan kata kunci "Amnion", "Burns", dan "Wound healing". Kriteria inklusi mencakup penelitian yang mengevaluasi aplikasi membran amnion pada luka bakar. Parameter luaran yang dianalisis meliputi waktu rata-rata penyembuhan, laju penyembuhan luka, insidensi infeksi luka, frekuensi pergantian balutan, skor nyeri, dan lama rawat inap.
Hasil: Sebanyak sebelas uji klinis (n=971) yang diterbitkan antara tahun 1989 hingga 2023 diidentifikasi, terdiri dari delapan uji acak terkontrol (RCT) dan tiga uji non-RCT (NRCT). Analisis gabungan *risk ratio* (RR) menunjukkan perbedaan yang signifikan secara statistik antara kelompok yang menggunakan membran amnion dibandingkan dengan kelompok kontrol dalam hal waktu rata-rata penyembuhan (RR -4,52 [95% CI; -6,93, -2,11]; p=0,0002), laju penyembuhan luka (RR 1,60 [95% CI; 1,09, 2,33]; p=0,02), insidensi infeksi luka (RR 0,48 [95% CI; 0,30, 0,77]; p=0,002), dan frekuensi pergantian balutan (RR -1,64 [95% CI; -2,48, -0,79]; p=0,0002).
Kesimpulan: Meta-analisis ini menunjukkan bahwa membran amnion memberikan keuntungan sebagai balutan biologis bagi pasien luka bakar.

Kata kunci: *Membran Amnion; Luka Bakar; Frekuensi Pergantian Balutan; Insidensi Infeksi Luka; Lama Rawat Inap; Waktu Penyembuhan Rata-rata; Skor Nyeri; Laju Penyembuhan Luka.*

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Conflicts of Interest Statement:
The author(s) listed in this manuscript declare the absence of any conflict of interest on the subject matter or materials discussed.

INTRODUCTION

Burn injuries represent a significant clinical challenge, especially the management to optimize wound healing and minimize complications. Traditional burn wound care has evolved over the years, with various strategies being employed to enhance recovery and improve patient outcomes.[1] The use of biological dressings, such as the amniotic membrane has gained considerable attention.

The amniotic membrane, a natural tissue derived from the inner layer of the placenta, has been explored for its potential benefits in wound healing due to its unique biological properties.[2] It has been reported that amnion membrane could promotes epithelization as it possess several significant growth factor for epithelial regrowth such as TGFs (EGF, KGF, HGF, bFGF, TGF-α, and TGF-β1, TGF-β2, and TGF-β3) and some identified mRNA expression and protein concentration of the epidermal, keratinocyte, hepatocyte, and basic fibroblast growth factor.[2] Other investigated properties of amniotic membrane includes immunogenicity, analgesic properties, and protection from protein or fluid loss.[3] Furthermore, amniotic membrane is a biological waste that generally can be obtained easily and does not require invasive procedure which minimizes ethical problems. This has led to its investigation as a promising biological dressing for burn wounds.

Amniotic membranes have been utilized in the treatment of burn wounds for decades with initial application documented in 1912 by Sabella et al.[4] Since then, several trials had reported the application of amniotic membrane as biological dressing for burn patients. The amniotic membrane preparations are also varied. Trials using fresh, cryopreserved, alcohol and glycerol preserved, cell cultured, dried and dehydrated, and animal derived amniotic membrane have been conducted over the years. Hence, our study aimed to provide updates regarding recent trials to analyze the efficacy of amniotic membrane in burn care, and its outcome in wound healing.

METHOD

Inclusion Criteria

The inclusion criteria for this review includes clinical studies assessing the application of human amniotic membrane on burn wounds. Studies with electrical and chemical burns, amniotic membrane derived from animals, dry and dehydrated amnion, studies evaluating graft take and donor site as the outcome were excluded in this study. Studies included were limited to articles published in English only. There was no time restriction applied.

Search Technique

A systematic review was conducted using the terms "Amnion", "Burns", and "Wound Healing" as keywords, in multiple databases including Pubmed, Cochrane, and ScienceDirect. This review is conducted according to PRISMA guidelines. Study selection process shown in **Figure 1**. All included studies were critically appraised and reviewed.

Data Extraction

Reviewers extracted data independently and assessed its methodological quality using predefined criteria. Extracted data consists of study year, study design, patients, treatment compared, side effects, mean healing time, wound healing rate, wound infection, dressing renewal frequency, pain score, and length of hospital stay. We extracted information regarding the trials methodological quality using Cochrane's criteria and resolved disagreement among reviewers by discussion.

Risk of Bias Assessment

RCT risk of bias assessment was conducted using Cochrane risk of bias tool for randomized trials (RoB 2) for RCT trial. The Newcastle–Ottawa Scale (NOS) was used to assess non-randomized clinical trials (NRCT**)**. A score of 0–9 (described by stars) was assigned to each study. Studies with seven or higher stars were considered as high quality studies.

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Statistical Analysis

The primary outcome measures were mean healing time and wound healing rate. The secondary outcome measures of this study were incidence of wound infection, dressing renewal frequency, pain score, and length of hospital stay. We used Review Manager 5.4.1. to analyze the outcome data. A random-effect model used to pool the results and reported the relative risk (RR) for binary outcomes and mean difference for continuous outcomes with 95% CI. Heterogeneity was evaluated with the I^2 statistic. Differences of the overall effects between randomized and non-randomized studies were assessed by a test of interaction. The publication bias was performed to analyze the distribution of studies using Review Manager 5.4.1.

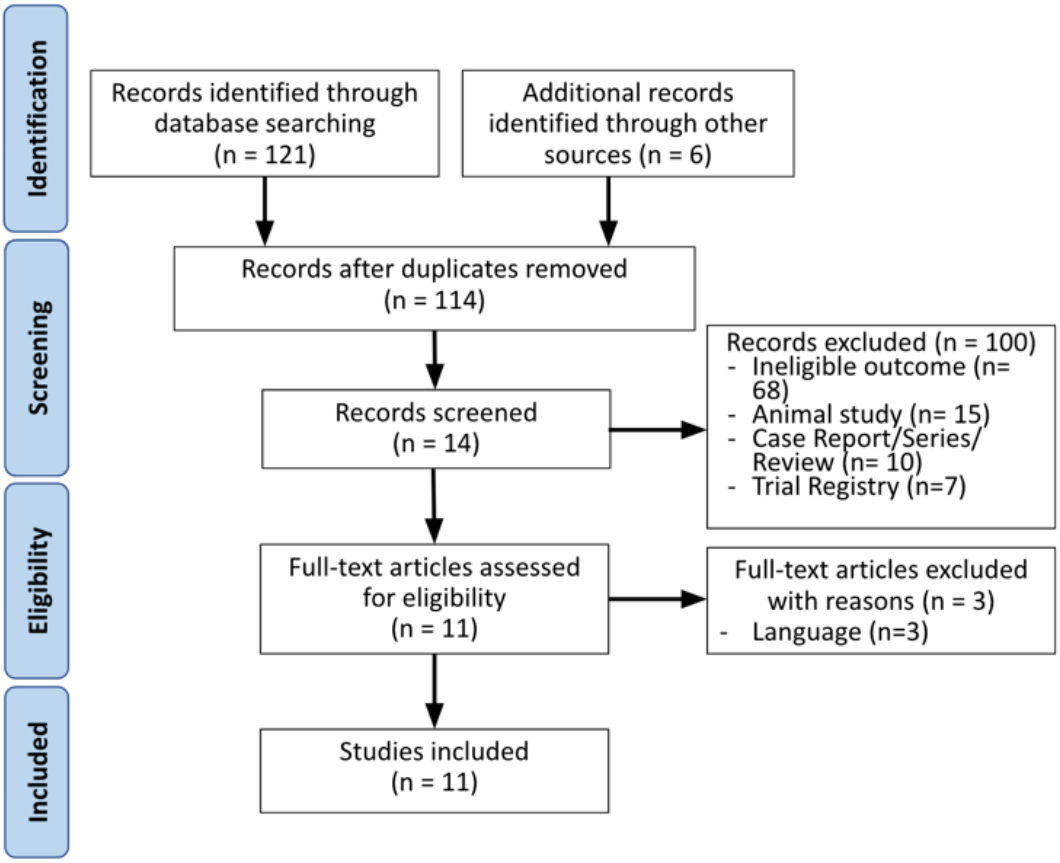

Figure 1. PRISMA Diagram for Study Selection Process

RESULTS

Included Studies

From the total of 127 candidate studies, 13 studies were excluded due to duplication. On the screening of the abstracts, 114 studies were excluded, most often because they had ineligible outcomes (n=68), such as non-burn wounds amnion studies, amniotic membrane derived from animals, dry and dehydrated amnion, and studies evaluating graft take and donor site as the outcome. In the full text article assessment, we excluded three studies due to language limitation.

We identified eleven trials (n=971) ranging from the year 1989 to 2023, containing eight randomized clinical trials and three non-randomized controlled trials. These studies assessed comparison between amniotic membranes as the treatment group on burn wounds to several other dressing and treatment used in burns. All included studies were critically appraised and reviewed.

Table 1 summarizes the study design, intervention, control group description, and

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side effects reported. The total sample of patients treated with the amnion membrane and control group is 480 and 491 patients, respectively.

Amnion intervention in these studies varied between non-preserved amnion, preserved amnion, and amniotic membrane product in sterilized packaging. Control treatment also varies between studies, which are topical antibiotics, silver sulfadiazine, honey, nitrofurazone, and conventional standard treatment of burn including excision and skin grafting as control.

Randomized Study Quality

RoB assessment in **Figure 2** shows that overall RCT studies have some concerns of bias. In terms of outcome and data reporting, the quality of all randomized studies was generally good. However most of the studies did not mention methods regarding randomization process, allocation concealment, and blinding. The eight randomized studies accounted for 86.1% of the total patients in the meta-analysis.

The total numbers of patients in these eight studies range from 50 to 211 (**Table 1**). There was a small, old study but had generally a low risk of bias, even though this study was published 30 years ago. Despite randomization, small size studies tend to be more vulnerable with the risk of poor outcome.

Only two studies mentioned the method of randomization process, while the other studies did not. Patients were assigned to the treatment group by Random Allocation Software and simple randomization method. Allocation concealment was accomplished in one study with a numbered envelope containing patient allocation sheets. The remaining studies had unspecified methods about their allocation concealment. In blinding, only two studies explained the blinding methods either for the participant or personnel, and four studies described the blinding of the outcome assessor. The blinding process was unspecified for the other studies. One study had a missing outcome for both groups because two and three patients dropped out in the intervention and control group, respectively. Another study reported missing outcomes due to withdrawal of ten patients in the control group and eight patients in the intervention group. However, they were not related to the true outcome. Other studies did not report any missing outcomes.

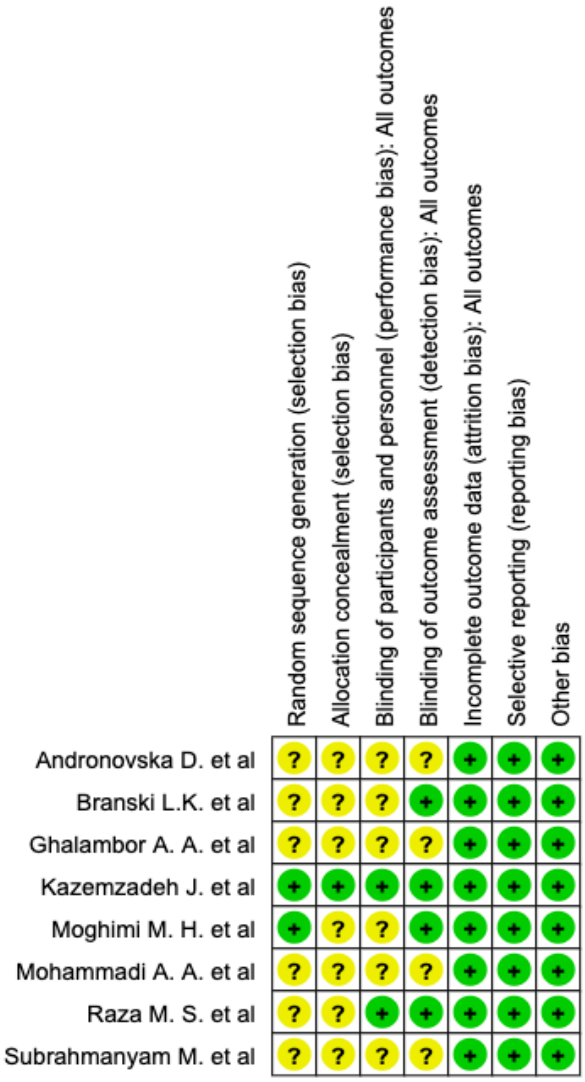

Figure 2. Risk Of Bias Assessment using RoB 2 for RCT Trials

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Table 1. Summary of Eligible Studies

Study, Year	Study Design	Inclusion Criteria	Amnion (n)	Amnion Intervention	Control (n)	Control	Side effect reported
Randomized Studies							
Moghimi M. H. et al[5], 2023	RCT	Patients above 18 years old Less than 20% TBSA second degree heat burn wounds	25	• Non-preserved amniotic membrane covered with vaseline gauze followed by saline-moisturized gauze and wrapped using a crepe. • Wound inspection and secondary dressing changes on day 4 or 5.	25	• 20 grams of Silver sulfadiazine ointment per percentage of the wound, soaked in gauze. • Dressing changes were done daily.	No infection signs present in both groups.
Kazemzadeh J. et al[6], 2022	RCT	Second degree burns Less than 10% TBSA	35	• Amniotic membrane product in sterilized packaging, applied and covered by petroleum impregnated gauze and wet gauze moistened by saline. Dressing changes once a week.	35	• 20 grams nitrofurazone ointment per TBSA soaked in gauze. • Dressing changes were done daily.	No side effects reported.
Raza M. S. et al[7], 2020	RCT	Superficial partial thickness facial burns No comorbid	34	• Preserved amnion membrane in 30% Glycerol. Wounds evaluated daily until healed, and also evaluated at 1 month, 3 months, and 6 months intervals.	28	Application of ointment containing Polymyxin B and Bacitracin. Wounds evaluated daily.	No side effects reported.
Mohammadi A.A. et al[8], 2009	Single-blind RCT	Less than 20% TBSA Second and Third degree of burns	104	• Non-preserved amniotic membrane covered with Vaseline gauze and dry gauze dressing. • Dressing changed every 3-4 days until healed.	107	• Application of silver sulfadiazine ointment. • Daily dressing changes until healed.	Fever in some patients, treated with antibiotics.
Andronovska D. et al[9], 2008	RCT	Dermal and subdermal burns Acute burn patients less than 24 hours	30	• Preserved Amnion membrane in 76% alcohol, no outer dressings mentioned. • Wound evaluation on the seventh day.	30	Conventional standard treatment of burns: exposition, occlusive dressing and initial excision with skin grafting. No information of dressing changes.	No side effects reported.
Ghalambor A. A. et al[10], 2000	RCT	Less than 20% TBSA Second degree	100	• Non-Preserved Amnion membrane dressings. • Inspected daily but the membrane changes when necessary up to 3-4 days	100	Conventional topical antibiotics (nitrofurazone ointment) and classical dressings (cotton bandage). Wound was evaluated daily for up to 10 days.	No side effects reported.
Subrahma-nyam M. et al[11], 1994	RCT	Less than 40% TBSA Admitted within 6h of injury	24	• Non-preserved amniotic membrane. • First wound inspection on day 8 when the dressing was changed and then every second day until healed.	40	• Honey impregnated gauze (unprocessed and undiluted honey) covered with an absorbent dressing. • These wounds were inspected every 2 days until healed.	There are no allergic reactions in any of the patients in either group.
Branski L.K. et al[12], 2007	RCT	• Partial-thickness burns of the face, head and neck • Less than 40% TBSA	61	• Cryopreserved amniotic membrane and topical antimicrobial creme • Re-application of amnion, if needed	59	Topical facial antimicrobial cream: 1% nystatin and 2% polymyxin B/ bacitracin Daily dressing changes, wound cleaning, and re-application of topical antibiotics	No side effects reported.
Non-randomized Studies							
Liu D. et al[13], 2010 (AA)	Case control	No information.	12	• Non-preserved acellular Amniotic Membranes. • No information of dressing	12	No information on control treatment and dressing changes.	No evidence of immunological rejection and

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				changes.			inflammatory reaction.
Liu D. et al[14], 2010 (BM)	Case control	No information.	10	• Non-preserved acellular Amniotic Membranes. • No information of dressing changes.	10	No information on control treatment and dressing changes.	No evidence of immunological rejection and inflammatory reaction.
Sawhney C. P. et al[15], 1989	Case Control	Superficial dermal, intermediate depth dermal, deep dermal burns	45	• Non preserved amnion membrane. • The dressing was changed daily, if the membrane dissolved it was reapplied, subsequent dressing was changed every 3 days until healed.	45	Application of 1% silver sulfadiazine cream. Dressing changes daily until wound healing.	No side effects reported.

Table 2. Patient's Characteristics

Study, Year	Age (years old)		TBSA (%)		Burn Depth
	Amnion	Control	Amnion	Control	
Moghimi M. H. et al, 2023	26.72 ± 7.49	27.16 ± 8.13	13.64 ± 2.60	14.72 ± 2.50	Partial thickness
Kazemzadeh J. et al, 2022	20.05 ± 3.60	21.60 ± 2.02	12.72 ± 8.99	12.61 ± 9.44	Partial thickness
Raza M. S. et al, 2020	26.5 ± 12.2		34.9 ± 10.9	35.6 ± 9.1	Superficial partial thickness
Mohammadi A.A. et al, 2009	17.30 ± 12.42	19.10 ± 11.56	11.90 ± 3.80	12.30 ± 4.14	Partial to Full thickness
Andronovska D. et al, 2008	NI	NI	10-20% (12/40)*	<10% (13/40)*	Partial to Full thickness
Ghalambor A. A. et al, 2000	<10 years old (96/200)*		<20*	<20*	Partial thickness
Subrahma- nyam M. et al, 1994	25*	24.6*	18.5*	19.4*	Partial thickness
Branski L. K. et al, 2007	7±4	7±4	12±7	11±6	Partial thickness

**No mean and standard deviation data available.*
Abbreviation: NI = No Information; TBSA = Total Body Surface Area

Table 3. Risk Of Bias Assessment using NewCastle Ottawa Scale (NOS) for non-RCT Trial

Study, Year	Selection				Compara-bility	Outcome			Score
	Adequate definition of cases	Representa-tiveness of the cases	Selection of Controls	Definition of Control	Compara- bility of cases and controls on the basis of the design or analysis	Ascertain-ment of exposure	Same method of ascertain-ment for cases and control	Non-response rate	
Liu D. et al, 2010 (AA)	*	-	-	-	*	*	*	*	*****
Liu D. et al, 2010 (BM)	*	-	-	-	*	*	*	*	*****
Sawhney C. P. et al, 1989	*	*	-	*	**	*	*	*	********

Patients' characteristics shown in **Table 2**. Age differences were varied between studies. One study did not mention the age range nor the mean age of their patients. Four studies had similar mean age in adults, while three studies had lower mean age and age range in pediatrics. However, the imbalance was proportional between amniotic membrane and

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control groups. No statistical judgement was applied for this imbalance.

Mean % TBSA was higher in one study, but the rest of the studies had similar mean % TBSA under 20% of total body surface area. Despite there was a higher mean % TBSA reported in one study, it marked proportional in both amniotic membrane and control group.

Most of the studies included partial thickness burns as their inclusion criteria, while two studies also included full thickness burns. Andronovska D. et al[9] mentioned initial excision and skin grafting as their standard control treatment while Mohammadi A. A. et al[8] did not mention excisional debridement in their methods.

Non-randomized Study Quality

The Newcastle–Ottawa Scale (NOS) was applied to assess three non-randomized controlled trials. Among three studies, only one study scored eight stars indicating a high quality study. The risk of bias using the NOS scale is detailed in **Table 3.**

Three non-randomized controlled trials published from 1989 to 2010, accounted for 13.9% of the total patients in this meta-analysis. These were small studies with a high risk of bias. One study published 35 years ago, was a high quality study, limited by its study design, a case control study.

All of these case control studies had no information regarding the patient's baseline characteristics, such as the age, total body surface area, nor burn depth. One of them had silver sulfadiazine as their control treatment, while two of them had unknown control treatment and had no information regarding dressing changes , assuming standard burn treatment using moist dressing applied in those studies. No missing outcomes were reported in these studies.

Mean Healing Time

One of the primary outcomes of this study was mean healing time. Data for mean healing time was available in three randomized controlled trials and three non-randomized trials. Healing time data were available in Subrahmanyam M. et al[11] study (9.4 vs 17.5 days) but we excluded it in this meta-analysis due to different unit measurements used. The effects of mean healing time between amnion and control treatment shown in **Figure 3.**

Two randomized studies had 58.4% weight effect for this meta-analysis compared to 41.6% weight effects from three non-randomized studies. The pooled RR showed statistically significant differences between amniotic membrane group and control group in mean healing time (RR -4.52 [95% CI; -6.93, -2.11]). The forest plot showed lower mean healing time by days with the overall effects between groups favoring amnion treatment than control treatment (p=0.0002).

Non-randomized studies' overall effect separately had statistically significant results compared to randomized studies, precisely (p = 0.0002 vs p = 0.07). There was also a significant Heterogeneity test (p<0.00001) with I^2 = 97%. The heterogeneity could be potentially referred to as study design per trials, since RR for mean healing time differs significantly between RCTs and NRCTs. Higher heterogeneity tests were found in randomized studies (I^2 = 98%; p<0.00001) compared to heterogeneity tests in non-randomized studies (I^2 = 50%; p = 0.13). Inconsistent results between studies may contribute to the overall effect of the outcome, such as the imbalance baseline of % TBSA and burn depth. Raza M. S. et al[7] study had higher mean %TBSA compared to mean %TBSA in Mohammadi A.A. et al[8] study. Mohammadi A. A. also included full thickness burns in their inclusion criteria, compared to Raza M. S. et al[7] only included partial thickness burns. Higher inhalation injury was also found in the amnion group in Branski L.K. et al.[15]

Wound Healing Rate

One of the primary outcomes of this study was wound healing rate. Data for wound healing rate were available in three RCTs by day 7, day 14, and day 30. Data of wound healing rate by day 7 was reported from all studies, while data of wound healing rate by day 14 and 30 only provided by two and one study, respectively **(Table 4)**.

From **Table 4**, wound healing rate was faster in the amnion group compared to the control group by day 7. All three studies reported p value <0.05 showing statistically significant results in wound healing rate by day 7 in the amnion group. Faster wound healing rate was also found in the amnion group by day 14 and day 30 in Moghimi M. et al[5] study, even though there was missing data of wound healing rate in day 14 and 30 due to drop out

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patients. Complete wound healing rate found in both groups by day 14 in Kazemzadeh J. et al[6] study.

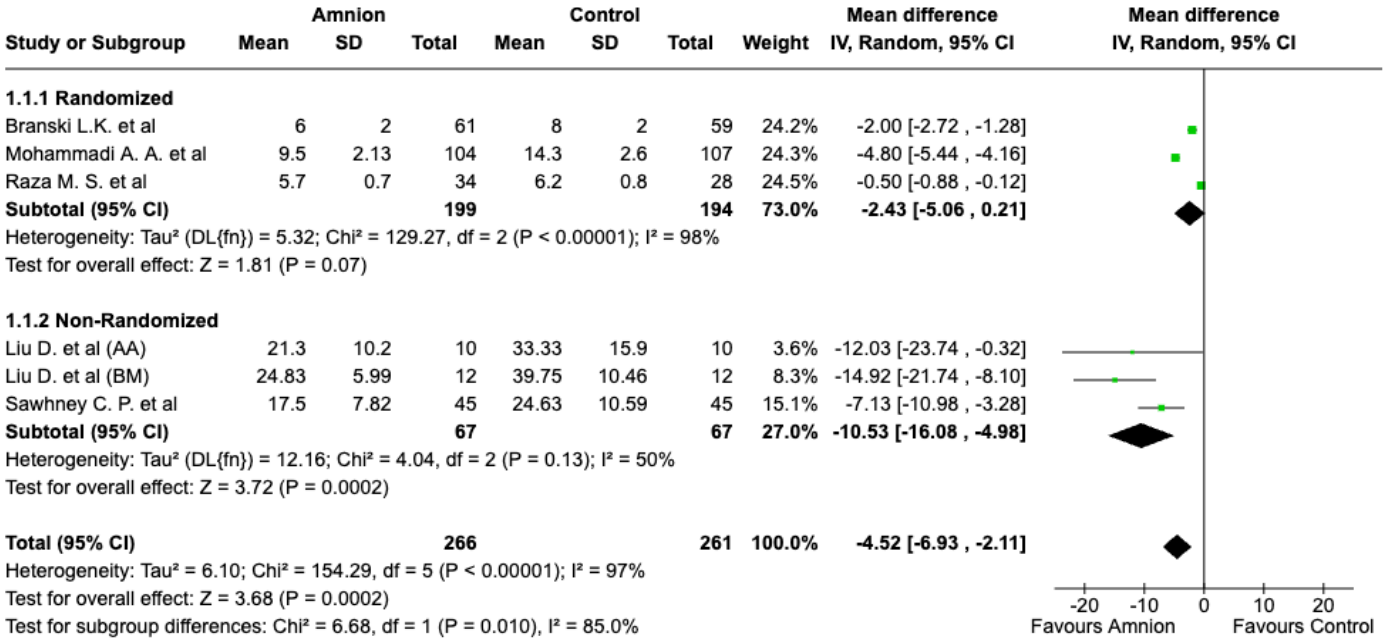

Figure 3. *Mean Healing Time in Amniotic Membrane and Control Treatment in Burn Wound*

Table 4. Wound Healing Rate between Amnion and Control Treatment in Burn Wound

Study, Year	Wound Healing Rate Day 7		P-value	Wound Healing Rate Day 14		P-value	Wound Healing Rate Day 30		P-value
	Amnion Group	Control Group		Amnion Group	Control Group		Amnion Group	Control Group	
Andronovska D. et al, 2008	22/30	14/30	p=0.035	-	-	-	-	-	-
Moghimi M. H. et al, 2023	23/25	10/25	p<0.001	23/23	14/22	p=0.001	23/23	22/22	p=0.1
Kazemzadeh J. et al, 2022	35/35	27/35	p=0.002	35/35	35/35	-	-	-	-

The pooled RR showed statistically significant differences between amnion group and control group in wound healing rate (RR 1.60 [95% CI; 1.09, 2.33]).

Figure 4 showed the overall effects favours amnion treatment than control treatment (p=0.02). There was also a significant Heterogeneity test (p = 0.04) even though the I^2 = 69% showing inconsistent results between studies.

Moghimi M. H. et al[5] and Kazemzadeh J. et al[6] had similar patient's characteristics. However, Andronovska D. et al[9] had different patient's characteristics baseline compared to the other two studies. Andronovska D. et al[9] had no information regarding their patient's mean age and included full thickness burns patients. This may have contributed to the inconsistent results of the outcome.

Incidence of Wound Infection

One of the secondary outcomes of this study was incidence of wound infection. Incidence of wound infection were available in five RCTs. **Figure 5** showed the effects of amnion treatment compared to control treatment in incidence of wound infection. Incidence of wound infection was generally found less in the amnion group compared to the control group.

The pooled RR showed statistically significant differences between groups (RR 0.48 [95% CI; 0.30, 0.77]; p = 0.002). The forest plot favours the control group due to higher incidence cases of wound infection compared to amnion group.

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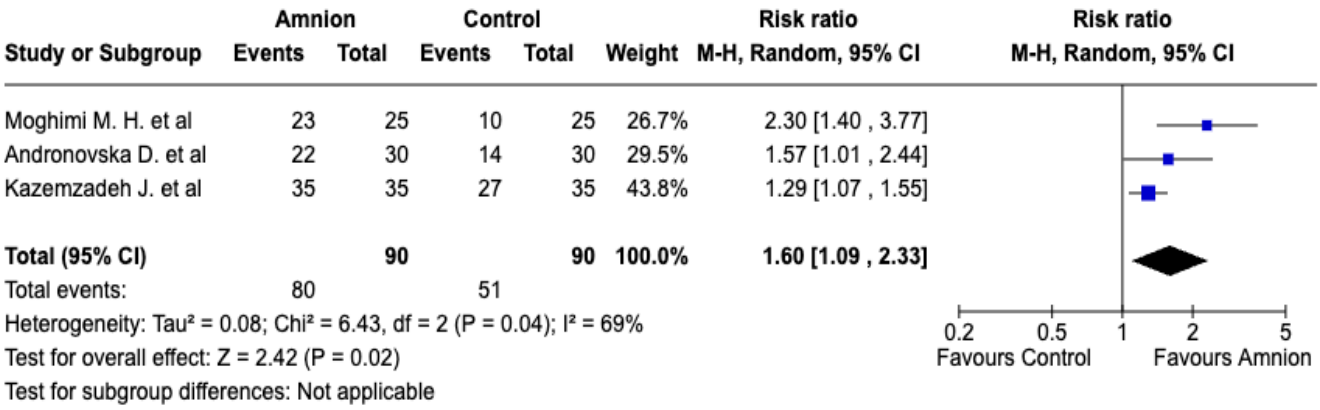

Figure 4. *Wound Healing Rate by Day 7 in Amniotic Membrane and Control Treatment in Burn Wound*

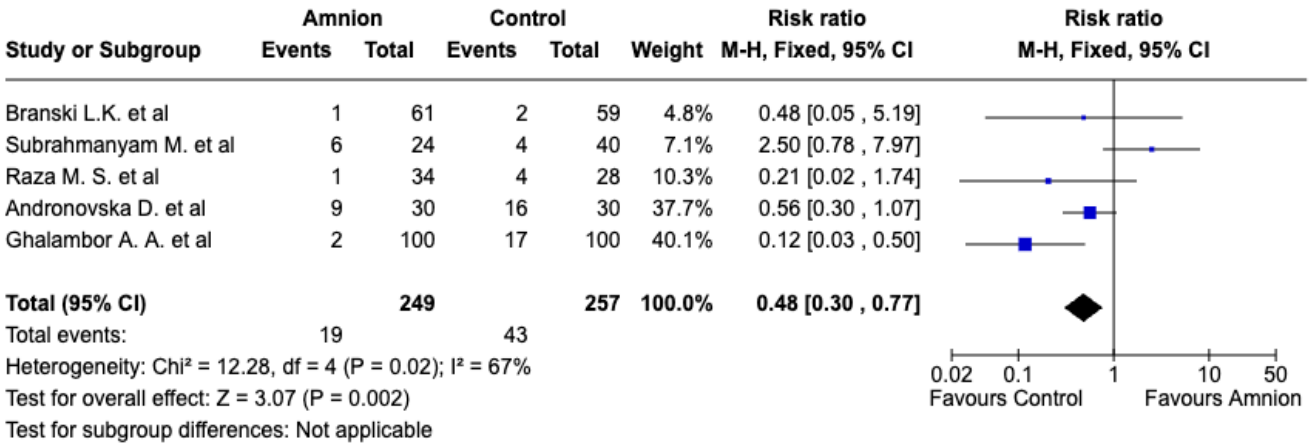

Figure 5. *Incidence of Wound Infection in Amniotic Membrane and Control Treatment in Burn Wound*

There was a significant Heterogeneity test (p = 0.02) even though the I^2 = 67% showing inconsistent results between studies. Higher mean %TBSA in Raza M. S. et al[7] and full thickness burn patients in Andonovska D. et al[9] may contribute to inconsistent results between studies.

Dressing Renewal Frequency

Dressing renewal frequency is assessed in this study as one of the secondary outcomes. Data for dressing renewal frequency were available in two RCTs shown in **Figure 6** Dressing renewal frequency was available in Kazemzadeh J. et al study[6] (1 vs 7 days) but we excluded it in this meta-analysis due to different unit measurements used.

The mean of dressing renewal frequency was found lower in the amnion group and the pooled RR showed statistically significant differences between groups (RR -1.64 [95% CI; -2.48, -0.79]; p = 0.0002). The forest plot favours the amnion group due to lower dressing renewal frequency. Low heterogeneity test was achieved in this meta-analysis (I^2 = 0%;p=0.44) showing consistent results between studies.

Pain Score

The other secondary outcome of this study was pain score. Data for mean pain score were available in four randomized controlled trials, but we excluded two studies from this meta-analysis due to different pain scales used.

There are several types of pain assessment tools in adults from Numerical Rating Scale (NRS), Visual Analog Scale (VAS), and any other pain assessment tools used to assess the degree of pain in patients.[16] Two studies used Visual Analog Scale (VAS) as their pain assessment tools, while the other two studies did not mention their pain assessment tools.

Subrahmanyam M. et al[11] used categorical pain assessment from none, mild, moderate, and severe pain, while Mohammadi A.A. et al[8]

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used numerical scale from 0 means no pain to 10 means severe pain. The assessment of pain itself is similar between studies, the pain score judged by the patients. We included studies with VAS pain scale to be assessed in this meta-analysis.

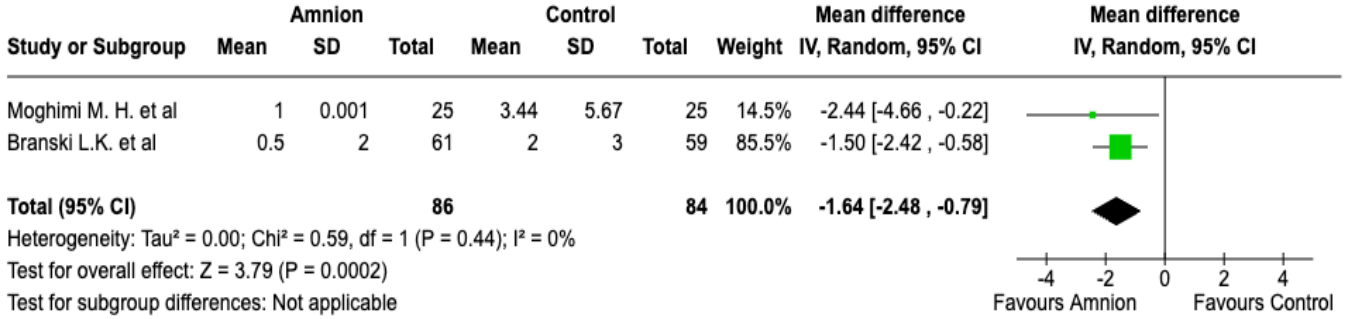

Figure 6. *Dressing Renewal Frequency in Amniotic Membrane and Control Treatment in Burn Wound*

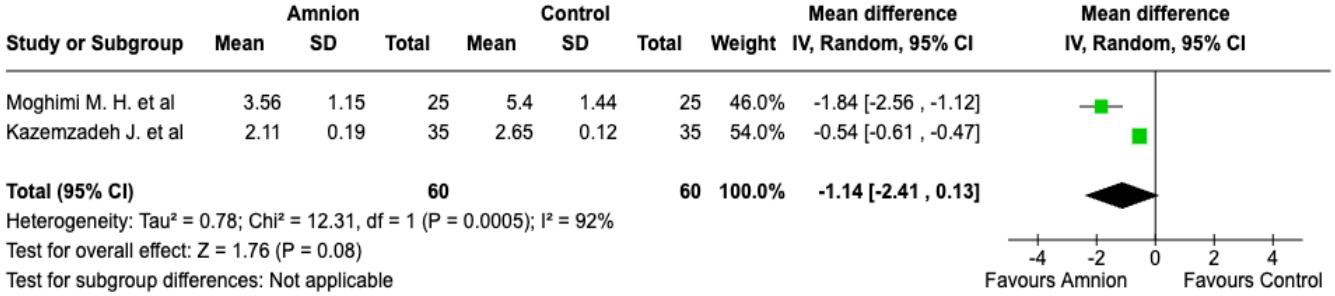

Figure 7. *Pain Score in Amniotic Membrane and Control Treatment in Burn Wound*

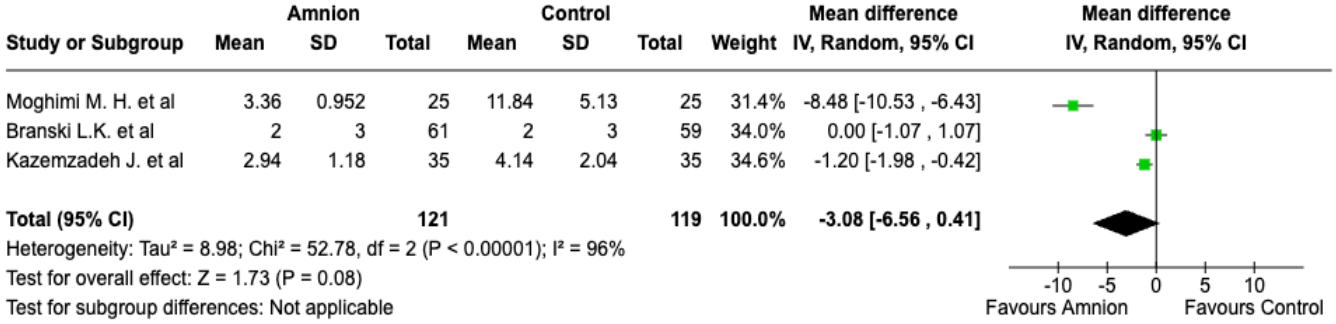

Figure 8. *Length of Hospital Stay in Amniotic Membrane and Control Treatment in Burn Wound*

The pain score assessed was found less in the amnion group compared to the control group, but the pooled RR did not have statistically significant differences between groups (RR -1.14 [95% CI; -2.41,0.13]; p = 0.08). The forest plot favours amnion treatment due to lower pain scale was reported in the amnion group. High heterogeneity test (I^2 = 92%; p = 0.0005) may not be attributed since the RR in pain score did not differ significantly between groups.

Length of Hospital Stay

Length of hospital stay is also assessed in this study as one of the secondary outcomes. Data for mean length of hospital stay were available in three RCTs shown in **Figure 7**.

The mean length of hospital stay was found lower in the amnion group, but the pooled RR did not have statistically significant differences between groups (RR -3.08 [95% CI; -6.56, 0.41]; p = 0.08). The forest plot favours the amnion group due to lower mean length of hospital stay. High

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heterogeneity test (I^2 = 96%;p<0.00001) may not be attributed since the RR in length of hospital stay did not differ significantly between groups.

Publication Bias

Funnel Plot was assessed to evaluate the distribution of studies in this meta-analysis. **Figure 9** shows the distribution of studies in mean healing time data, while **Figure 10** shows the distribution of studies in wound healing rate. It is evident that the studies are asymmetrically distributed, with a less balanced distribution to the left of the center line in **Figure 9** and to the right of the center line in **Figure 10**. This suggests a potential publication bias that could affect the accuracy of the results, as it may not fully represent the body of research conducted on the topic.

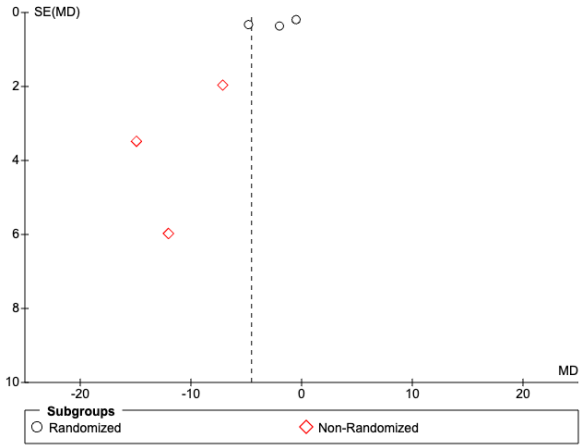

Figure 9. Funnel Plot in Mean Healing Time

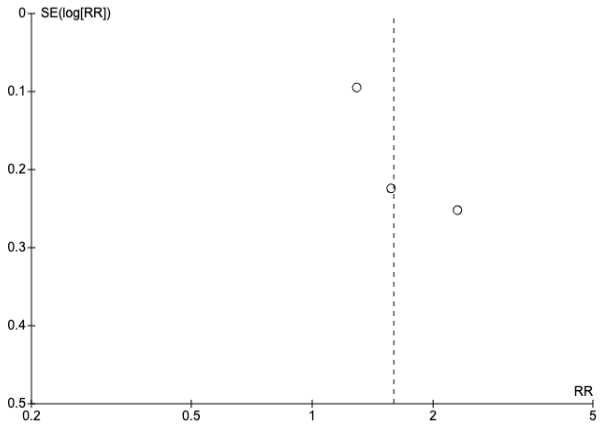

Figure 10. Funnel Plot in Wound Healing Rate

DISCUSSION

In this systematic review, we identified a total of eleven studies, with eight RCTs and three NRCTs. Evaluating the application of amniotic membrane on burn wounds. While the results varied across the studies, we were able to assess several outcomes, including mean healing time, wound healing rate, incidence of wound infection, pain score, length of hospital stay, and dressing renewal frequency

This meta-analysis suggests that, based on the totality of currently available evidence, amniotic membranes may reduce the mean healing time on burn patients, resulting in low wound infection incidence, reduce pain scores, shortened the hospital length of stay, and resulting in fewer dressing changes frequency. However, this meta-analysis indicates some weakness in the existing studies. The RCTs lack sufficient information regarding randomization, allocation concealment, and the blinding of participants, personnel, and outcome assessors. The RCTs have a lack of information given in terms of randomization, allocation concealment, and blinding of participant, personnel, and outcome assessor. Only one study provided complete information regarding this matter. Nevertheless, the quality of reported outcomes is generally good.

In patient's characteristics, baseline imbalances were observed in TBSA percentage in one study. Two studies included full-thickness burns in their randomized clinical trials, but only Andronovska et al.[9] utilized the gold standard treatment of skin excision and skin grafting for these burns in their control group. Despite these imbalances, no statistical adjustments were made.

As outlined in the results section, the preparation methods for amniotic membranes varied among the studies reviewed. Seven studies utilized non-preserved amniotic membranes, with two of these cultured with cells. Most non-preserved membranes underwent microbiological testing prior to application to ensure they were free from bacterial contamination. The review also included preserved amniotic membranes, which were preserved using various methods, such as glycerol, alcohol, and cryopreservation. One study employed amniotic membrane products in sterile packages. Regardless of the preservation methods used, all amniotic membranes were

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human amniotic membranes. No animal-derived amnion was included in this study.

The control groups in the reviewed studies varied widely. Three studies used silver sulfadiazine, while four studies applied topical antibiotics, including nitrofurazone ointment, polymyxin B, bacitracin, and nystatin. One study utilized honey as the control. Additionally, one study compared conventional burn treatments such as occlusive dressings, early excision, and skin grafting to amniotic membrane therapy. The two remaining studies by Liu et al.[12,13] did not specify the interventions used in the control group.

Most of the studies reported no adverse effect found in patients. Only Mohammad A.A. et a[8]l reported fever in some patients following amniotic membrane application, however it was relieved by antibiotics treatment with no evidence of local infection or sepsis. Immunological rejection and inflammatory reaction was not present in any of the studies. This is in accordance with low immunogenicity and anti-inflammatory properties in amniotic membranes. Human amniotic epithelial cells (hAECs) and human amniotic mesenchymal stromal cells (hAMSCs) found in the human amniotic membrane exhibit low to moderate levels of human leukocyte antigen (HLA) and costimulatory molecules (CD80, CD86) on their surfaces. These characteristics of the human amniotic membrane reduce the likelihood of transplant rejection.[17]

Our meta-analysis demonstrated a benefit of amniotic membrane on mean healing time (RR -4.52 [95% CI; -6.93, -2.11]), statistically significant (p=0.0002). This is consistent with a meta-analysis performed by Yang. et al in 2021[18], that concluded amniotic membrane results in overall shorter healing time compared to conventional methods. Another literature review conducted by Kesting. et al[7], found that amniotic membrane outperformed silver sulfadiazine cream, nitrofurazone-embedded gauze, and topical antibiotics in terms of wound healing time. Notably, in our meta-analysis on mean healing time, Mohammadi. et al[8] and Sawhney. et a[14]l, also used silver sulfadiazine as control group, while Raza M. S. et al[7], and Branski L. K. et al[15] used topical antibiotics. No information regarding the control group in both studies conducted by Liu. et al.[12,13] Heterogeneity in the mean healing time is most likely due to imbalance in baseline characteristics in studies including higher mean of TBSA in one study. and inclusion of full thickness burns in another study. Rittenhouse B.A. et al reported that the higher TBSA percentage and the deeper the burn wounds, the longer mean healing time was reported.[19]

The other primary outcome in this meta-analysis was the wound healing rate by day 7. The result from the pooled RR showed statistically significant differences (p=0.02) between amniotic membrane group and control group in wound healing rate (RR 1.60 [95% CI; 1.09, 2.33]) indicating that the amnion group has faster healing rate by day 7. Nevertheless, this data also shows high heterogeneity. Andronovska D. et al.[8] included full thickness burns in their study as likely a contributing factor. Shin Chen Pan et al reported that the deeper the burn wound, the longer the wound healing rate reported.[20]

We analyzed wound infection as a secondary outcome. The result was significantly different, superior for the amniotic membrane group, with pooled RR 0.48 [95% CI; 0.30, 0.77]; p=0.002. Amniotic membrane has been reported to have antimicrobial activity in vivo. King et al. and Buhimschi et al. reported in their study that hAECs in human amniotic membrane express natural antimicrobials, such as human β-defensins, elafin, and secretory leukocyte protease inhibitor in vivo.[21,22] In another study by Kim et al, it was found that histones H2A and H2B, which possess antimicrobial and endotoxin-neutralizing activity, were localized in the cytoplasm and extracellular surface of hAECs.[23] These microbial properties of amniotic membrane prevent wound infection. The meta-analysis for wound infection showed high heterogeneity with most likely contributing factors were higher mean TBSA percentage in the study conducted by Raza et al[7] than the other studies and Andronovska D. et al.[9] included full thickness burn in their study. Jeschke et al reported that the deeper the burn wound, the higher the risk of wound infection.[24]

In this parameter, Different amniotic membrane types and preparation methods used in the studies reviewed. Subrahmanyam. et al[11] and Moghimi. et al[5] used non-preserved amniotic membranes, Branski L. K. et al[15] used cryopreserved amniotic membrane, while Raza M. S. et al[7], and Ghalambor. et al[10], preserved the

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amniotic membrane in glycerol and alcohol, respectively. In one study, it is also reported that the amniotic membrane was stored up to three months prior to the application[9].

However, despite using non-preserved amniotic membrane, Subrahmanyam, et al.[11] presented different results than the other studies with infection higher in the amnion group. This incoherent result might be biased from the control group which were honey. In their other randomized clinical trial, Subrahmanyam et al compared the use of honey and silver sulfadiazine as burn treatment and found patients treated with honey had rendered infection more than the silver sulfadiazine group[25]. This shows that honey has higher antimicrobial activity than silver sulfadiazine. Due to this antimicrobial activity, the incidence of wound healing treated with honey is higher than the amniotic membrane.

The pain VAS score between groups was found less in the amnion group, but was not statistically significant (RR -1.14 [95% CI; -2.41,0.13]; p = 0.08). Two of the studies that were included in the forest plot showed the amniotic membrane is less painful compared to silver sulfadiazine and nitrofurazone ointment as their control group. In previous studies, amniotic membranes had been found to decrease pain and provide more comfort for patients. In their randomized clinical trial, Mohseni et al[26] proved amniotic membrane dressing can be effective in reducing pain after cesarean section and can eliminate the patients' need towards analgesics. In another study conducted by Adly et al, the patient's tolerance to pain during burn wound dressing change was significantly improved by the use of amniotic membrane.[27] All of these studies supported our recent finding regarding amniotic membrane in reducing pain.

Another secondary outcome discussed in this study is dressing renewal, described as the frequency of dressing changes. The result from two studies showed significant results (RR -1.64 [95% CI; -2.48, -0.79]; p = 0.0002). Amnion group required less dressing renewal frequency compared to the control group, with low heterogeneity. Less dressing renewal can enhance comfort for patients, reduce the risk of infection, and lower healthcare cost. Both of the studies assessing dressing renewal used topical treatment as their control treatment and dressing changes daily for control groups. On the other hand, Moghimi M. H.[5] et al used non-preserved amniotic membrane, which changed every 4-5 days, while Branski L.K. et al[15] used cryopreserved amniotic membrane, which is the dressing changes if needed. Despite these differences of dressing changes in the amnion group, both studies show lower frequency of dressing changes in the amnion group compared to control group.

The last secondary outcome was the patients' hospital length of stay. Although not statistically significant, the mean length of hospital stay was found lower in the amnion group, (RR -3.08 [95% CI; -6.56, 0.41]; p = 0.08) in three studies reviewed. In a case control study with 350 patients involved, Ramakrishnan K. et al, also discovered the length of stay in the amnion group was shorter compared to conventional dressings in superficial and deep partial thickness with hospital stay less than 3 weeks in the amnion group and 15 days to more than 4 weeks in the control group. They also stated that this reduction of length of stay is beneficial in reducing treatment cost.[28]

The limited reporting of outcomes, such as residual scarring and analgesic dosage prevented the pooling of data. Four studies assessed residual scarring between groups; however, they employed different scoring methods and observation times. Moghimi M. H. et al[5] and Kazemzadeh J. et al[6] both utilized the Vancouver Scar Scale (VSS) to evaluate wound scarring. Moghimi M. H. et al[5] assessed scars on day 30 post-treatment, reporting that none of the patients in the amnion group had severe scarring, and 38% were scar-free. In contrast, 20% of the control group had severe scars by day 30, a difference that was statistically significant ($p < 0.001$). Kazemzadeh J. et al[6] conducted scarring assessments on days 14 and 30, with results favoring the amnion group; on day 14, they found a statistically significant mean score of 2.02 ± 0.96 for the amnion group compared to 3.28 ± 0.85 for the control group ($p = 0.001$). Subrahmanyam M. et al[11] performed a three-month follow-up to evaluate residual scarring by testing the range of motion in the affected area, classifying scars as major or minor. They found that 16.6% of patients in the amnion group had residual scars, with one patient exhibiting a major contracture, though this result was not statistically significant. Lastly, Branski L. K. et al[15] used the Hamilton Scar Scale to compare residual scarring over a 12-month

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period, but found no statistically significant differences between the groups.

Analgesic dosage was examined in three studies. Moghimi M. H. et al[5] administered pethidine as a painkiller, leading to a significantly lower dosage for the amniotic group (72 ± 29.15 mg vs. 806 ± 49.3 mg, $p < 0.001$). Kazemzadeh et al[6] also found that morphine intake was significantly lower in the amnion group compared to the control group. Similarly, Mohammadi A.A. et al[8] reported results favoring the amnion group, with a statistically significant p-value ($p < 0.001$), although they did not specify the drug used in their study. Due to the variations in the drugs administered, we did not plot this parameter. Raza M.S. et al[7] compared the frequency of analgesic administration and found it to be less frequent in the amnion group than in the control group ($p = 0.002$).

Our review has several strengths, such as employing a comprehensive search strategy and performing duplicate screening, eligibility assessments, and data extraction. Additionally, we evaluated the methodological quality of the included trials using established criteria, highlighting the aspects that we considered methodologically important. We acknowledge that quality assessment has its challenges.

Our meta-analysis has some limitations which must be taken into account. Firstly, this review encompassed studies with different control interventions. Instead of comparing the effect of amniotic membrane to specific outcomes, we focused on providing a general description of its effects. Differences in control interventions could introduce bias. Furthermore, two of the NRCT did not mention control group definition. Secondly, as previously explained, even though all of the studies use human amniotic membranes, the preparation method differs. The third limitation is most of the RCTs included did not mention the method of randomization, allocation concealment, blinding of patients, personnel, and outcome assessor leaving the risk of bias unclear.

CONCLUSION

This meta-analysis indicates that amniotic membrane is advantageous as a biological dressing for burn patients. However, further high-quality trials are necessary because the existing evidence is limited in scope and quality.

Correspondence regarding this article should be addressed to:
Name : Nandita Melati Putri. Plastic Reconstructive and Aesthetic Division, Department of Surgery, Cipto Mangunkusumo Hospital, Universitas Indonesia, Jakarta, Indonesia. E-mail: nandita.putri@ui.ac.id

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