RESEARCH PAPER

Comparative Study of Collagen Density in Reconstruction of Trachea Defects with Aponeurosis Patch of External Oblicus Muscle and Dry Amnion Membrane (A Study with New Zealand Rabbits)

Antonio Akbar Santoso[1]*, Marjono Dwi Wibowo[2], Dwi Hari Susilo[2]

[1]*Departement of surgery, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia*
[2]*Division of Head and Neck Surgery, Departement of Surgery, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia*

ABSTRACT
Background The Reconstruction Of Tracheal Abnormalities Due To Various Clinical Conditions, Such As Trauma, Cancer, And Other Illnesses, Remains A Significant Problem For Surgeons.Dry Amniotic Membrane (Dam) Has Long Been Regarded As A Promising Source Of Scaffold Material, An Essential Element In Tissue Engineering That Serves As A Supportive Matrix For Cellular And Tissue Viability.The Aponeurosis Fascia Is Frequently Employed By Surgeons As A Surgical Patch To Enhance The Efficacy Of Wound Healing In Both Acute And Chronic Wounds.

Objectives To Analize Collagen Density In Rabbits Undergoing Tracheal Reconstruction With The External Oblique Muscle Aponeurosis Patch Alone And Those With Dam Added.

Methods This Study Is An Experimental Randomized Controlled Trial Conducted On Male New Zealand White Rabbits.The Rabbits Underwent Acclimatization For Seven Days, After Which Randomization Was Executed Via Permuted Block Randomization To Partition The Samples Into Two Groups.Following The Conclusion Of The Proliferation Period (14 Days), Collagen Density Was Assessed In The Surgical Wound Region Subsequent To Tracheal Restoration.The Data Analysis Was Conducted With Spss Software.

Results There Were 16 Samples In Each Group, Group With Dam Procedure And Control Without Mak. Collagen Density Was Obtained In Group With Dam Procedure (1.00 (±0.73) Cm) Compared To The Control Group Without Dam (1.85 (±1.2) Cm). There Were Differences In Collagen Density In The Control And Treatment Groups (P = 0.05) Which Showed A Significant Relationship.

Conclusion There Was An Increase In Collagen Density In Tracheal Defects Reconstructed With An External Oblique Muscle Aponeurosis Patch Combined With Dry Amniotic Membrane (Dam) Compared To Tracheal Defects Reconstructed With An External Oblique Muscle Aponeurosis Patch Alone..

Keywords: primary tracheal repair, Dry Amniotic Membrane (DAM), collagen density.
How to cite this article: Santoso AA, Wibowo MD, Susilo DH, Comparative Study of Collagen Density in Reconstruction of Trachea Defects with Aponeurosis Patch of External Oblicus Muscle and Dry Amnion Membrane (A Study with New Zealand Rabbits)..Int J Drug Deliv Technol. 2026;16(2s): 279-286; DOI: 10.25258/ijddt.16. 279-286

Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Tracheal injury is a clinically significant disorder that necessitates particular care because to its potential origins in trauma, pathological processes, or iatrogenic factors.In patients with blunt chest and neck trauma, tracheobronchial damage occurs in around 0.5–2% of cases, but penetrating chest trauma has an incidence of less than 2%.Traumatic tracheal injury is more commonly reported in men, with an average age of around 20 years.Iatrogenic tracheal damage associated with endotracheal intubation may occur in around 1 in 20,000 to 75,000 elective intubation operations..[1]

The trachea has a complicated structure made of fibrocartilage that keeps the airway open while breathing, helps the neck move, and allows speech. Small tracheal defects can be closed fairly easily, but larger ones, especially those that are more than 50% of the tracheal circumference, usually need reconstructive surgery.[2] Despite advances in airway surgery, reconstruction of large tracheal defects remains a major challenge, and an ideal replacement material has not yet been established. Multiple reconstructive strategies have been explored, including stents, prostheses, implants, autografts, allografts, and tissue engineering techniques.[3], [4]. Tracheal substitution

**Author for Correspondence: antoniosantoso@ymail.com*

options have also been described to include allotransplantation, autologous tissue reconstruction, tissue engineering, and aortic homograft reconstruction,.[5]

Clinical and experimental experiences indicate that grafts and prosthetic materials used for tracheal reconstruction frequently fail, with ischemia, tissue rejection, and stenosis representing key mechanisms of failure. These limitations are particularly relevant to synthetic prostheses, which may behave as foreign bodies, become chronically infected, induce granulation tissue formation, and lead to anastomotic complications or even erosion into major vessels. Therefore, a reconstructive material should be well tolerated by the host, cause minimal inflammatory response, and be adaptable in shape and size, while preserving essential functional goals of tracheal repair.[6], [7] For optimal functional outcomes, three core components should be evaluated after graft implantation: sustained airway patency, epithelial coverage of the luminal surface, and adequate vascularization.[8] Collectively, these requirements highlight why durable tracheal reconstruction remains difficult in cases of large-segment or circumferential defects.[3]

A central biological determinant of post-reconstruction outcomes is wound healing and its regulation of extracellular matrix deposition, particularly collagen. Wound healing proceeds through overlapping phases including hemostasis, inflammation, granulation or proliferation, and also remodeling. Hemostasis occurs immediately after injury happened, followed by inflammation typically within days, proliferation over subsequent weeks, and remodeling that may continue for months to years.[9] In the early period after tissue injury, platelets and inflammatory cells migrate rapidly and provide signaling cues that orchestrate cell influx, stromal activation, and neovascularization via cytokines and growth factors.[10], [11] During healing, tissue architecture changes to form scar tissue with distinct mechanical properties; tensile strength is low in the first 2–3 weeks, increases from around the third week as remodeling progresses, reaches approximately 50% by 6 weeks, and continues to rise gradually over 6–12 months, with maximal tensile strength typically not exceeding about 75% of uninjured tissue.[10]

Collagen synthesis and remodeling are pivotal to both effective repair and the risk of pathological scarring. Collagen is produced primarily by fibroblasts and constitutes roughly 30% of total protein in the human body, providing strength and structural integrity in normal tissues. After injury, collagen is required to restore tissue continuity and function; however, excessive collagen deposition can lead to fibrosis, altered mechanics, and luminal narrowing, while insufficient collagen may render the wound weak and prone to breakdown.[10], [11] In tracheal wound healing, collagen synthesis begins around day 3, accelerates markedly around weeks 2–4, and is regulated by collagenase and other factors that degrade collagen. In the maturation phase, remodeling depends on balance between ongoing synthesis and degradation; matrix metalloproteinases (MMPs) and collagenases remove excess collagen while newly formed collagen becomes more organized.[12] Importantly, prosthetic tracheal graft materials such as polypropylene mesh may stimulate granulation and fibrosis, increasing the risk of post-reconstruction tracheal stenosis.[13]

In this context, dried amniotic membrane (DAM), has been considered as a valuable scaffold material in tissue engineering because it provides a supportive matrix for tissue and cell survival. DAM has been used across diverse reconstructive and reparative applications, including burns, arthroplasty, tympanoplasty, bladder procedures, oral cavity reconstruction, vaginal reconstruction, and other interventions.[14] DAM has been reported to minimalize scar formation and inflammation, accelerate wound healing, and it has role as a scaffold for cellular growth and differentiation.[15] Mechanistically, endogenous MMP genes expression by human amniotic membrane participate in extracellular matrix degradation in fetal membranes and have been associated with membrane rupture in pregnancy.[16] This biological profile supports the concept that DAM may modulate extracellular matrix remodeling when applied to reconstructive patches, potentially limiting excessive collagen accumulation during foreign-body-associated healing responses.[13], [16]

One proposed approach is to coat an aponeurotic patch with DAM to influence inflammation and collagen dynamics in tracheal defect repair. DAM may reduce excessive collagen formation through several mechanisms, including decreasing fibroblast activity, inhibiting the transforming growth factor-β (TGF-β) pathway, and suppressing collagen mRNA synthesis, thereby potentially lowering the risk of post-reconstruction tracheal stenosis.[13] Because luminal stenosis is strongly linked to excessive fibrosis and dense scar formation, an intervention that modulates collagen deposition and remodeling may be clinically meaningful in tracheal reconstruction.[12], [13] Therefore, evaluating collagen density after reconstructive interventions provides a relevant histologic endpoint aligned with mechanisms of stenosis risk.[10]

Experimental models are essential to test reconstructive strategies and healing modulation under controlled conditions. The rabbit trachea is a suitable experimental airway model because it shares key anatomical characteristics with the human trachea and provides practical advantages including accessibility and cost-effectiveness. In rabbits, the trachea connects the larynx to the bronchi and is composed of mucosa, submucosa, cartilage, and adventitia layers that support airway stability and flexibility. Reported rabbit tracheal length is approximately 6–7 cm with a diameter of 4–6 mm, and it consists of C-shaped cartilaginous rings with a posterior trachealis muscle that contributes to airway caliber changes. Additional advantages include the relatively long cervical trachea permitting surgical access and transplantation procedures, and a mapped immunologic background relevant to graft tolerance studies. Notably, tracheal size does not differ substantially between male and female rabbits, supporting consistent model selection in experimental design.[4] However, evidence specifically addressing collagen modulation in tracheal reconstruction remains limited

Based on these considerations, the current study aims to evaluate collagen density after reconstruction of a tracheal defect in a rabbit model using an external oblique muscle aponeurosis patch coated with dried amniotic membrane (DAM). The study compares collagen density following tracheal defect reconstruction by an external oblique muscle aponeurosis patch coated with DAM versus reconstruction using an external oblique muscle aponeurosis patch alone. The underlying hypothesis is that coating the aponeurotic patch with DAM can reduce collagen density during healing, thereby potentially lowering the risk of post-reconstruction stenosis associated with excessive fibrotic response.[13]

METHODS

This study was executed as a laboratory-based experimental investigation employing a randomized controlled trial design including male New Zealand White rabbits.The animals were acclimatized for seven days prior to any intervention. Eligible rabbits were selected based on inclusion criteria of age (6–9 weeks), body weight (1900–2500 g), and absence of visible anatomical abnormalities. Rabbits were excluded if they exhibited aggressive behavior during adaptation, died during the adaptation period or before sampling, and were classified as drop-outs if death occurred between defect creation and biopsy, or if they developed illnesses such as poor general condition, abscess, infection, or sepsis.The sample size was determined using the Federer formula with two treatment groups ($T = 2$), applying the criterion $(T-1)(n-1) \geq 15$, resulting in a minimum of 16 animals. To account for potential attrition, an additional 10% per group (2 rabbits) was included, leading to 18 rabbits assigned to the treatment group and 18 rabbits to the control group, totaling 36 animals.The study was conducted at the Experimental Animal Laboratory, Faculty of Veterinary Medicine, Airlangga University, with histopathological examinations performed at the Department of Anatomy–Histology, Faculty of Medicine, Airlangga University, from March to May 2024.

Rabbits were acquired from Pusat Veterinerina Farma (PUSVETMA) and subjected to clinical assessment prior to inclusion to confirm the absence of disease and any transmissible diseases.Following screening, animals were randomly allocated to control or treatment groups by permuted block randomization.Eight blocks were constructed with coded allocations A (control) and B (treatment) (e.g., AABB, ABAB, BAAB), and sequential rabbit identification numbers were aligned with the pre-generated block sequences to guarantee equal probability of assignment.The histology examiner was blinded to group allocation to enhance the impartiality of outcome assessment.All surgeries were conducted under general anesthesia utilizing injectable ketamine at a dosage of 20–40 mg/kg.The anterior neck region was shaved and disinfected with 10% povidone-iodine.A standardized tracheal defect was established at the 8th tracheal ring, consisting of a square full-thickness defect of 0.6×0.6 cm², utilizing a No. 15 scalpel blade.In the control group, the lesion was rebuilt utilizing a 0.6×0.6 cm² external oblique muscular aponeurosis patch, affixed with simple interrupted nylon 6-0 sutures.In the treatment group, the defect was reconstructed using a patch of external oblique aponeurosis of the same size, coated with dried amniotic membrane (DAM) of identical dimensions, arranged in a total of six layers (three layers above and three layers below the aponeurosis patch), and secured with simple interrupted nylon 6-0 sutures.

Amniotic membrane was aseptically harvested from the placenta after an elective cesarean section utilizing sterile devices and techniques.The membrane was rinsed with saline containing 50 μg/mL of streptomycin and 50 μg/mL of penicillin, after which the inner layer was detached from the chorion.The amnion was subsequently immersed in transport media and preserved at 4°C, with extraction conducted within 24 hours; specimens were transferred to the laboratory in sterile tubes with dry ice.The membrane was dried on absorbent paper, weighed to determine its wet weight, and its volume was determined prior to being stored in a deep freezer for approximately 24 to 48 hours.Freeze-drying was subsequently conducted until adequate desiccation was attained (about 7–8 hours), followed by packaging under laminar airflow conditions and chemical sterilization using gamma irradiation (25 kGy).For the fabrication of the aponeurosis patch, fresh aponeurosis tissue was procured and customized to about 1.5 times the anticipated luminal diameter, thereafter sutured to the tracheal edge as previously delineated.Postoperatively, rabbits received intramuscular penicillin at a dosage of 50,000 units/kg, commencing at the time of surgery and continuing daily for five days. Analgesia was administered using ketorolac, and the animals were provided with routine feeding and hydration, with the placement of food and water containers adjusted lower to minimize movement of the muscles surrounding the trachea.On postoperative day 14, coinciding to the proliferative phase, the animals were euthanized via decapitation, their corpses burned, and tracheal specimens were obtained from the surgical site, including neighboring tissue.

For histopathological assessment of collagen density, tracheal tissue specimens were obtained from a cranio-caudal segment that included both macroscopically healthy and affected tissue, encompassing the full thickness of the tracheal wall, and were subsequently fixed in 10% neutral buffered formalin.The specimens were processed into paraffin blocks and underwent histological staining assessment utilizing hematoxylin and eosin; supplementary reagents for Masson's trichrome staining were generated for collagen evaluation.Collagen density was evaluated by quantifying collagen cells in three high-power fields with a light microscope at 400× magnification.Data were modified, coded, and input into SPSS version 26.0 for Windows, followed by cleaning before analysis.Descriptive statistics were computed as mean and standard deviation, and group distributions were illustrated using box plots.Between-group comparisons for numerical outcomes were conducted using an independent two-sample t-test for normally distributed data, or the Mann–Whitney test where distributional assumptions were not satisfied.

Ethical approval was required prior to the commencement of the study from the Animal Care and Use Committee, Faculty of Veterinary Medicine, Airlangga University.Animal welfare monitoring encompassed the observation of feeding and drinking behaviors, mental status and alertness, as well as clinically significant indicators such as body weight, body temperature, respiratory patterns, shock, anaphylaxis, hemorrhaging, diarrhea, vomiting, neurological disturbances, blindness, and seizures, in addition to environmental monitoring of temperature, humidity, ventilation, noise, pollution, and other housing conditions.Nutritional sufficiency was achieved in accordance with rabbit norms by a diet including 20–25% protein, 5–12% fat, 2.5% crude fiber, and 45–60% carbs.Potential adverse effects and complications following tracheal defect creation were monitored throughout the study period. Waste management, including food remnants, feces, carcasses post-euthanasia, and tissue remnants, was executed in accordance with applicable standards through decontamination and incineration to avert environmental contamination.

RESULTS

Characteristics of the Study Samples

This study employed an experimental randomized controlled trial design utilizing male New Zealand white rabbits as animal models.A total of 32 rabbits that satisfied the inclusion criteria were enrolled in this investigation.All animals experienced a 7-day acclimation period before the intervention.Subsequent to acclimatization, the rabbits were randomly allocated by permuted block randomization into two groups: a control group and a treatment group.The treatment group underwent tracheal defect reconstruction utilizing an external oblique muscle aponeurosis patch covered with dried amniotic membrane (DAM), whereas the control group underwent reconstruction with an external oblique muscle aponeurosis patch devoid of DAM.The dried amniotic membrane utilized in this investigation was a freeze-dried specimen procured from the tissue bank of Dr. Soetomo General Hospital, Surabaya.

Collagen Density Measurement Results

Samples of tissue were taken on the fourteenth day following surgery, following the proliferative phase. Specimens were obtained from the tracheal surgical wound area measuring 1.2 cm in the craniocaudal direction, including both healthy tissue (0.6 cm) and pathological tissue (0.6 cm).The specimens had a mediolateral width of 0.6 cm and encompassed the entire thickness of the tracheal wall.After the specimens were fixed in 10% buffered formalin, they were shaped into paraffin blocks and histopathologically evaluated using Masson's Trichrome staining procedure. To determine the density of collagen, the deposition of collagen was examined using a light microscope at a magnification of 400× in three high-power fields.

[Table 1]

Table 1 shows the individual collagen density measurements.The mean collagen density in the treatment group using DAM was 1.00 (±0.73) cm, whereas the control group without DAM demonstrated a higher mean collagen density of 1.85 (±1.2) cm. As summarized in Table 1, collagen density was consistently lower in the DAM-treated group. These findings indicate that tracheal defects reconstructed with an aponeurosis patch covered by DAM exhibited lower collagen density compared to reconstruction without DAM.

[Figure 1]

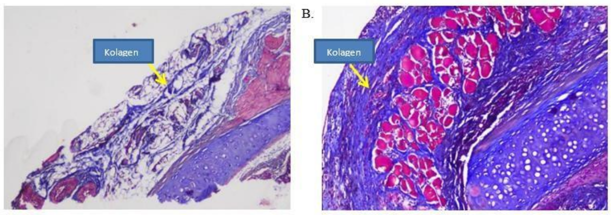
Histopathological findings are illustrated in Figure 1. The DAM-treated group (Figure 1A) demonstrated a less dense and more loosely distributed collagen arrangement compared to the control group without DAM (Figure 1B), which showed thicker and denser collagen deposition.

Figure 1. Microscopic appearance of collagen density using Masson’s Trichrome staining at 400× magnification

A) External oblique muscle aponeurosis patch with DAM

External oblique muscle aponeurosis patch without DAM

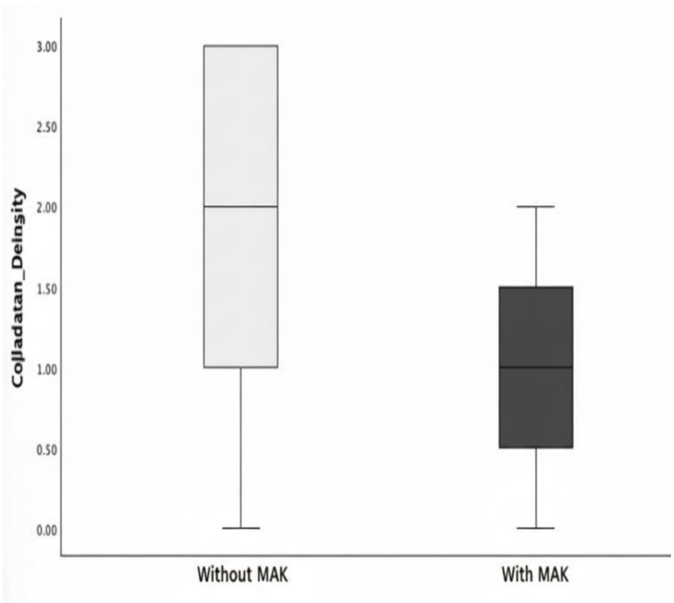
A. B.



[Figure 2]

The comparison of collagen density distribution between groups is further illustrated using a boxplot diagram in Figure 2. The graph shows a higher median density of collagen in the control group without DAM compared to the DAM-treated group.

Figure 2. Graph comparing the mean collagen density between the treatment and control groups



Association Between DAM Use and Collagen Density

The Shapiro Wilk test was used to check for normality in the data before doing a comparative statistical analysis because the sample size was less than 50.The results indicated that the collagen density values were not regularly distributed ($p = 0.001$; $p < 0.05$).

Consequently, data were presented as median (minimum–maximum), and intergroup comparisons were conducted using the Mann Whitney U test.Table 2 shows the findings of the statistical study

[Table 2]

Table 1. Comparison of Collagen Density Between Treatment and Control Groups

Treatment group with DAM		**Control Group without DAM**	
Sample No.	**Collagen Density (cm)**	**Sample No.**	**Collagen Density (cm)**
1	2	17	3
2	2	18	2
3	1	19	3
4	0	20	3
5	1	21	3
6	1	22	2
7	1	23	0
8	1	24	3
9	2	25	3
10	1	26	2
11	0	27	0
12	0	28	2
13	2	29	0
14	0	30	2
15	1	31	2
16	1	32	0
Mean Collagen Density	**1.00 (±0.73) cm**	**Mean Collagen Density**	**1.85 (±1.2) cm**

Table 2. Association Between the Use of Dried Amniotic Membrane (DAM) and Collagen Density Using Mann-Whitney U Test

Group	**Mean Collagen Density (cm)**	**Collagen Density (Median [Min–Max]) (cm)**	**p-value**
Treatment group with DAM	1.00 (±0.73)	1.00 (0.00–2.00)	
Control group without DAM	1.85 (±1.2)	2.00 (0.00–3.00)	0.023*

The median collagen density in the DAM-treated group was 1.00 (0.00–2.00) cm, while the control group without DAM demonstrated a median collagen density of 2.00 (0.00–3.00) cm. The Mann–Whitney U test revealed a statistically significant difference between the two groups ($p = 0.023$; $p < 0.05$).

These results show that the use of dried amniotic membrane in tracheal defect reconstruction is significantly associated with reduced collagen density compared to reconstruction without DAM.

DISCUSSION

Male New Zealand white rabbits that satisfied the inclusion criteria were utilized in this study.The inclusion criteria were rabbits aged 6–9 weeks with body weights between 1,900 and 2,500 grams.Before inclusion, all animals had clinical evaluation and were acclimatized for seven days to guarantee they were free from disease and not at danger of spreading infection.

Rabbits were chosen as the experimental model due to their comparatively lengthy cervical trachea, availability, ease of breeding, and closer evolutionary relationship to primates than smaller laboratory animals.Significantly, rabbit tracheal architecture closely parallels that of humans, rendering it an appropriate model for tracheal reconstruction studies.Furthermore, the expense associated with rabbit maintenance is comparatively lower than that of bigger animal models.[4]

The rabbit's trachea measures around 6.5 cm in length with a diameter of 0.5 cm and consists of cartilaginous rings which are connected by anterior intercartilaginous ligaments and a posterior trachealis muscle.[4] In the control group, the defect was repaired solely with an external oblique muscle aponeurosis patch, whereas in the treatment group, the aponeurosis patch was augmented with a dried amniotic membrane (DAM) measuring 0.6 × 0.6 cm^2 and comprising six layers, with three layers positioned above and three layers below the aponeurosis patch.

Prior studies assessing the viability of heterologous aponeurosis grafts for abdominal wall closure in rabbits revealed positive results, including the absence of postoperative complications such as cellulitis, abscess formation, dehiscence, hematoma, or herniation.Histological investigation in these trials demonstrated inflammatory processes marked by myofibroblasts and mature collagen deposition, devoid of acute graft rejection or severe endothelial damage.These findings substantiate the biocompatibility and structural appropriateness of aponeurosis grafts for tissue restoration.[17]

Collagen density served as a metric for assessing the wound healing response and the impact of DAM on tissue repair.Tracheal wound healing adheres to the standard phases of wound healing, commencing with hemostasis, succeeded by inflammation, proliferation, and maturation (remodeling).Collagen synthesis generally commences approximately three days post-injury and significantly escalates during the second to fourth weeks.The maturity phase typically commences around the third week and may endure for months or even years, contingent upon the type of wound, its location, and the biological circumstances present.

In the maturation phase, collagen remodeling relies on the equilibrium between collagen production and breakdown, regulated by collagenases and matrix metalloproteinases (MMPs).While the overall collagen content rises during this period, the arrangement and quality of collagen fibers are more significant than mere number.Excess collagen is eliminated as freshly generated collagen becomes increasingly ordered and aligned, resulting in tissue that is both stronger and more flexible.[12]

In this study, the mean collagen density was lower in tracheal defects reconstructed with an external oblique muscle aponeurosis patch covered with DAM compared to reconstruction using an aponeurosis patch alone. These findings are consistent with existing literature indicating that DAM reduces excessive scar formation and inflammation, accelerates wound healing, and serves as a natural scaffold for proliferation and differentiation of the cells.[15] The extracellular matrix components of DAM contain various growth factors, making it a promising biomaterial for tissue engineering.[14] Histopathological evaluation further supported these findings, showing less dense collagen deposition in the DAM-treated group. This suggests that DAM modulates fibroblast activity and collagen synthesis, leading to a more regulated healing process rather than excessive fibrotic response.

Statistical analysis demonstrated a significant association between the use of DAM and reduced collagen density in reconstructed tracheal defects ($p < 0.05$). The DAM-treated group exhibited significantly lower collagen density compared to control group without DAM.

The amniotic membrane is recognized for its abundant composition of cytokines, growth factors, and extracellular matrix components that facilitate wound healing and tissue regeneration.It functions as a biological scaffold that facilitates cell migration and proliferation while concurrently inhibiting excessive inflammatory reactions.Additional benefits of amniotic membrane encompass anti-inflammatory, antibacterial, anti-fibrotic, and low immunogenic qualities, along with its capacity to alleviate pain and inhibit excessive scar formation.[14]

The anti-inflammatory properties of the amniotic membrane are facilitated by the inhibition of pro-inflammatory cytokines, including IL-1α and IL-1β, as well as the synthesis of natural metalloproteinase inhibitors.In vitro investigations have shown that amniotic membrane-derived cells do not elicit xenogeneic or allogeneic immunological responses, actively decrease T-lymphocyte proliferation, and block monocyte development.[14]

Dried amniotic membrane was first utilized as a skin graft in 1910 and has since been employed in various reconstructive procedures, including burn treatment, oral cavity reconstruction, bladder and vaginal repair, tympanoplasty, arthroplasty, and conjunctival defect repair (Fernandes et al., 2005).Its capacity to diminish fibrosis and inflammation while facilitating epithelialization has been extensively documented..[15]

In the context of tracheal restoration, the incorporation of DAM into an aponeurosis patch seems to diminish collagen density relative to aponeurosis alone.This effect may be attributed to a mechanical mismatch between the amniotic membrane and tracheal tissue, as well as the influence of DAM in postponing the synthesis of robust type I collagen, which is crucial for tissues necessitating significant structural integrity, such as the trachea.The tracheal wound healing process resembles tunnel remodeling, with collagen deposition serving as structural reinforcement.Collagen is essential for preserving tissue integrity; yet, excessive accumulation may constrict the tracheal lumen, akin to too thick tunnel walls.In this setting, the application of desiccated amniotic membrane serves as a modulator of wound healing by regulating fibroblast activity and collagen deposition, thus preserving a balance between tissue integrity and luminal patency.

CONCLUSIONs

According on the results of this comparative study evaluating collagen density in reconstruction of tracheal defect using an external oblique muscle aponeurosis patch with and without dried amniotic membrane (DAM), it can be concluded that the use of DAM is associated with a statistically significant difference in collagen density. Tracheal defects reconstructed with an aponeurosis patch covered by dried amniotic membrane appeared to demonstrated significantly lower collagen density compared to those reconstructed using an aponeurosis patch alone or without DAM. These findings suggest that dried amniotic membrane plays a beneficial role in modulating collagen deposition during tracheal wound healing, potentially reducing fibrosis and contributing to improved structural and functional outcomes following tracheal reconstruction.

Although this study provides evidence supporting the effectiveness of dried amniotic membrane in reducing collagen density in tracheal reconstruction, several limitations should be considered. The relatively small sample size may alter the generalizability of the findings; therefore, future studies with larger numbers of experimental animals are recommended. Additionally, this study only compared dried amniotic membrane with an external oblique muscle aponeurosis patch. Further research is needed to evaluate and compare other reconstructive materials, including fascia lata, perichondrium or autologous cartilage, biological pericardium, and biodegradable synthetic biomaterials, to determine their relative effectiveness in controlling collagen deposition and preventing fibrosis. Furthermore, collagen assessment in this study was limited to the second postoperative week; thus, future investigations should include serial and longer-term evaluations of collagen remodeling to better understand the temporal dynamics of tracheal wound healing.

Acknowledgements

General: The authors wish to acknowledge the support of the Faculty of Medicine, Airlangga University and the Surgery Department of Dr. Soetomo General Hospital for their assistance in carrying out this study.

Funding Statement: The research did not receive any specific funding.

Approval: Ethical approval was granted by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Airlangga University. Ethical Number: 2.KEH.65.05.2025

Financial support and sponsorship

Not applicable.

Conflict of Interest

The authors declare that there are no conflicts of interest to report.

Ethical Declaration

The authors confirm that the study adhered to the principles outlined in the Declaration of Helsinki and appropriate institutional review board approval was obtained prior to the commencement of the study.

REFERENCE

[1] Kolimi P, Narala S, Nyavanandi D, Youssef AAA, Dudhipala N. Innovative treatment strategies to accelerate wound healing: trajectory and recent advancements. Cells. 2022;11(15):2439. doi:10.3390/cells11152439.

[2] Sindhutomo A, Samsarga GW, Suyata AL, Sudarsa SD. Polyphenol in olive oil: a promising adjuvant therapeutic agent in wound healing management and wound complication prevention. Intisari Sains Medis. 2023;14(1):553–9. doi:10.15562/ism.v14i1.1614.

[3] Krupová L, Pokorná A. Quality of life in patients with non-healing wounds, with particular focus on assessment tools: a literature review. Cent Eur J Nurs Midw. 2020;11(2):94–103. doi:10.15452/CEJNM.2020.11.0010.

[4] Amtha R, Marcia M, Aninda AI. Plester sariawan efektif dalam mempercepat penyembuhan stomatitis aftosa rekuren dan ulkus traumatikus. Maj Kedokt Gigi Indones. 2017;3(2):69. doi:10.22146/majkedgiind.22097.

[5] Chew WZ, Tan ELY, Teo BSX. In vivo evaluation of topical marine gel from ethanolic extract of Eucheuma cottonii for burn wound healing activity on healthy mice. Int J Med Toxicol Legal Med. 2018;21(3–4):23–6. doi:10.5958/0974-4614.2018.00020.7.

[6] Yulianti E, Sunarti, Wahyuningsih MSH. The effect of Kappaphycus alvarezii fraction on plasma glucose, advanced glycation end-products formation, and renal RAGE gene expression. Heliyon. 2021;7(1):e05978. doi:10.1016/j.heliyon.2021.e05978.

[7] Teo BSX, Gan RY, Abdul Aziz S, Sirirak T, Mohd Asmani MF, Yusuf E. In vitro evaluation of antioxidant and antibacterial activities of Eucheuma cottonii extract and its in vivo evaluation of the wound-healing activity in mice. J Cosmet Dermatol. 2021;20(3):993–1001. doi:10.1111/jocd.13624.

[8] Hidayah MN, Feranisa A, Septiana L. Efektivitas nanoemulgel ekstrak alga merah (Eucheuma cottonii) 5% terhadap jumlah fibroblas pada proses penyembuhan ulkus traumatikus (kajian pada tikus Wistar). Prosiding KIMU 7. 2022;102–10.

[9] Sohn JT. Resource equation method for sample size calculation in animal studies. Am J Emerg Med. 2023;63:175–6. doi:10.1016/j.ajem.2022.10.041.

[10] Sharun K, Banu SA, Mamachan M, Subash A, Mathesh K, Kumar R, et al. Comparative evaluation of Masson's trichrome and Picrosirius red staining for digital collagen quantification using ImageJ in rabbit wound healing research. J Exp Biol Agric Sci. 2023;11(5):822–33. doi:10.18006/2023.11(5).822.833.

[11] Hong JH, Kim DH, Rhyu IJ, Kye YC, Ahn HH. A simple morphometric analysis method for dermal microstructure using color thresholding and moments. Skin Res Technol. 2020;26(1):132–6. doi:10.1111/srt.12776.

[12] Meyerholz DK, Beck AP. Fundamental concepts for semiquantitative tissue scoring in translational research. ILAR J. 2018;59(1):13–17. doi:10.1093/ilar/ily025.

[13] Muñoz-Torres JR, Garza-Veloz I, Velasco-Elizondo P, Martinez-Fierro ML. HEALS-A and GRADES: novel histological and clinical scales for assessing skin regeneration in murine wound healing models. Diagnostics. 2025;15(3):387. doi:10.3390/diagnostics15030387.

[14] Chang S, Lim HH, Su F, HuangFu J, Teo S. The wound-healing effect of Kappaphycus alvarezii: in vitro and in vivo. Next Res. 2025;2:100246. doi:10.1016/j.nexres.2025.100246.

[15] Almadani YH, Vorstenbosch J, Davison PG, Murphy AM. Wound healing: a comprehensive review. Semin Plast Surg. 2021;35(3):141–4. doi:10.1055/s-0041-1731791.

[16] Kangal MKO, Kopitnik NL. Physiology, wound healing. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535406/

[17] Chandimali N, Bak SG, Park EH, et al. Free radicals and their impact on health and antioxidant defenses: a review. Cell Death Discov. 2025;11:19. doi:10.1038/s41420-024-02278-8.

[18] Pramesti D, Harlita T, Aina G. Efektivitas antibakteri kombinasi ekstrak daun binahong (Anredera cordifolia) dan keji beling (Strobilanthes crispus) terhadap Escherichia coli pada ulkus diabetikum. JI-KES. 2024;8:67–75. doi:10.33006/jikes.v8i1.790.

[19] Zanchetta FC, De Wever P, Morari J, Gaspar RC, Prado TP, De Maeseneer T, et al. In vitro and in vivo evaluation of chitosan/HPMC/insulin hydrogel for wound healing applications. Bioengineering. 2024;11(2):168. doi:10.3390/bioengineering11020168.

[20] Yang L, Shi F, Cao F, Wang L, She J, He B, et al. Neutrophils in tissue injury and repair: molecular mechanisms and therapeutic targets. MedComm. 2025;6(5):e70184. doi:10.1002/mco2.70184.

[21] Hakim EP, Siahaan JM, Anto EJ, Eyanoer PC. Effect of ethanol extract of suruhan leaves (Peperomia pellucida) on blood sugar levels and macroscopic wounds in male Wistar rats diabetic ulcer model. Indones J Med. 2024;9(2):192–206. doi:10.26911/theijmed.2024.09.02.06.

[22] Kanber AA, Kadhim EF. Efficacy of locally applied κ-carrageenan on the healing of thermal burn in rat: experimental histological study. J Res Med Dent Sci. 2022;10(9):160–5.

[23] Edo GI, Ndudi W, Makia RS, Ainyanbhor IE, Yousif E, Gaaz TS, et al. Carrageenan-based hydrogels for advanced wound healing and controlled drug delivery in tissue engineering. J Biomed Mater Res B Appl Biomater. 2025;113(5):e35594. doi:10.1002/jbm.b.35594.

[24] Jongsomchai K, Pudgerd A, Sakaew W, Wongprasert K, Kovensky J, Rudtanatip T. Sulfated galactan derivative from Gracilaria fisheri improves histopathology and alters wound healing-related proteins in excision rat skin. Front Biosci (Landmark Ed). 2024;29(11):388. doi:10.31083/j.fbl2911388.

[25] Mokhtari H, Tavakoli S, Safarpour F, Kharaziha M, Bakhsheshi-Rad HR, Ramakrishna S, et al. Recent advances in chemically-modified and hybrid carrageenan-based platforms for drug delivery, wound healing, and tissue engineering. Polymers. 2021;13(11):1744. doi:10.3390/polym13111744.

[26] Ulagesan S, Krishnan S, Nam TJ, Choi YH. The influence of κ-carrageenan–R-phycoerythrin hydrogel on in vitro wound healing and biological function. Int J Mol Sci. 2023;24:12358. doi:10.3390/ijms241512358.

[27] Gounden V, Singh M. Hydrogels and wound healing: current and future prospects. Gels. 2024;10(1):43. doi:10.3390/gels10010043.

[28] Masson-Meyers DS, Andrade TAM, Caetano GF, Guimaraes FR, Leite MN, Leite SN, et al. Experimental models and methods for cutaneous wound healing assessment. Int J Exp Pathol. 2020;101(1–2):21–37. doi:10.1111/iep.12346