

“The Effect of Freeze-Dried Human Amnion Compared to Freeze-Dried Human Amnion Combined with Secretome on Tendon Healing in New Zealand Rabbits”

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ABSTRACT

Background: Tendon injuries have limited healing capacity due to their hypovascular nature, often resulting in reduced biomechanical strength and an increased risk of re-rupture. Tissue engineering approaches, such as the use of amnion and secretome, have been developed to enhance tendon healing.

Objective: This study aims to evaluate the effect of freeze-dried human amnion compared with a combination of freeze-dried human amnion and secretome on tendon healing in New Zealand rabbits.

Methods: An experimental study using a randomized post-test-only control group design was conducted on 18 male New Zealand rabbits. In the control group, tendon repair was performed using the modified 4-strand Kessler technique for the core suture and a continuous suture for the peripheral tendon, followed by wrapping with freeze-dried amnion. In the treatment group, topical secretome was additionally applied. Evaluation was performed after 3 weeks and included tensile strength, fibroblast count, and collagen density. Statistical analysis was performed using an independent t-test and Mann–Whitney test, with statistical significance defined as $p < 0.05$.

Results: The mean tensile strength was higher in the treatment group than in the control group (4.83 ± 2.13 vs. 4.59 ± 3.21 ; $p = 0.849$). The fibroblast count was also higher in the treatment group (126.38 ± 60.32 vs. 105.38 ± 53.53 ; $p = 0.508$). Histologically, collagen density appeared to be higher in the treatment group.

Conclusion: The combination of freeze-dried human amnion and secretome showed a tendency to improve tendon healing parameters, although the differences were not statistically significant.

org/licenses/by/4.0/).Keywords: tendon healing, amnion, secretome, tensile strength, fibroblasts, collagen.

Introduction

Tendon injuries are common musculoskeletal conditions which can result in substantial functional impairment, particularly when involving the hand, and other structures essential for movement. Despite advances in surgical repair techniques, tendon healing remains a challenging process because tendon tissue is relatively low vascularization and has limited intrinsic regenerative capacity. Consequently, repaired tendons may develop scar tissue with inferior biomechanical properties compared with native tendon, contributing to impaired function and the risk of re-rupture. Previous studies have demonstrated the continuing clinical burden of tendon injuries and the risk of reoperation following tendon repair.^{1,2}

Strategies that promote effective tissue regeneration while minimizing excessive fibrosis and adhesion formation remain an important area of investigation. Tendon healing is a complex biological process involving inflammatory, proliferative, and remodeling phases. Fibroblasts play an essential role during the proliferative phase by synthesizing extracellular matrix components, particularly collagen. Collagen type I is the predominant structural component of mature tendon and is critical for restoration of its mechanical properties. However, excessive extrinsic healing may result in adhesion formation, which can interfere with tendon gliding and ultimately compromise functional recovery.³⁻⁵

Tissue engineering approaches have emerged as potential strategies to enhance tendon healing. Amniotic membrane has attracted considerable interest as a biological scaffold because of its biocompatibility, anti-inflammatory and antifibrotic properties, and ability to support cellular proliferation and tissue regeneration. The membrane contains extracellular matrix components and biologically active molecules, including growth factors and cytokines, that may facilitate tissue repair.⁶⁻⁸ Previous experimental research using freeze-dried human amnion in a New Zealand rabbit tendon model demonstrated improvements in tensile strength, fibroblast proliferation, and collagen density.⁸ Clinically, wrapping repaired

flexor tendons with amniotic membrane has also been associated with improved tendon gliding and total active motion.⁹

Secretome-based therapy represents another promising cell-free approach for tissue regeneration. Secretome consists of a complex collection of bioactive molecules released by stem cells, including proteins, growth factors, cytokines, extracellular vesicles, exosomes, and microRNAs. These factors may regulate cellular proliferation, migration, angiogenesis, inflammation, extracellular matrix synthesis, and tissue remodeling.^{10,11} Secretome derived from mesenchymal stem cells has been reported to enhance tenocyte proliferation and promote collagen type I production while improving tendon microstructure and reducing inflammation in experimental models.^{12,13} These effects suggest that secretome may enhance the biological activity of a scaffold-based approach to tendon repair. Amniotic membrane can function as a biological scaffold and physical barrier, while secretome may provide bioactive signals that stimulate cellular activity and extracellular matrix deposition. This combination could potentially enhance fibroblast activity and collagen formation while supporting organized tendon regeneration. However, evidence regarding the combined use of freeze-dried human amnion and secretome in tendon healing remains limited. The potential synergistic effect of these two modalities therefore warrants further investigation.

This study aimed to evaluate the synergistic effects of combining these two modalities. By applying secretome topically in combination with amniotic membrane wrapping over the repaired tendon, it was expected to enhance fibroblast formation, collagen density, and tensile strength compared with the use of amniotic membrane alone. The integration of secretome and amnion is expected to serve as an innovative adjuvant therapy to optimize the tendon healing process.

Material and Method

Study Design and Animals

This experimental study employed a randomized post-test-only control group design. Eighteen healthy male New Zealand white

rabbits (*Oryctolagus cuniculus*), aged 2–3 months and weighing 2.5–4.0 kg, were included. The animals were randomly allocated into two groups of nine rabbits each: a control group receiving freeze-dried human amnion and a treatment group receiving freeze-dried human amnion combined with topical secretome. The rabbits were housed individually in wire-mesh cages measuring 30 × 40 × 30 cm under room ventilation at an ambient temperature of approximately 32°C. Food and water were provided *ad libitum*. The animals received PAR-G pellets supplemented with vegetables, including water spinach and carrots. Cages were cleaned every two days. The minimum sample size was determined using the Steel and Torrie formula, resulting in a minimum of seven samples per group. Considering an anticipated 20% mortality rate, 18 rabbits were included, with nine animals allocated to each group.

Surgical Procedure and Intervention

Anesthesia was induced with intramuscular ketamine at a dose of 150 mg/kg body weight. The hair surrounding the right Achilles tendon was shaved, followed by aseptic and antiseptic preparation. A longitudinal skin incision approximately 3 cm in length was made over the Achilles tendon, followed by layer-by-layer dissection until the tendon and paratenon were exposed. The paratenon was then incised to expose the Achilles tendon. The Achilles tendon was sharply transected using a No. 11 scalpel blade and subsequently repaired using a modified four-strand Kessler technique with nonabsorbable 4/0 nylon monofilament for the core suture. A continuous peripheral suture using nonabsorbable 6/0 nylon was subsequently performed. In the control group, the repaired tendon was wrapped with freeze-dried human amnion. In the treatment group, 0.2 cc of secretome was applied topically to the repaired tendon before wrapping with freeze-dried human amnion. The skin was closed layer by layer using interrupted 5/0 nylon sutures. The surgical wound was dressed with tulle and sterile gauze, and the operated extremity was immobilized using a splint. The freeze-dried human amnion used in this study was obtained from the Tissue Bank of Dr. Soetomo General Hospital, Surabaya, and had undergone freeze-drying and gamma irradiation sterilization. Secretome was also obtained from the Tissue Bank of Dr. Soetomo General Hospital and applied topically to the repaired tendon in the treatment group.



Figure 1. Transection and repair of the right Achilles tendon in New Zealand rabbits, followed by wrapping with freeze-dried human amnion (a), and Injection of secretome (b).

Outcome Assessment

All animals were evaluated 3 weeks after tendon repair. At day 21, the rabbits were euthanized using intraperitoneal phenobarbital at a dose of 60–100 mg/kg body weight. The repaired tendon and surrounding tissue were harvested *en bloc*. The primary outcomes were tensile strength, fibroblast count, and collagen density.

Tensile Strength

Tensile strength was assessed using a Universal Testing Machine (UTM) at the Material Physics Laboratory, Faculty of Science and Technology, Universitas Airlangga. Each tendon specimen was secured at both ends of the testing apparatus and subjected to tensile loading until rupture. The maximum load at rupture (F_{max}) and tendon dimensions were recorded. Tensile strength was calculated as the maximum force divided by the tendon cross-sectional area and expressed in MPa.

Fibroblast Count

Following tensile testing, tendon specimens were fixed in 10% formalin and processed for histopathological examination. Sections were stained with hematoxylin and eosin (H&E) and examined using light microscopy at 400× magnification. Fibroblasts were counted in five microscopic fields for each specimen.

Collagen Density

Collagen density was evaluated microscopically using H&E staining at 400× magnification. Collagen density was graded using a semiquantitative scoring system from 0 to 4 according to the proportion of collagen fibers

within the wound area: score 0, no collagen fibers; score 1, low density (<10%); score 2, moderate density (10–50%); score 3, dense collagen (50–90%); and score 4, very dense collagen (90–100%) per microscopic field

Statistical Analysis

Statistical analysis was performed using SPSS. The distribution of continuous data was assessed using the Shapiro–Wilk test. Normally distributed variables were analyzed using an independent-samples *t* test, whereas non-normally distributed variables were analyzed using the Mann–Whitney test. Statistical significance was defined as $p < 0.05$.

Ethical Approval

The study received ethical approval from the Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Universitas Airlangga, under approval number 2.KEH.76.05.2025, dated 20 May 2025. The study was conducted at the Animal Laboratory, Faculty of Veterinary Medicine; Material Physics Laboratory, Faculty of Science and Technology; and Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya, from February to March 2026.

Result

A total of 18 male New Zealand rabbits weighing 2.5–4.0 kg were included in the study and randomized into two groups, with nine rabbits in each group. No animal deaths occurred during the study. All animals were sacrificed at the end of the 3-week observation period. Tensile strength was assessed using a Universal Testing Machine, while histopathological evaluation was performed using hematoxylin and eosin staining.

Tensile Strength

The mean tensile strength was higher in the treatment group receiving freeze-dried human amnion combined with topical secretome than in the control group receiving freeze-dried human amnion alone (4.83 ± 2.13 MPa vs. 4.59 ± 3.21 MPa). The tensile-strength data were normally distributed in both groups and demonstrated homogeneous variances based on Levene's test ($F = 2.049$, $p = 0.172$). An independent-samples *t* test showed no statistically significant difference in tensile strength between the two groups ($p = 0.849$).

Fibroblast Count

Histopathological examination at 400× magnification showed a visually higher number of fibroblasts in the treatment group compared with the control group. Fibroblasts were quantified in five microscopic fields for each specimen. The mean fibroblast count was 126.38 ± 60.32 in the treatment group and 105.38 ± 53.53 in the control group. Despite the higher mean fibroblast count in the treatment group, the difference was not statistically significant ($p = 0.508$) based on the Mann–Whitney test.

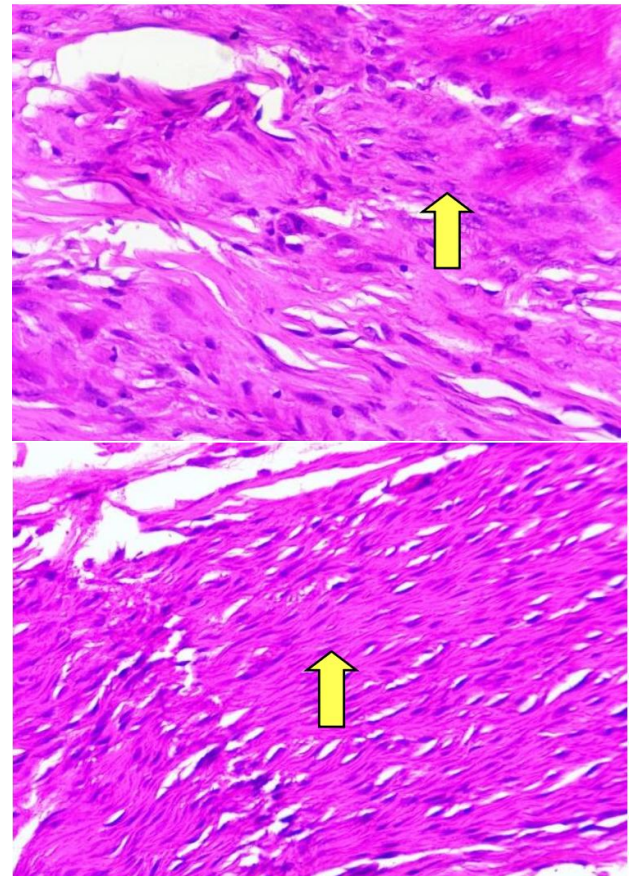


Figure 2. Microscopic appearance of fibroblast cells using a Nikon Eclipse Ci microscope at 400× magnification in the control group (a) and treatment group (b). Yellow arrows indicate fibroblast cells.

Collagen Density

Histopathological evaluation demonstrated a higher collagen density in the treatment group. Collagen density was assessed in five microscopic fields using a semiquantitative scoring system ranging from 0 to 4. The mean collagen-density score was 3.13 in the treatment group compared with 2.00 in the control group.

Table 1. Comparison of Tensile Strength, Fibroblast Count, and Collagen Density Between the Control and Treatment Groups

Outcome	Control	Treatment	P-value
Tensile strength (MPa)	4.59 ± 3.21	4.83 ± 2.13	0.849
Fibroblast count	105.38 ± 53.53	126.38 ± 60.32	0.508
Collagen density score	2.00	2.00	0.004

Statistical analysis using an independent-samples *t* test demonstrated a statistically significant difference in collagen density between the two groups ($p = 0.004$). Thus, unlike tensile strength and fibroblast count, collagen density was significantly higher in rabbits receiving the combination of freeze-dried human amnion and topical secretome.

Discussion

Tendon healing is a complex biological process involving inflammatory, proliferative, and remodeling phases. Due to the limited vascularity, Tendon healing capacity is lower than that of many other tissues, and restoration of mechanical strength may take considerably longer than the initial formation of repair tissue. In the present study, the combination of freeze-dried human amnion and topical secretome resulted in higher mean values of tensile strength, fibroblast count, and collagen density than freeze-dried human amnion alone. However, only the difference in collagen density reached statistical significance at 3 weeks.

The mean tensile strength was slightly higher in the combination group than in the freeze-dried human amnion group (4.83 ± 2.13 MPa vs. 4.59 ± 3.21 MPa); however, this difference was not statistically significant ($p = 0.849$). This finding indicates that the addition of secretome did not produce a measurable improvement in the mechanical strength of the repaired tendon at the 3-week evaluation point. The absence of a significant difference may be related to the relatively early timing of evaluation. During the early stages of tendon healing, newly deposited collagen is still undergoing organization and maturation. Although collagen deposition may increase the amount of collagen within the repair site, the mechanical properties of the tendon depend not only on the quantity of collagen but also on its organization, alignment, and maturation.

Therefore, an increase in collagen density does not necessarily translate into a proportional increase in tensile strength at an early time point. This interpretation is consistent with the biological progression of tendon healing described in the literature. Amadio described tendon healing as a process in which restoration of tendon nutrition and tissue organization contributes progressively to recovery of mechanical properties.³ Hope and Saxby similarly emphasized that tendon strength develops progressively during healing rather than being restored immediately after repair.⁴

The relatively large variability in tensile-strength measurements in both groups may also have contributed to the lack of statistical significance. Individual tensile-strength values varied considerably between animals, indicating heterogeneity in the mechanical response of the repaired tendons. In addition, the use of only nine animals per group may have limited the statistical power to detect a relatively small difference between interventions.

The treatment group demonstrated a higher mean fibroblast count than the control group (126.38 ± 60.32 vs. 105.38 ± 53.53), although the difference was not statistically significant ($p = 0.508$). Fibroblasts have an important role during the proliferative phase of tendon healing because they contribute to extracellular matrix synthesis and collagen production. Therefore, the higher mean fibroblast count observed following secretome administration may indicate a potential biological response to the treatment, although this effect was insufficient to produce a statistically significant difference in the present study. Secretome contains a complex mixture of soluble bioactive molecules, including growth factors, cytokines, and other signaling molecules that may influence cell proliferation, migration, and extracellular matrix synthesis.^{6,9} Goodarzi et al. described the regenerative potential of mesenchymal stem cell-derived extracellular products, while Chen et al. demonstrated that mesenchymal stem cell-derived conditioned medium can promote tenocyte proliferation and migration.^{10,12} The nonsignificant result suggests that the effect of secretome on fibroblast proliferation may not be sufficiently strong or sustained to produce a significant difference at 3 weeks. Furthermore, fibroblast number alone may not fully represent fibroblast activity or the quality of the extracellular matrix produced.

Secretome may influence fibroblast function, collagen synthesis, or cellular behavior without necessarily causing a marked increase in the absolute number of fibroblasts.

In contrast to tensile strength and fibroblast count, collagen density was significantly higher in the treatment group than in the control group, with mean scores of 3.13 and 2.00, respectively ($p = 0.004$). This was the only parameter demonstrating a statistically significant difference between the groups and suggests that the addition of topical secretome to freeze-dried human amnion may enhance collagen deposition during tendon healing.

The finding is biologically plausible because secretome-derived bioactive factors may stimulate fibroblast activity and extracellular matrix production.^{6,9,11,12} Sam et al. specifically reported the potential effectiveness of mesenchymal stem cell secretome in chronic tendon injury and evaluated its effect using procollagen analysis and histopathological assessment.¹¹ The present findings therefore provide further support for the potential role of secretome as a cell-free biological approach to enhance tissue repair. The freeze-dried human amnion may also contribute to the observed improvement in collagen density. Amniotic membrane has been investigated as a biological scaffold because of its structural properties and its potential to modulate the local wound-healing environment. Liu et al. demonstrated the potential application of decellularized amnion in

tendon sheath repair, particularly in relation to tendon adhesion.⁵ Previous work by Iustitiati et al. also investigated freeze-dried human amnion in a New Zealand rabbit tendon-healing model.⁷ Prakash et al. reported the use of amniotic membrane in flexor tendon repair, further supporting its potential application in tendon regeneration.⁸ The combination of a structural biological material and secretome may therefore provide complementary effects: the amnion can function as a local scaffold or tissue interface, while secretome provides bioactive signals that may influence cellular and extracellular matrix responses. However, the present study was not designed to directly demonstrate a synergistic interaction between the two modalities.

Conclusion

The combination of freeze-dried human amnion and topical secretome showed a favorable trend across all three evaluated parameters. The treatment group had higher mean tensile strength, fibroblast count, and collagen density than the freeze-dried human amnion group, with a statistically significant improvement observed only in collagen density. These findings suggest that the combination may enhance aspects of the biological tendon-healing response, particularly extracellular matrix deposition, during the early healing period.

Reference

- Miranda BH, Spilsbury ZP, Hallas AR. Hand trauma: a prospective observational study reporting diagnostic concordance in emergency hand trauma which supports centralised service improvements. *J Plast Reconstr Aesthet Surg*. 2016;69(10):320-02.
- Svingen J, Wiig M, Turesson C, Farnebo S, Arner M. Risk factors for reoperation after flexor tendon repair: a registry study. *J Hand Surg Eur Vol*. 2022;47(10):320-02.
- Amadio PC. Tendon nutrition and healing. In: Tang JB, Amadio PC, Guimberteau JC, Chang J, editors. *Tendon Surgery of the Hand*. Philadelphia: Elsevier Saunders; 2012. p.320-02.
- Hope M, Saxby T. Tendon healing. *Foot Ankle Clin*. 2007;12(4):320-02.
- Liu C, Yu K, Bai J, Tian D, Liu G. Experimental study of tendon sheath repair via decellularized amnion to prevent tendon adhesion. *PLoS One*. 2018;13(10):e0205811.
- Ahangar P, Mills SJ, Cowin AJ. Mesenchymal stem cell secretome as an emerging cell-free alternative for improving wound repair. *Int J Mol Sci*. 2020;21(19):7038.
- Iustitiati MAA, Rizaliyana S, Budi AS. The effect of freeze-dried human amnion on New Zealand rabbit tendon healing. *Bali Med J*. 2022;11(3):320-02.
- Prakash SK, Kalra P, Dhal A. Flexor tendon repair with amniotic membrane. *Int Orthop*. 2020;44(10):320-02.

- Md Fadilah NI, Mohd Abdul Kader Jailani MS, Badrul Hisham MA, Sunthar Raj N, Shamsuddin SA, Ng MH, et al. Cell secretomes for wound healing and tissue regeneration: next generation acellular based tissue engineered products. *J Tissue Eng.* 2022;13.
- Goodarzi P, Larijani B, Moghadam SA, Beik AT, Jahani FM, Ranjbaran N, et al. Mesenchymal stem cells-derived exosomes for wound regeneration. *Adv Exp Med Biol.* 2018;1119:320-02.
- Sam ADP, Warsingih W, Usman MA, Johan M, Suroto H, Saleh MR, et al. Mesenchymal stem cell secretome effectiveness in healing chronic tendon injury: procollagen analysis and histopathology in rat tendons. *Stem Cells Cloning.* 2025;18:320-02.
- Chen Q, Liang Q, Zhuang W, Zhou J, Zhang B, Xu P, et al. Tenocyte proliferation and migration promoted by rat bone marrow mesenchymal stem cell-derived conditioned medium. *Biotechnol Lett.* 2018;40(1):320-02.