ORIGINAL ARTICLE

Journal of Dentomaxillofacial Science (J Dentomaxillofac Sci) October 2025, Volume 10, Number 4: 194-197
P-ISSN.2503-0817, E-ISSN.2503-0825

Post–tooth extraction supportive therapy with Limosilactobacillus reuteri probiotic and hyaluronic acid gel combination on collagen density

Desak MH. Wijayanti,[1] Armelia S. Widyarman,[2*] Melanie H. Sadono,[2] Firstine K. Hartanto,[3] Komariah,[4] Wiwiek Poedjiastuti[4]

Abstract

Objective: This study aimed to analyze the differences in collagen density during wound healing process after tooth extraction with the administration of supportive therapy by combining HA and probiotic L. reuteri in Wistar rats (Rattus norvegicus).

Material and Method: Collagen density was measured using Masson's trichrome staining. Observations were performed with light microscope at 400× magnification and Fiji/ImageJ software. Collagen measurement was observed on the blue-colored surface of the specimen. The unit of collagen density obtained in a single field of view was expressed as an area percentage (area%).

Results: One-way ANOVA showed significant differences in collagen density between the groups on days 3 ($p = 0.005$), 7 ($p<0.001$), and 14 ($p= 0.009$). In the combination therapy group (T3), the average collagen density was significantly higher than in the other groups on days 7 and 14 ($p <0.05$).

Conclusion: Supportive therapy combining HA and L. reuteri probiotic combination therapy can increase collagen density during wound healing in Wistar rats, with the strongest effects observed on days 7 and 14.

Keywords: Collagen, Hyaluronic acid, Limosilactobacillus reuteri, Probiotic, Wound healing process
DOI: 10.15562/jdmfs.v10i4.2024

[1]Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia
[2]Department of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia
[3]Department of Oral Medicine and Oral Pathology, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia
[4]Department of Oral & Maxillofacial Surgery, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia

*Correspondence to:
Armelia S. Widyarman, Department of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia
armeliasari@trisakti.ac.id

Received 10 June 2025
Revised 25 September 2025
Accepted 27 September 2025
Available online 30 October 2025

Introduction

Wound healing post-surgical procedures, such as tooth extraction, is a complex process involving tissue regeneration, inflammation, and collagen synthesis. Post-extraction complications can cause pain and delayed healing, leading to increased patient visits to dental practice, and disruption of normal daily activities. One of key factors determining the success of wound healing is collagen formation, a structural protein that supports integrity and strength of the healed tissue.[1-3] During the post-extraction healing process, optimal collagen formation is crucial for accelerating periodontal tissue regeneration and preventing complications such as infection or healing failure.[4,5]

Probiotics are known to provide various health benefits, including improving the balance of gut microflora and enhancing immune system function. Several studies have also shown that probiotics can promote wound healing by regulating inflammation and collagen synthesis.[6,7] Limosilactobacillus reuteri is a probiotic bacterium widely used for oral cavity health due to its advantage of producing reuterin, a compound with antimicrobial properties that supports wound healing.[8] HA is known for viscoelastic properties, which support tissue hydration and enhance cell migration during healing process.[9] HA has been shown to accelerate reepithelialization and stimulate changes in protein expression in vivo in incision wounds in the human dermal layer. However, it does not have a significant effect on the inflammation process, as measured by assessing erythema conditions. Gels offer a practical and effective means for the local delivery of probiotics to support wound healing.[10] The synergistic combination of L. reuteri and HA is believed to improve tissue regeneration, particularly by increasing collagen density, after tooth extraction.[11] However, in vivo studies on the effectiveness of probiotics as supportive therapies for wound healing are limited, and more research is needed.[12,13]

The combination of HA and probiotics is expected to create synergistic effect in improving tissue repair, especially by increasing collagen density in wounds after tooth extraction. This research is expected to provide new insights into the use of probiotics in dental wound healing therapy and offer solid scientific basis for developing more effective and safer topical therapies to accelerate recovery after tooth extraction procedures.

Material and Methods

The study was approved by the Ethical Clearance Commission of the Faculty of Dentistry of Trisakti University, Indonesia (No. 640/S2/KEPK/FKG/4/2023).

Probiotic Gel Preparation

Probiotic L. reuteri was prepared in gel form based on a commercially available lozenges tablet formulation. The lozenge tablets (1 tablet = 400 mg) contained L. reuteri DSM 17938 and L. reuteri ATCC PTA 5289 at concentration of 2 × 100,000,000 CFU.

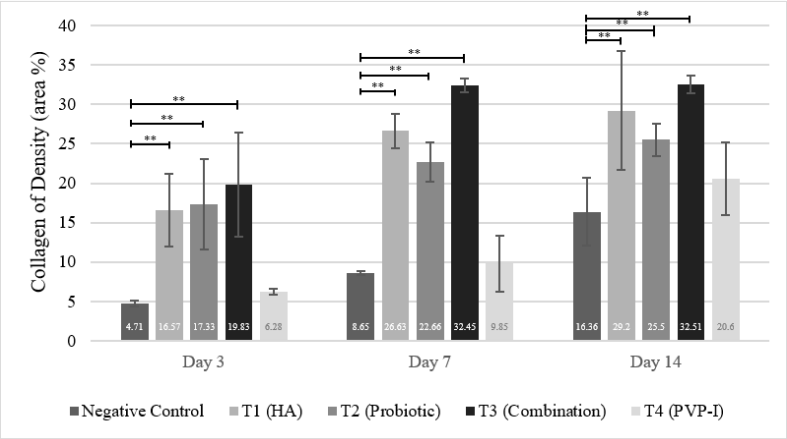

Figure 1. Graph of mean collagen density in each group on days 3, 7, and 14 based on histopathological examinations. NC: negative control group; T1: HA treatment group; T2: L. reuteri treatment group; T3: HA–L. reuteri treatment group; T4: PVP-I treatment group. *p<0.05 (significant compared to the negative control) and **p<0.01 (highly significant compared to the negative control).

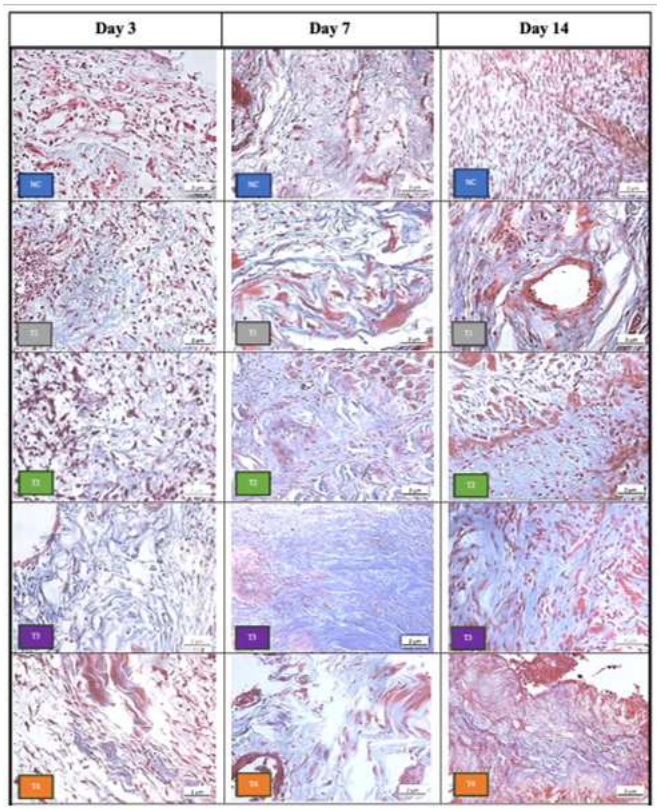

Figure 2. Histopathological findings. Changes in collagen density on days 3, 7, and 14 are indicated by blue coloration using Masson's trichrome staining (400× magnification). NC: negative control group; T1: HA treatment group; T2: L. reuteri treatment group; T3: HA–L. reuteri treatment group; T4: PVP-I treatment group.

Carboxymethyl cellulose sodium (CMC-Na; 1.5 g) was dissolved in 50 ml of hot distilled water (70°C) and allowed to sit for 15 min. Subsequently, 50 ml of cold water was added, and the mixture was stirred with a magnetic stirrer until it expanded. Furthermore, 1.75 g of CMC-Na was placed in a beaker, and 3.15 ml of glycerin, 0.3 ml of propylene glycol, and 45 ml of distilled water were added. Subsequently, 800 mg (two tablets) was added, and the mixture was stirred until it became homogeneous. A culture was then performed using de Man–Rogosa–Sharpe agar to confirm that the bacteria in the gel remained viable.

Rat Preparation

The study sample consisted of 45 male Wistar rats aged 2–3 months. The rats underwent a seven-day adaptation period in wire mesh cages lined with wood shavings. Food and water were provided ad libitum, and the rats were continuously monitored until the study was completed. The treatment of the rats was supervised by specialized staff, including a veterinarian, at an animal hospital.

Treatment Procedure

The left lower incisor of each rat was extracted following general anesthesia with ketamine (50–80 mg/kg; Kepro B.V., Deventer, Netherlands) and xylazine (20 mg/kg; Agrovet Market, Lima, Peru), administered via intraperitoneal injection. After severing the periodontal ligament with a scalpel, the tooth was gently removed using a needle holder as a substitute for extraction forceps. The depth of the socket post-extraction was measured using a periodontal probe to determine the volume of gel required and to ensure even distribution throughout the socket. Specifically, 0.1 ml of gel was administered using a sterile syringe with a blunt plastic tip.

Preparation of Staining Slide

The unstained slide was placed in oven at 64°C for 25 minutes, then immersed in xylene solution twice. The slide was then immersed in following alcohol solutions: absolute alcohol, 96% alcohol and 70% alcohol for 5 minutes. Preheated Bouin's solution was dropped onto slide surface and incubated for 1 hour. After rinsing, the next steps were performed: Weigert's solution A and Weigert's solution B were mixed to create Iron Hematoxylin solution. The solution was applied to slide, rinsed with running water and distilled water. Biebrich Scarlet/Acid Fuchsin was dropped onto slide surface, incubated for 5 minutes, and rinsed. Phosphomolybdic/Phosphotungstic Acid was dropped onto slide surface and left for 15 minutes. Aniline Blue was applied to slide surface for 8 minutes, then rinsed. Acetic Acid was applied to slide surface and incubated for 4 minutes. The slide was placed in same alcohol solutions as before, then cleared and covered with cover glass.[14,15]

Collagen density examination

Collagen density was measured using density quantitative measurement method by performing color thresholding with color deconvolution plugin in Fiji/ImageJ (developed by the National Institutes of Health, Bethesda, MD, USA). Masson's

trichrome staining was identified by blue color under 400× light microscope. The unit of collagen density in single field of view was expressed as the percentage area (area%).[14]

Statistical Analysis

Data Analysis was performed using SPSS IBM statistics version 29. Cell counts were obtained from observations of three interpreters. Inter-rater Reliability was assessed using Intraclass Correlation Coefficient (ICC) to evaluate reliability among interpreters. Normality and homogeneity tests were performed. One-way ANOVA test (parametric hypothesis test) was performed with 95% confidence level ($p < 0.05$). Least Significant Difference (LSD) post hoc test was conducted to identify significant and non-significant differences between groups.

Results

The calculation of mean collagen density during wound healing process was measured using the same method. The reliability test result was 0.912, indicating excellent agreement. The presentation of mean collagen density in Rattus norvegicus on days 3, 7, and 14 are shown in figure 1. The histopathological appearance is shown in figure 2.

The Shapiro–Wilk test confirmed that collagen density data on days 3, 7, and 14 were normally distributed ($p > 0.05$), and Levene's test showed homogeneous variances ($p > 0.05$). One-way ANOVA revealed significant differences in collagen density between groups on days 3 ($p = 0.005$), 7 ($p < 0.001$), and 14 ($p = 0.009$).Post Hoc LSD analysis showed that on day 3, treatment groups T1 (HA), T2 (probiotic), and T3 (combination) had significantly higher collagen density than the control groups ($p < 0.05$), but no significant differences were observed among T1, T2, and T3. On day 7, T3 exhibited significantly higher collagen density than all other groups ($p < 0.05$). T1 showed higher values than T2, T4 (PVP-I), and the negative control, but lower than T3. T2 was higher than control groups but lower than T1 and T3. On day 14, T3 again showed significantly higher collagen density compared to T2, T4, and the negative control ($p < 0.05$), but not significantly different from T1 ($p > 0.05$).

Discussion

Collagen density is an important indicator of tissue healing during the remodeling and maturation phases. Collagen fiber formation supports tissue strength at the wound site, with synthesis increasing during maturation.[3,15] Healing begins when fibrillar collagen interacts with blood, triggering platelet aggregation and chemotactic factor release that attract fibroblasts. Collagen forms the extracellular matrix, facilitating granulation tissue and wound contraction.[16,18]

In this study, collagen density was measured quantitatively using Fiji software on Masson's trichrome-stained samples.[19] On day 3, the combination therapy group (T3) showed higher collagen density than other groups but without statistical significance ($p > 0.05$), consistent with previous findings that collagen forms thinly at this stage.[20] On day 7, T3 exhibited significantly higher collagen density compared to all other groups ($p < 0.05$), including highly significant differences versus untreated and PVP-I groups ($p < 0.01$). On day 14, T3 maintained significantly higher density than probiotic (T2), negative control, and PVP-I (T4) groups ($p < 0.05$), but was not significantly different from HA (T1) ($p > 0.05$). This suggests probiotics enhance HA's effect on collagen synthesis. Previous studies show tissue remodeling follows proliferation phase, leading to maturation of dense collagen.[18,21] The combination of HA and L. reuteri works synergistically to accelerate healing by modulating inflammation, promoting cell migration, and stimulating fibroblast activity, resulting in increased collagen density on days 7 and 14.[22]

Conclusion

The results of this study support the theory that hyaluronic acid (HA) and L. reuteri probiotics can enhance collagen density during wound healing process through different mechanisms, with HA accelerating extracellular matrix formation and L. reuteri improving immune response and tissue regeneration. The combination therapy of both provides stronger synergistic effect, increasing collagen synthesis and accelerating wound healing compared to control groups.

Acknowledgments

None.

Conflict of Interest

The authors report no conflict of interest.

References

1. Rezandaru F, Syamsudin E, Hadikrishna I, et al. Effectiveness of the application of bawang Dayak (Eleutherine palmifolia L. Merr) extracts on healing process in osteitis alveolar post tooth extraction through fibroblast examination, collagen density and amount of osteogenesis (experimental study on Sprague Dawley mice). J Dentomaxillofac Sci 2020;5: 28-33.
2. Suryadewi NPI, Utomo RB, Bramanti I. The effect of green robust coffee gel ((Coffea canephora) to collagen density on gingival wound healing (an in-vivo study in Wistar rats). J Dentomaxillofac Sci 2023;8: 34-38.
3. Binlateh T, Thammanichanon P, Rittipakorn P, et al. Collagen-Based biomaterials in periodontal regeneration:

Current Applications and future perspectives of plant-based collagen. Biomimet 2022;7: 1-16.
4. Chen Y, Huang X, Liu A, et al. Lactobacillus reuteri vesicles regulate mitochondrial function of macrophages to promote mucosal and cutaneous wound healing. Adv Sci 2024;11: 1-16.
5. Udeabor SE, Heselich A, Al-Maawi S, et al. Current knowledge on the healing of the extraction socket: A narrative review. Bioeng 2023;10: 1-11.
6. Dovedytis M, Liu ZJ, Bartlett S. Hyaluronic acid and its biomedical applications: A review. Engineer Reg 2020;1: 102-113.
7. Widyarman A, Pranoto S, Theodorea C, et al. Isolation and identification of Indonesian lactobacillus reuteri strain from the saliva of young adults. Scient Dent J 2018;2: 67.
8. Hurtado-camarena A, Serafín-higuera N, Pitones-rubio V. Lactobacillus probiotics and their impact on periodontal diseases. JDOM 2023;1849: 111-115.
9. Chang W, LiRu C, KuoHu C. The Bioengineering Application of hyaluronic acid in tissue regeneration and repair. Int J Biol Macromolec 2024;270.
10. Gardeazabal L, Izeta A. Elastin and collagen fibres in cutaneous wound healing. In Experiment Dermatol 2024;33: 1-14.
11. Widyarman AS, Bachtiar EW, Bachtiar BM, et al. Inhibitory effect of probiotic lactobacilli against. Scient Dent J 2019: 50-55.
12. Bekiaridou A, Karlafti E, Oikonomou IM, et al. Probiotics and their effect on surgical wound healing: A systematic review and new insights into the role of nanotechnology. Nutr 2021;13.
13. Mu Q, Tavella VJ, Luo XM. Role of lactobacillus reuteri in human health and diseases. Front Microbiol 2018;9: 1-17.
14. Sharun K, Banu SA, Mamachan M, et al. Comparative evaluation of masson's trichrome and picrosirius red staining for digital collagen quantification using imagej in rabbit wound healing research. J Experiment Biol Agriculture Sci 2023;11: 822-833.
15. Chen Y, Yu Q, Xu CB. A convenient method for quantifying collagen fibers in atherosclerotic lesions by imagej software. Int J Clin Experiment Medic 2017;10: 14904-14910.
16. Moretti L, Stalfort J, Barker TH, et al. The interplay of fibroblasts, the extracellular matrix, and inflammation in scar formation. J Biologic Chem 2022;298.
17. Amita K, Balqis U, Iskandar CD. Histopathological description of wound healing in mice (Mus musculus) using binahong leaf extract (Anredera cordifolia (tenore) steenis). Jimvet 2017;1: 584 591.
18. Toma AI, Fuller JM, Willett NJ, et al. Oral wound healing models and emerging regenerative therapies. Translational research. J Lab Clin Medic 2021;236: 17-34.
19. Suvik, Effendy. the use of modified masson ' s trichrome staining in collagen evaluation in wound healing study. Malay J Veter Res 2012;3: 39-47.
20. Xu J, Chen C, Gan S, et al. The potential value of probiotics after dental implant placement. Microorg 2023;11: 1-25.
21. Feizabad SJ, Jafari P, Marjani A, et al. Novel wound dressing based on postbiotic/chitosan film accelerates cutaneous wound healing. J Microbiol 2021;14.