RESEARCH PAPER

Comparison of Type I Collagen Expression in Prolotherapy Using Nanochitosan and Dextrose in Rat Ligament Injury

Alif Fariz Jazmi[1,2], Belindo Wirabuana*[1,2], Prananda Surya Airlangga[1,2], Christrijogo Sumartono Waloejo[1,2], Dedi Susila[1,2], Atika[3]

[1]Department of Anesthesiology and Reanimation, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Anesthesiology and Reanimation, Dr Soetomo General Academic Hospital, Surabaya, Indonesia
[3]Department of Public Health – Preventive Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Received: 14th Aug, 2025; Revised: 15th Sep 2025; Accepted: 15th Nov, 2025; Available Online: 30th Nov, 2025

ABSTRACT

Introduction: Ligament injury is a musculoskeletal problem th at requires effective regenerative therapy. Prolotherapy with dextrose has been widely used, while nanochitosan has been developed as a new alternative due to its higher biological activity, including anti-inflammatory, antioxidant, and fibroblast and epithelial stimulation effects. This study aimed to compare the effects of 0.4% nanochitosan with 12.5% dextrose prolotherapy on type I collagen expression in wistar rat patellar ligament injury.

Methods: The study design was a true experimental randomized post-test only control group. Thirty-three rats were divided into three groups: Control (KK), nanochitosan prolotherapy (KN), and dextrose prolotherapy (KD). Type I Collagen expression was measured on days 8, 14, and 21, calculated from the time of injury or 24 hours, 7 days, and 14 days after prolotherapy using the ELISA method.

Results: The results showed that both dextrose and nanochitosan can increase the expression of type I collagen through inflammatory mechanisms and fibroblast activation, supporting ligament tissue remodeling. Nanochitosan exhibits bioactive activities including stimulation of fibroblast proliferation and anti-inflammatory effects that support tissue regeneration. Although there was no statistically significant difference between the administration of nanochitosan and dextrose, there was a significant temporal change from day 8 to 21, indicating stimulation and accumulation of type I collagen expression due to prolotherapy treatment.

Conclusion: There was no difference between prolotherapy agents. Both prolotherapy agents stimulated type I collagen expression in ligament injury healing. The numerical trends and additional biological properties of nanochitosan warrant further investigation as an alternative prolotherapy agent.

Keywords: Prolotherapy, Nanochitosan, Chitosan, Dextrose, Type I Collagen, Ligament.

How to cite this article: Jazmi AF, Wirabuana B, Airlangga PS, Waloejo CS, Susila D, Atika. Comparison of Type I Collagen Expression in Prolotherapy Using Nanochitosan and Dextrose in Rat Ligament Injury. Int J Drug Deliv Technol. 2025;15(4): 2002-2008, DOI: 10.25258/ijddt.15.4.53

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Musculoskeletal injuries, particularly ligament damage, represent a significant global health concern affecting millions annually and contributing substantially to disability and reduced quality of life[1]. Ligament and tendon injuries comprise approximately 50% of all musculoskeletal injuries, with knee ligament injuries being among the most common, frequently resulting in chronic pain and functional impairment[2,3].

The normal healing process of ligament injuries progresses through three distinct phases: the inflammatory phase (days 0-7), characterized by hemostasis, immune cell recruitment, and initial cytokine production; the proliferative phase (days 7-14), marked by fibroblast activation and collagen deposition; and the remodeling phase (beyond day 21), involving collagen maturation and cross-linking[4]. During this cascade, type I collagen synthesis is fundamental to restoration of ligament strength and biomechanical properties[5].

Treatment approaches for ligament injuries include surgical and non-surgical modalities. While surgical interventions address severe injuries, non-surgical approaches encompassing pharmacotherapy, regenerative injection techniques, and physical rehabilitation have gained prominence due to favorable risk-benefit profiles and cost-effectiveness[6]. Among emerging regenerative therapies, prolotherapy has demonstrated promising results in clinical and preclinical settings[7].

Prolotherapy, or proliferative injection therapy, involves strategic injection of irritant solutions into damaged ligamentous and tendinous structures to stimulate endogenous healing cascades through controlled localized

**Author for Correspondence: belindowirabuana@fk.unair.ac.id*

inflammation[8]. Hypertonic dextrose (12,5-25%) represents the most widely used prolotherapy agent globally due to its safety profile, water solubility, cost-effectiveness, and documented capacity to promote tissue regeneration[9]. The mechanism involves osmotic cell stress, selective cell lysis, and subsequent recruitment of inflammatory cells and growth factors that enhance fibroblast proliferation and collagen synthesis[10].

Despite dextrose's established efficacy, investigation of alternative prolotherapy agents may yield superior outcomes. Chitosan, a naturally occurring polysaccharide derived from crustacean exoskeletons, possesses multiple bioactive properties including antimicrobial, anti-inflammatory, antioxidant, and pro-regenerative effects[11]. Nanochitosan, characterized by markedly reduced molecular weight (<3900 Da) and nanoscale particle dimensions (10-1000 nm), represents an advanced formulation with enhanced tissue penetration and bioavailability[12].

Type I collagen comprises approximately 85% of total collagen in ligaments and provides primary tensile strength and structural integrity[13]. Enhanced Type I collagen synthesis directly correlates with improved healing outcomes and restored biomechanical function[14]. The role of Type I collagen as a biomarker for regenerative therapy efficacy is well-established in ligament healing research[15].

Limited comparative data currently exist regarding nanochitosan versus dextrose prolotherapy effects on Type I collagen expression during ligament healing. This study addresses this research gap through rigorous comparison of these prolotherapy agents using a validated in vivo ligament injury.

METHODS

Study Design

This research applied a true experimental randomized post-test only control group design. Out of 40 Wistar rats induced with patellar ligament injury, seven were excluded during adaptation, leaving 33 rats assigned into KK (control group, n=9), KN (0.4% nanochitosan prolotherapy group, n=13), and KD (12,5% dextrose prolotherapy group, n=11).

Procedure of Ligament Injury

Rats were anesthetized intramuscularly with a rat cocktail containing 2.5% ketamine and 1% xylazine at 0.1 ml per 100 grams of body weight. The patellar ligament injury was created by shaving and disinfecting the right hind knee, making a 1 cm vertical incision from the patella to the tibial tuberosity, followed by a horizontal transection across 30% of the ligament's diameter. The wound was sutured and treated with 2% topical mupirocin. This procedure can be seen in Figure 1.

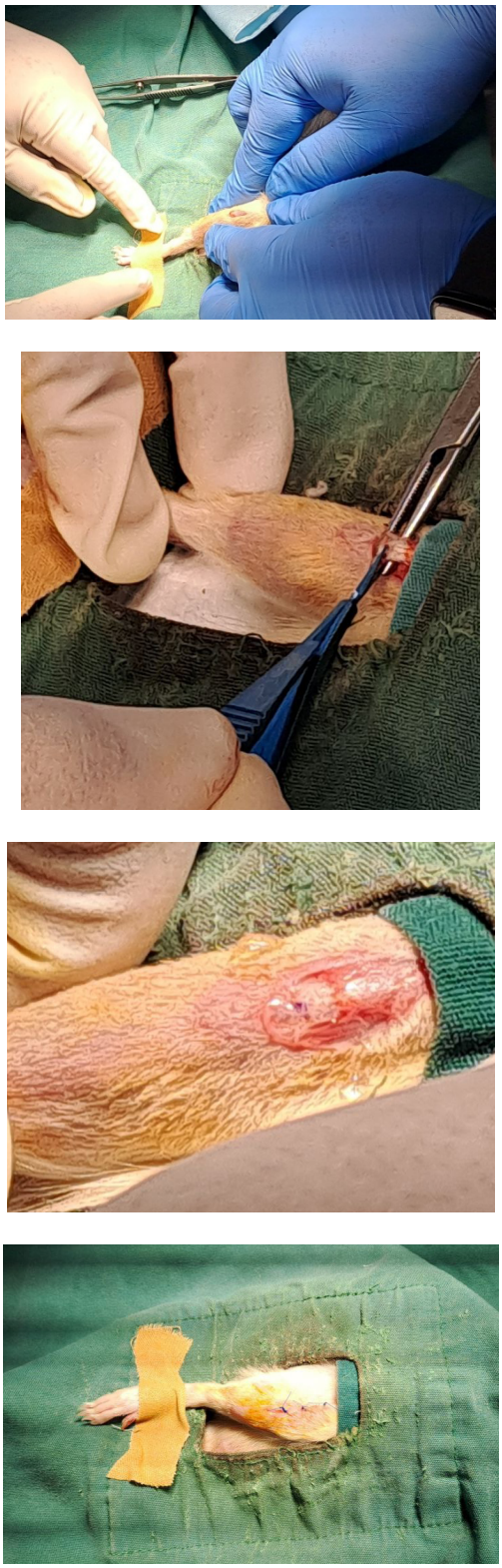

Figure 1. Ligament transection process in Wistar rats

Prolotherapy Preparation and Administration

Sterile solutions of dextrose and nanochitosan were prepared at required concentrations. Nanochitosan was produced at the Mineral and Material Processing Laboratory, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia, using shrimp shell as the raw material.

Nanochitosan salts were synthesized using microwave pyrolysis from shrimp shell without chemical additives, producing particles averaging 60.73 nm, a molecular weight of 244–258 Da, and a 98.99% degree of deacetylation. The 0.4% nanochitosan solution was made by dissolving 400 mg of nanochitosan salt in 100 ml sterile distilled water. Seven days post-injury, 0.1 ml of the assigned prolotherapy (dextrose or nanochitosan) was injected directly into the injured ligament.

Sample Collection and Collagen Type 1 Measurement

Ligament tissues were collected on days 8, 14, and 21 after injury. Sampled on days 8, 14, and 21 post injury, coded as "i", "p", and "r" respectively. Samples were homogenized and analyzed for collagen type I measurement using ELISA following the manufacturer's guidelines.

Statistical Analysis

Normality and homogeneity were assessed using Shapiro-Wilk and Levene's tests. One-way ANOVA was conducted for normally distributed data, with significance at $p < 0.05$. For not normally distributed data, the Kruskal Wallis test was used.

Table 1. Comparative Test of Rat Body Weight in Each Group

Weight (gram)	Day- 8 Mean ± SD) or (Median (Min.-Max.))	Day – 14 Mean ± SD or Median (Min.-Max.)	Day – 21 Mean ± SD) or Median (Min.-Max.)	p-value
KK	252 ± 46 232 (220 – 305)	232,5 ± 30,6 229 (204 – 268)	261,5 (221 – 302)	0,176^
KN	233,3 ± 14,5	232,2 ± 22,2	259,3 ± 25,8 259,5 (228 – 290)	0,177v
KD	278 ± 32,7	255,8 ± 16,7	232,5 ± 30,7 229 (204 – 268)	0,186v
p-value	0,198v	0,083v	0,226^	

^) Kruskal-Wallis test

v) ANOVA test

RESULTS

Demographic Characteristics

A total of 40 male Wistar rats were initially included in this study. However, seven animals were excluded from the final analysis due to mortality (six rats) and infection signs (one rat), resulting in 33 rats for analysis. The rats had an average body weight of 248.21 ± 29.882 grams, ranging from 204 to 308 grams. Normality and homogeneity tests on body weight across the groups and sampling days showed normal distribution (Shapiro-Wilk $p > 0.05$) except for one group with only two samples where the test was not applicable. Levene's test confirmed homogeneity of variances ($p > 0.05$). Comparisons of body weight between groups showed no significant differences ($p > 0.05$), indicating no confounding influence of weight (Table 1)

Type I Collagen Expression

Type I collagen expression patterns are illustrated across the three groups: control (KK), 0,4% nanochitosan prolotherapy (KN), and 0,25% dextrose prolotherapy (KD), sampled on days 8, 14, and 21 post-injury, coded as "i", "p", and "r" respectively. Normality testing using Shapiro-Wilk confirmed normal distribution for all groups except control day 21 (KKr, n=2, N/A : not analyzed), with $p > 0.05$ across KKi, KKp, KNi, KNp, KNr, KDi, KDp, and KDr. Levene's test verified homogeneity of variances ($p>0.05$) at each comparison.

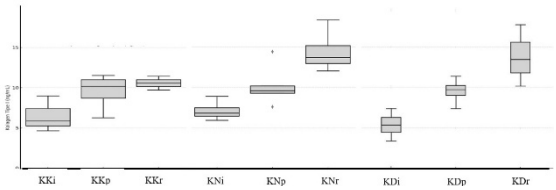

Figure 2. Type I Collagen Expression in Control and Prolotherapy Group

Type I collagen expression increased progressively across all groups from day 8 to day 21 post-injury (Figure 2). At day 8 (24 hours post-prolotherapy), mean expression was highest in the nanochitosan group (KNi: 7.127 ± 1.259 ng/ml), followed by control (KKi: 6.394 ± 2.088 ng/ml) and dextrose (KDi: 5.400 ± 1.697 ng/ml), with no significant inter-group differences (ANOVA, $p = 0.383$). At day 14 (7 days post-prolotherapy), nanochitosan showed the highest mean (KNp: 10.221 ± 2.527 ng/ml), followed by dextrose (KDp: 9.581 ± 1.650 ng/ml) and control (KKp: 9.517 ± 2.336 ng/ml), but inter-group differences remained non-significant (ANOVA, $p = 0.871$). On day 21 (14 days post-prolotherapy), median values were highest in nanochitosan (KNr: 13.677 ng/ml (12.030–18.293)), followed by dextrose (KDr: 13.470 ng/ml (10.201–17.763)) and control (KKr: 10.557 ng/ml (9.697–11.417)), with no significant differences (Kruskal-Wallis, $p = 0.194$) (Table 2).

Table 2. Differences in Type I Collagen Expression in Control and Prolotherapy Groups

Collagen Type I Expression	Day- 8 (i) Mean ± SD) or Median (Min. – Max)	Day - 14 (p) Mean ± SD or Median (Min. – Max.)	Day - 21 (r) Mean ± SD) or Median (Min. – Max.)	p-value
KK	6,394 ± 2,088 5,860 (4,624 – 8,968)	9,517 ± 2,336 10,152 (6,246 – 11,519)	10,557 (9,697 – 11,417)	0,108^

KN	7,127 ± 1,259	10,221 ± 2,527	14,419 ± 2.717 13,677 (12,030 – 18,293)	0,004[v]
KD	5,400 ± 1,697	9,581 ± 1,650	13,811 ± 3,792 13,470 (10,201 – 17,763)	0,005[v]
p-value	0,383[v]	0,871[v]	0,194[^]	

^) Kruskal-Wallis test
[v]) ANOVA test

However, within-group temporal analyses revealed significant increases in prolotherapy groups. Nanochitosan showed progression from 7.127 ng/ml (day 8) to 10.221 ng/ml (day 14) to 14.419 ± 2.717 ng/ml (day 21) (ANOVA, p = 0.004), while dextrose prolotherapy also showed the same pattern, increasing from 5.400 ng/ml (day 8) to 9.581 ng/ml (day 14) to 13.811 ± 3.792 ng/ml (day 21) (ANOVA, p = 0.005). Control showed non-significant increases (Kruskal-Wallis, p = 0.108) (Table 2).

Significant results in both prolotherapy groups were reconfirmed with post hoc Tukey tests. The test confirming significance in nanochitosan prolotherapy between days 8 and 21 (p = 0.003) (Table 3). On the other hand, dextrose prolotheraphy also showed significant between days 8 and 21 (p = 0.004) (Table 4).

These results suggest a natural increase in type I collagen expression in the control group over time, but a more pronounced and statistically significant increase in groups receiving prolotherapy with nanochitosan or dextrose, indicating their efficacy in enhancing collagen synthesis during ligament healing.

Table 3. Post Hoc Tukey Test on nano chitosan (KN) group

KNi Mean ± SD	KNp Mean ± SD	p-value
7,127 ± 1.259	10,221 ± 2,527	0,159
KNi Mean ± SD	KNr Mean ± SD	Nilai p
7,127 ± 1.259	14,419 ± 2,717	0,003
KNp Mean ± SD	KNr Mean ± SD	Nilai p
10,221 ± 2.527	14,420 ± 2,717	0,051

Table 4. Post Hoc Tukey Test on Dextrose Group (KD)

KDi Mean ± SD	KDp Mean ± SD	p-value
5,400 ± 1,697	9,581 ± 1,650	0,87
KDi Mean ± SD	KDr Mean ± SD	Nilai p
5,400 ± 1,697	13,811 ± 3,792	0,004
KDp Mean ± SD	KDr Mean ± SD	Nilai p
9,581 ± 1,650	13,811 ± 3,792	0,110

DISCUSSION

This study evaluated the effect of prolotherapy using nanochitosan (KN) compared with 12.5% dextrose on type I collagen expression in a rat patellar ligament injury. Type I collagen is the predominant structural protein in ligaments and tendons, constituting approximately 80% of dry weight and providing essential tensile strength for functional healing[16]. Across all observation points (days 8, 14, and 21 after injury), type I collagen expression was assessed to characterize the temporal dynamics of the healing response and to determine the potential of nanochitosan as an alternative prolotherapy agent to dextrose.

At day 8 after ligament injury and prolotherapy administration, there was no statistically significant difference in type I collagen expression among the groups (KK, KN, and KD; p > 0.05). These results align with ligament healing biology where inflammation dominates days 1-7, with collagen synthesis just initiating[17,18,19]. Pro-inflammatory cytokines (TNF-α, IL-1, IL-6) and immune cell infiltration predominate, while fibroblast activation remains suboptimal. Dextrose acts as osmotic irritant triggering chemotaxis; nanochitosan begins anti-inflammatory/fibroblast stimulation but requires time for detectable ELISA changes (24 hours post-injection).

By day 14, all groups (KKp, KNp, KDp) All groups showed physiological collagen increases versus day 8. Nanochitosan exhibited highest mean (10.221 ng/ml) though non-significant (p=0.871). Fibroblasts actively produce matrix, but immature fibrils and natural control increases reduce group contrast[17,19]. Dextrose promotes PDGF/TGF-β release via controlled inflammation when nanochitosan adds antioxidation optimizing microenvironment[10]. Biological variability and sample size likely masked trends[20].

On day 21, Peak expression occurred as expected[21]. Nanochitosan showed highest median (13.677 ng/ml), followed by dextrose (13.470 ng/ml) and control (10.557 ng/ml), but non-significant (p=0.194). Collagen reorganizes into aligned fibrils; nanochitosan's multifaceted effects (antioxidation, angiogenesis, fibroblast proliferation) support superior matrix maturation versus dextrose's inflammation reliance[10,19,22,23]. ELISA limitations (total quantity, not organization) and variability were contributed to non-significance[24].

When temporal changes were analyzed within each prolotherapy group across days 8, 14, and 21, distinct patterns emerged. Control showed gradual non-significant increase (p=0.108), reflecting natural healing peaking ~day 21[17,25]. Nanochitosan demonstrated significant progression (p=0.004; day 8 vs 21 p=0.003), indicating enhanced remodeling[23]. Dextrose similarly increased significantly

(p=0.005; day 8 vs 21 p=0.004) via osmotic shock/cell lysis stimulating collagen transition[10,21].

The significant increase in type I collagen expression from day 8 to day 21 in both nanochitosan (KN) and dextrose (KD) groups highlights their effectiveness in supporting ligament healing mechanistically and functionally. Nanochitosan's bioactive properties stimulate fibroblast proliferation, reduce oxidative stress, and enhance angiogenesis, promoting efficient tissue repair[23,27,28]. In contrast, dextrose induces a local inflammatory response and osmotic shock that elevate growth factors like PDGF and TGF-β, leading to increased collagen synthesis[10,21].

While dextrose prolotherapy acts primarily through inflammation-driven fibroblast activation and matrix remodeling, nanochitosan's biochemical mechanisms involve progressive depolymerization releasing bioactive compounds that modulate inflammatory mediators and promote higher fibroblast activity and survival[26,29,30]. Prior studies also report nanochitosan's role in enhancing fibroblast migration and angiogenesis, supporting the formation and organization of type I collagen matrix[26,29,30]. Despite no significant difference observed between KN and KD at individual time points, both prolotherapys exhibited significant temporal increases, indicating meaningful stimulation and accumulation of collagen type I over time. Clinically, the trend toward higher collagen levels with nanochitosan suggests potential for improved long-term ligament strength and mechanical properties, as collagen type I offers superior tensile strength[22]. Thus, nanochitosan emerges as a promising alternative to dextrose prolotherapy, warranting further studies with larger samples and longer follow-up to confirm its clinical benefits.

The study has limitations including the short observation period (days 8, 14, and 21), which does not cover the full remodeling phase essential for collagen maturation and ligament strength. These limits capturing long-term healing effects and the peak collagen synthesis phase. Body weight was only measured before prolotherapy and not during sample collection, which may affect healing outcomes due to nutritional status changes. The use of ELISA for measuring collagen type I provides quantitative data but lacks qualitative information on collagen structure, organization, and cross-linking, which are important for mechanical strength. ELISA results may also be influenced by antibody cross-reactivity and protein degradation. Future studies should include longer observation times, repeated body weight monitoring, and complementary methods such as immunohistochemistry and biomechanical testing to provide more comprehensive insight into prolotherapy effects.

CONCLUSION

This experimental study demonstrates that prolotherapy with either 0,4% nanochitosan or 12,5% dextrose successfully stimulates type I collagen expression in rat patellar ligament injury healing. There was no difference between prolotherapy agents. Both increased collagen expression between day 8 and 21 after injury, confirming successful activation of the regenerative cascade. Nanochitosan demonstrated numerically superior collagen expression at all measured timepoints and possesses distinctive biological properties (anti-inflammatory, antioxidant, direct fibroblast activation) beyond the osmotic-inflammatory mechanism of dextrose. Although superiority was not definitively demonstrated, these findings support the feasibility and potential efficacy of nanochitosan as a viable alternative prolotherapy agent.

ETHICAL CLEARANCE

The study protocol was approved by the Animal Care and Use Committee (ACUC) of Universitas Airlangga, Surabaya, Indonesia.

CONFLICT OF INTEREST

The authors declare no conflicts of interest concerning this work.

FUNDING

No financial support was received by the authors for the research or publication of this article.

REFERENCE

1. Gill TK, Mittinty MM, March LM, Steinmetz JD, Culbreth GT, Cross M, Kopec JA, Woolf AD, Haile LM, Hagins H, Ong KL. Global, regional, and national burden of other musculoskeletal disorders, 1990–2020, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. The Lancet Rheumatology. 2023 Nov 1;5(11):e670-82. https://www.thelancet.com/pdfs/journals/lanrhe/PIIS2665-9913(23)00232-1.pdf
2. Leong NL, Kator JL, Clemens TL, James A, Enamoto-Iwamoto M, Jiang J. Tendon and ligament healing and current approaches to tendon and ligament regeneration. Journal of Orthopaedic Research®. 2020 Jan;38(1):7-12. doi:10.1002/jor.24475
3. Camarda L, D'Arienzo A, Morello S, Guarneri M, Balistreri F, D'Arienzo M. Bilateral ruptures of the extensor mechanism of the knee: a systematic review. Journal of orthopaedics. 2017 Dec 1;14(4):445-53. doi:10.1016/j.jor.2017.07.008
4. Chamberlain CS, Crowley E, Vanderby R. The spatio-temporal dynamics of ligament healing. Wound repair and regeneration. 2009 Mar;17(2):206-15. https://doi.org/10.1111/j.1524-475x.2009.00465
5. Naomi R, Ridzuan PM, Bahari H. Current insights into collagen type I. Polymers. 2021 Aug 9;13(16):2642. https://doi.org/10.3390/polym13162642
6. Yang SM, Chen WS. Conservative treatment of tendon injuries. American Journal of Physical Medicine & Rehabilitation. 2020 Jun 1;99(6):550-7. doi: 10.1097/phm.0000000000001345
7. Zhao AT, Caballero CJ, Nguyen LT, Vienne HC, Lee C, Kaye AD. A Comprehensive Update of Prolotherapy in the Management of Osteoarthritis of the Knee. Orthopedic Reviews. 2022 May

31;14(3):33921. doi: 10.52965/001c.33921
8. Distel LM, Best TM. Prolotherapy: a clinical review of its role in treating chronic musculoskeletal pain. PM&R. 2011 Jun;3:S78-81. https://www.prolotherapy.com/pdf-general-articles/GA-012-Prolotherapy-a-clinical-review-of-its-role-in-treating-musculoskeletal-pain-(2011).pdf
9. Setiarini, Rohmania. Proloterapi Dextrosa pada Osteoartritis Lutut. Medika Kartika Jurnal Kedokteran dan Kesehatan, 2021 .4(5): 521-530. doi: 10.35990/mk.v4n5.p521-530.
10. Wilson M., Topping A., and Praet A. Dextrose Prolotherapy - Mechanism of Action. Acta Scientific Orthopaedics, 2021. 5(10):149-156. doi: 10.31080/ASOR.2022.05.0585
11. Singh R, Shitiz K, Singh A. Chitin and chitosan: biopolymers for wound management. International wound journal. 2017 Dec;14(6):1276-89. doi: 10.1111/iwj.12797
12. Kim Y, Zharkinbekov Z, Raziyeva K, Tabyldiyeva L, Berikova K, Zhumagul D, Temirkhanova K, Saparov A. Chitosan-based biomaterials for tissue regeneration. Pharmaceutics. 2023 Mar 1;15(3):807. doi: 10.3390/pharmaceutics15030807
13. Kim Y, Zharkinbekov Z, Raziyeva K, Tabyldiyeva L, Berikova K, Zhumagul D, Temirkhanova K, Saparov A. Chitosan-based biomaterials for tissue regeneration. Pharmaceutics. 2023 Mar 1;15(3):807. https://www.mdpi.com/1999-4923/15/3/807#
14. Liu SH, Yang RS, Al-Shaikh R, Lane JM. Collagen in Tendon, Ligament, and Bone Healing: A Current Review. Clinical Orthopaedics and Related Research (1976-2007). 1995 Sep 1;318:265-78. https://journals.lww.com/corr/abstract/1995/09000/collagen_in_tendon,_ligament,_and_bone_healing__a.34.aspx
15. Grana WA, Egle DM, Mahnken R, Goodhart CW. An analysis of autograft fixation after anterior cruciate ligament reconstruction in a rabbit model. The American journal of sports medicine. 1994 May;22(3):344-51. https://journals.sagepub.com/doi/abs/10.1177/036354659402200309
16. Rabago D, Ralston B, Zaharoff A. Prolotherapy: An Evidence-Based Adjunctive Therapy for Knee Osteoarthritis. American family physician. 2021 Apr 1;103(7):395-. https://www.aafp.org/pubs/afp/issues/2021/0401/p395.pdf
17. Riley G. Tendon and ligament biochemistry and pathology. Sports Injuries; Hutson, M., Speed, C., Eds. 2011 Mar 17:3-9. https://www.researchgate.net/profile/Graham-Riley-2/publication/236168496_Tendon_and_ligament_biochemistry_and_pathology/links/02e7e51ee43313059e000000/Tendon-and-ligament-biochemistry-and-pathology.pdf
18. Hauser RA, Lackner JB, Steilen-Matias D, Harris DK. A systematic review of dextrose prolotherapy for chronic musculoskeletal pain. Clinical medicine insights: arthritis and musculoskeletal disorders. 2016 Jan;9:CMAMD-S39160. doi: 10.4137/CMAMD.S39160
19. Chrisdianto A, Airlangga PS, Susilo I, Iskandar RP. Expression of M1 and M2 protein around incision wound area during wound healing process on mice model for diabetes mellitus. Journal of Medicinal and Pharmaceutical Chemistry Research. 2025 Jan 1;7(1):135-49. https://www.sid.ir/fileserver/je/39845-283043-x-1167343.pdf
20. Evi K, Naufal RP. Potensi Biopolimer Kitosan dalam Pengobatan Luka. MEDULA, medicalprofession journal of lampung university. 2019;9(3):459-64. http://repository.lppm.unila.ac.id/20996/1/Jurnal%2027.pdf
21. Reeves KD, Hassanein KM. Long-term effects of dextrose prolotherapy for anterior cruciate ligament laxity. Alternative therapies in health and medicine. 2003 May 1;9(3):58-63. https://oregenmed.com/wp-content/uploads/2014/07/KT1000-ACL-Laxity-Study-2003.pdf
22. Riley G. Tendinopathy—from basic science to treatment. Nature clinical practice Rheumatology. 2008 Feb;4(2):82-9. https://doi.org/10.1038/ncprheum0700
23. Azrianty A, Komariah K, Ranggaini MD, Anggraeni R, Halim J, Nugroho D. Antibacterial Efficacy of $AgNO_3$ Combined with Cymbopogon citratus Extract and Chitosan Nanocomposite Against Pseudomonas aeruginosa. Indonesian Journal of Pharmaceutical Education. 2025 Apr 11;5(2):158-67. https://ejurnal.ung.ac.id/index.php/ijpe/article/download/30641/10530
24. Nofiansyah R, Susila D, Kriswidyatomo P, Wirabuana B. Effectiveness of dextrose prolotherapy injection on chronic knee osteoarthritis patients. Bali Medical Journal. 2024 Mar 22;13(2):558-63.
25. Pérez-Prieto, F. J., Guadilla, J., Roldán, I., Pérez-Hernández, F. M.Intraosseous Prolotherapy: An Alternative Technique for Treatment of Sacroiliac Joint Syndrome. Journal of Pain & Relief, 5(1). 2016.
26. Wang W, Xue C, Mao X. Chitosan: Structural modification, biological activity and application. International Journal of Biological Macromolecules.

2020 Dec 1;164:4532-46. https://doi.org/10.1016/j.ijbiomac.2020.09.042

27. Setiyorini Y, Anggraeni A, Pintowantoro S. In-Vivo study of nano chitosan as therapeutic agent for toxic metal implant. Results in Engineering. 2022 Mar 1;13:100352. https://www.sciencedirect.com/science/article/pii/S2590123022000226
28. Joseph T, Jacob M. Removal of metal ions using Chitosan based electro spun nanofibers: A review. Наносистемы: физика, химия, математика. 2021;12(6):728-48. https://scholar.google.com/scholar?output=instlink&q=info:4AEUyC2J-fEJ:scholar.google.com/&hl=en&as_sdt=0,5&scillfp=10390671621200665766&oi=lle
29. Edition S, Reads T. Prolotherapy can restore joint and tissue function by enabling cells to regenerate and restore normal function without stem cells. https://doi.org/10.52211/asra080122.035
30. Qi L, Xu Z. In vivo antitumor activity of chitosan nanoparticles. Bioorganic & medicinal chemistry letters. 2006 Aug 15;16(16):4243-5., doi: 10.1016/j.bmcl.2006.05.078