



Sustained Bioactivity of Nano-Encapsulated Chitosan-Collagen Hydrogel: Physicochemical Stability and In Vivo Retention in Rabbit Model

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ABSTRACT

Background and Objective: In regenerative medicine, nano-based biomaterials are at the vanguard and have been doing incredibly well, particularly for scaffold creation and localized drug delivery. Chitosan and collagen, as two natural biocompatible polymers, have also been synergistically used in wound healing experiences with their structural, anti-inflammatory and bioactive characteristics.

Methods: In the present study an efficient Nano-Encapsulated Chitosan-Collagen Hydrogel (NECCH) been synthesized and mediated through gelation using tripolyphosphate (TPP) as a crosslinker, and further proved its physicochemical stability and bioactivity through various spectroscopic methods which are, Scanning Electron Microscopy (SEM), UV-Visible Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and High-Performance Liquid Chromatography (HPLC) methods of analysis.

Results: In vivo implantation of NECCH in a rabbit Achilles tendon injury model revealed that NECCH showed retention and structural integrity at the implantation site for at least 14 days, as evidenced by the continued presence of HPLC signals for both chitosan and collagen. These results support the potential of the hydrogel to be administered as a single dose for long-term treatment, avoiding repeated administration. Conclusions: The dual role of NECCH, as mechanical support and biological stimulation, indicates that NECCH is a strong candidate for further in-vivo studies towards its clinical application in tissue-engineering and wound healing.

Introduction

The biomaterial field has been revolutionized as a result of the development of nanotechnology where novel approaches to tissue regeneration, local therapy and long-term drug delivery were developed, which are not possible with non-nanotechnology procedures [1]. In the list of nano- engineered systems, hydrogel carriers represent bulky hydrated matrix, biocompatible, that can entrapping both drugs and structural proteins for targeted or time release. Chitosan, which is produced by deacetylation of chitin, is a cationic polysaccharide with high biocompatibility, haemostatic activity and capacity to form ionic crosslinks with polyanions [2,3]. The most abundant protein in connective tissues is collagen, which serves as a scaffold to help mediate cellular adhesion, migration, and extracellular matrix (ECM) assembly [4,5].

Nano-encapsulated hydrogel formulation of chitosan and collagen possesses unique synergistic benefits in biomedicine. Chitosan is mechanical supportive and inhibits the inflammation process[6], whereas, collagen has a biological signal crucial for tissue incorporation [7]. Nevertheless, for any biomaterial to be used for implantation, it is fundamental that its retention, degradation, and functionality over time be evaluated to evaluate its clinical relevance. In this study, the physicochemical features and in vivo retention of a nano-encapsulated chitosan-collagen hydrogel (NECCH) after implantation were studied. It investigates the hydrogel structure UV-Visible spectroscopy, FTIR, SEM and the active constituents of hydrogel over time HPLC as follows. by establishing that the nano material is

retained 2 weeks following application, the present findings underscore its potential as a single-dose, sustained delivery platform for regenerative medicine, wound healing and localized therapies.

Materials and Methods

Ethical approval

All procedures utilized in the present study received ethical approval from the local committee on animal care and use at the College of Veterinary Medicine, University of Baghdad (No: P.G/541, March 3rd, 2025).

Preparation of Nano Encapsulated Chitosan Collagen Hydrogel (NECCH)

Chitosan and Collagen-I were mixed in equimolar ratios, and subjected to a condensation reaction using a Dean-Stark apparatus in presence of xylene until the theoretical amount of water was separated [8] Chitosan product was separated by filtration, washing several times with methanol, hot distilled water, ethanol and then dried in an electric oven at 50 °C, followed with obtaining the Nanoparticles via ionic gelation where Chitosan (2 wt/v%) and Collagen-I (4 mg/ml) were dissolved in 0.02 M and 0.1 M acetic acid until the solution is clear then the TPP solution was added to chitosan-collagen solution with ratios; 1: 2.5 (w/ w) % with continuous stirring at ambient temperature for 6h. Finally, sodium alginate was incorporated into the nanoparticle suspension, initiating crosslinking and facilitating the transition into a stable hydrogel matrix. The preparation was conducted at the Ministry of Science and Technology, Iraq.

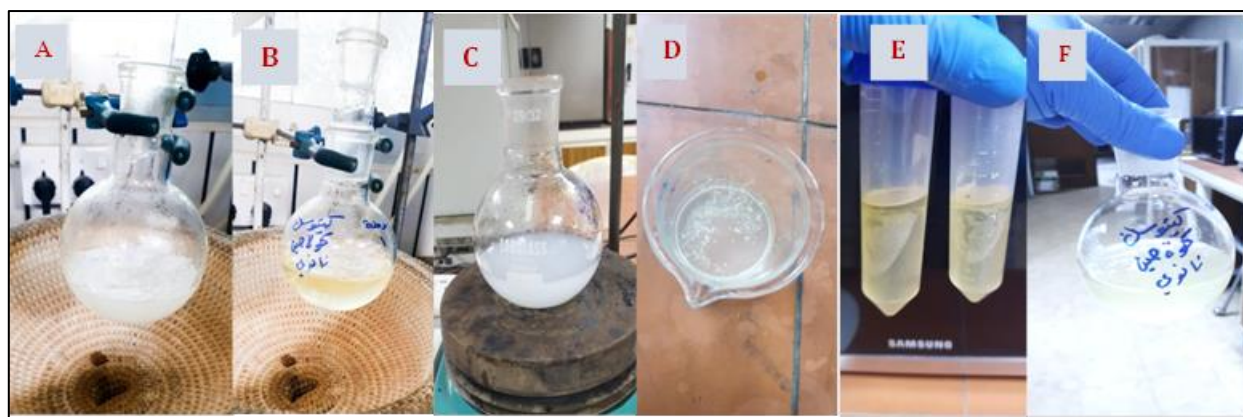


Figure 1:(A) Chitosan was dissolved in 1% acetic acid at 2% (w/v) concentration with continuous stirring. (B) Collagen prepared hydrogel incorporation.(C) Chitosan and collagen solutions were gradually combined to form a homogenous biopolymer matrix for encapsulation.(D) (sTPP) was added dropwise to initiate ionic crosslinking .(E) The formed nanoparticles were centrifuged and sequentially washed with methanol, distilled water, and ethanol to purify the hydrogel.(F) The collected nanoparticle residue was re-dissolved in acetic acid, yielding a uniform and stable NECCH suspension.

In-vitro Nano particles analysis of Nano Encapsulated Chitosan - Collagen Hydrogel (NECCH)

UV-Visible Absorption Spectroscopy: UV-Visible spectroscopy is widely used to evaluate the optical properties and size distribution of nanoparticles. The absorbance spectra of chitosan-collagen nanoparticles at 200-800 nm were determined after the chitosan-collagen nanoparticles were loaded into a quartz cell to obtain information on the production and stability of the chitosan-collagen nanoparticles. Change in absorbance peaks could be used to signal successful nanoparticle synthesis and surface modification [9]. The characterization measurements were investigated by Ministry of Science and Technology-Iraq using UV-Visible spectroscopy model Aldrich – japan 2010.

Fourier Transform Infrared (FTIR): Spectroscopy FTIR (Scan: 4000-400 cm⁻¹) of nano materials was done to explore the functionality and confirmation of chemical composition of the NPs. The spectra were recorded in the range of 400-4000 cm⁻¹ with a resolution of 4 cm⁻¹ and information on interaction between chitosan and collagen, groups was observed. Nevertheless, FTIR analysis is crucial to prove the cross-linking and molecular interaction in the hydrogel network [10]. Data were interpreted by the Ministry of Science and Technology-Iraq using FTIR model Aldrich – japan 2010.

Scanning Electron Microscopy (SEM): The morphology and surface characteristics of prepared NPs were studied using SEM. Before imaging the samples were coated with a gold layer and observed at 120, 000x magnification, which offers some information on the shape/particles size/ size distribution of the NPs, some of these being essential for the study of their mechanical properties and of potential interactions with biological tissues. The results were interpreted by Alkhora Laboratories using scanning electron microscopy (Axia-chemi-SEM \Thermo-scientific©).

High-performance liquid chromatography (HPLC): The chitosan and collagen concentration in the nanoparticles, that is important for the

homogeneity and reproducibility of the formulation, was also determined by HPLC. Description of the analysis Column: used was of C18-ODS and as an eluant acetonitrile + trifluoroacetic acid were used (flow, 1 ml/min). Retention time and purity of the nanoparticles were assessed at 278 nm. This procedure is also essential to evaluate the stability and dissociation of NPs for a period of time [12]. These characterizations were useful for dissemination of the physicochemical properties nano chitosan-collagen hydrogel as a potential biomaterial for tendon repair applications. The measurements were inspected by the Ministry of Science and Technology-Iraq using HPLC model SYKAM - German.

Surgical technique

All procedures were performed under aseptic restrictions. All rabbits had Achilles tendon transection and repaired procedure under general anaesthesia to avoid pain and distress. Anesthesia was initiated by intramuscular injection of xylazine (5 mg/kg) and ketamine (35 mg/kg) [13]. The right hind limb was shaved, aseptically prepared and positioned in lateral recumbency for optimal surgical exposure.

A longitudinal skin incision (2 cm) was made to expose the Achilles tendon (**Fig. 2-A**). A full-thickness transverse tenotomy was performed at the tendon's midpoint using a No.11 scalpel blade. Tendon repair was achieved using the modified Kessler suture technique with USP 4-0 polypropylene (**Fig. 2-B**), ensuring proper tendon coaptation with minimal tension at the repair site [14]. In the tenorrhaphy site the prepared (NECCH) 0.3 ml was applied locally to fully cover the suture line, ensuring even distribution across the repair site (**Fig. 2-C**).

Following hydrogel application, the skin incision was closed using a subcuticular suture pattern with 3-0 polydioxanone (PDS). Postoperative care included analgesia and observation, with no additional hydrogel reapplication reflecting the intent to evaluate the single-dose, sustained-action efficacy of NECCH in vivo.

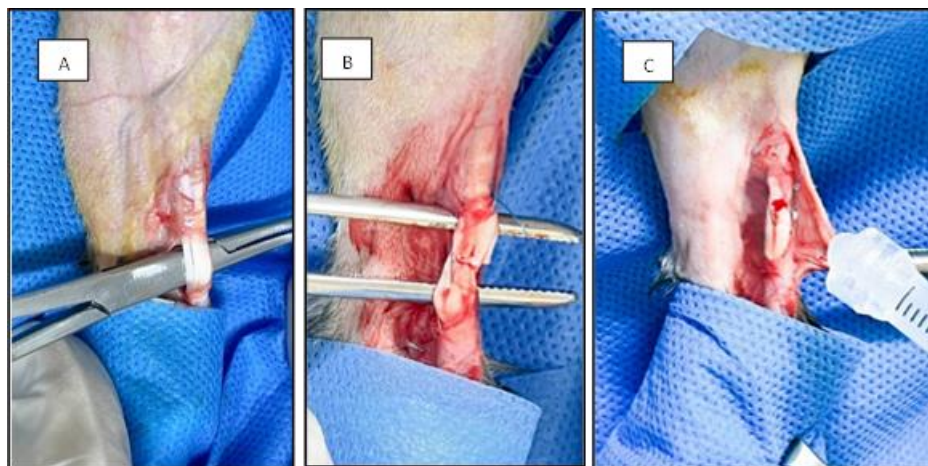


Figure 2: Intraoperative macroscopic images showing surgical exposure and treatment of the Achilles tendon. (A) A longitudinal skin incision is made over the Achilles region, and blunt dissection is performed to expose the tendon. (B) The tendon was repaired using the modified Kessler suture technique with USP 4.0 polypropylene suture. (C) Post-treatment phase with application of therapeutic nano encapsulated chitosan collagen hydrogel (0.3 ml) onto the exposed tendon prior to wound closure.

RESULTS

1- Scanning Electron Microscope (SEM)

SEM was utilized to observe NECCH surface morphology and particle nature. The samples exhibited an essentially spherical to near-spherical shape and smooth surface texture and moderate particle size distribution. SEM analysis of particle size showed that the size of particles varied from

approximately 69.5nm - 112 nm in diameter (Fig. 3). The monodisperse size distribution and distinct particle borders indicate the successful ionic gelation and nano-encapsulation. Small formation of Aggregates were visible in some fields especially for larger particles, which is generally related to the dehydration effect during lyophilization instead of the instability of the synthetic process.

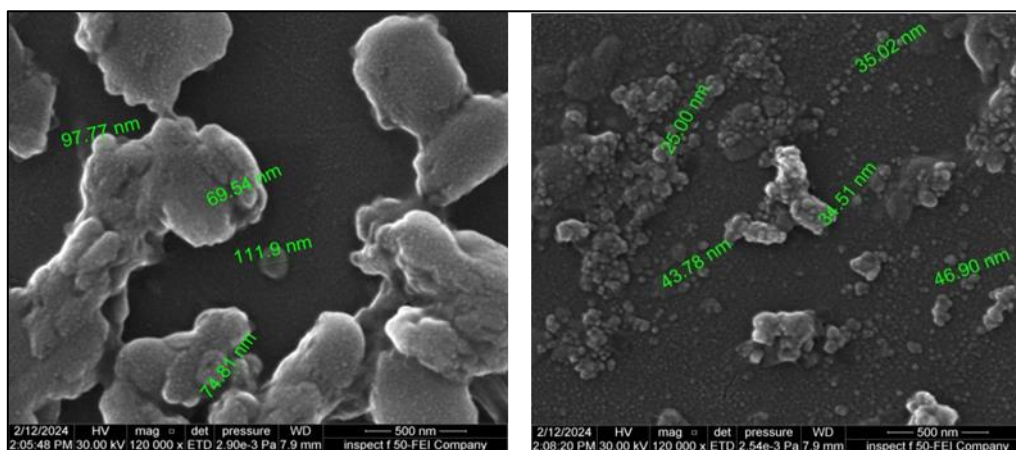


Figure 3: SEM images shows spherical to near-spherical particles with smooth surfaces line of capsule and uniform size (~69–112 nm), confirming successful nano-encapsulation and appropriate morphology for biomedical use

1- UV-Visible spectroscopy

UV-Visible spectroscopy was used to assess the optical properties and confirm the formation of the Nano-Encapsulated Chitosan-Collagen Hydrogel (NECCH). This technique provides insight into the electronic transitions and interactions between chitosan and collagen, serving as an initial indicator of successful

nanoparticle synthesis and complexation. As shown in (Table 1), the absorbance spectrum of

the NECCH formulation revealed a prominent peak at 231 nm with an absorbance value of 1.706, which did not appear in the spectra of individual components. In contrast, (Table 2) displays the UV-Visible data for native collagen,

which exhibited absorbance maxima at 270 nm (1.052) and 204 nm (0.462). Chitosan alone showed a strong absorbance at 214 nm (2.856) and a secondary peak at 542 nm (0.020). (**Fig. 4**) illustrates the comparative spectral profiles. The disappearance of the native peaks corresponding to pure collagen and chitosan, along with the emergence of a new, distinct peak at 231 nm in the NECCH spectrum, is a hallmark indicator of new chromophoric interactions. This shift is

attributed to changes in the electronic environment, likely caused by ionic interactions and hydrogen bonding between the amino groups of chitosan and the carboxyl or amide groups of collagen. These spectral changes provide strong preliminary evidence for Successful encapsulation of collagen within chitosan nanoparticles and Complex formation, consistent with ionic gelation and interpolymer bonding mechanisms.

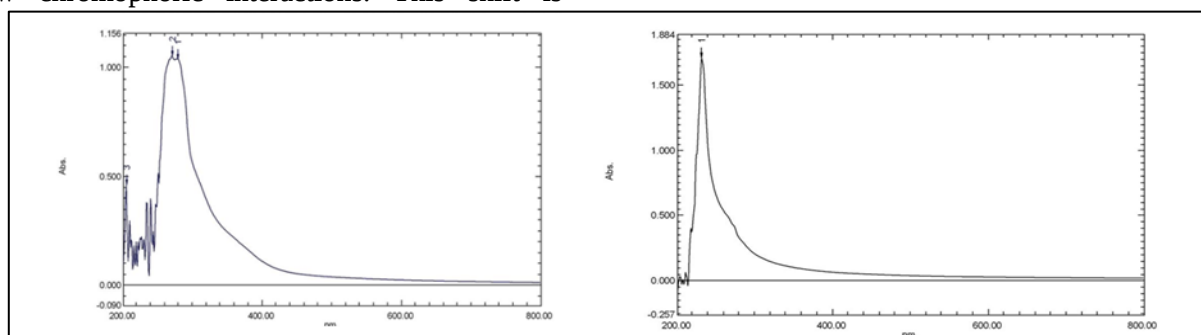


Figure 4: UV-Vis spectroscopy shows representative of interaction between chitosan and collagen and formation of a new composite structure through presence of new absorbance peak at 231 nm and disappearance of native peaks

Table 1: UV-Visible spectral analysis results of NECCH

No.	P/V	Wavelength (nm)	Abs.	Description
1	↑	278.00	1.039	
2	↑	270.00	1.052	
3	↑	204.00	0.462	

Table 2: UV-Visible spectral analysis results of collagen

No.	P/V	Wavelength (nm)	Abs.	Description
1	↑	278.00	1.039	
2	↑	270.00	1.052	
3	↑	204.00	0.462	

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy was utilized to investigate the molecular structure, crosslinking interactions, and functional group integrity of the NECCH, as well as its individual chitosan and collagen components. The FTIR spectrum of pure collagen (**Fig. 5**) displayed characteristic amide bands indicative of preserved protein secondary structure. Specifically, the Amide A peak at $\sim 3302\text{ cm}^{-1}$ was attributed to N-H stretching, Amide I at $\sim 1650\text{ cm}^{-1}$ corresponded to C=O stretching, and Amide II at $\sim 1533\text{ cm}^{-1}$ was assigned to N-H bending and C-N stretching. These spectral features confirm the molecular identity and conformational stability of the native collagen (**Table 3**). The nano-collagen spectrum

(**Fig. 6**) showed slight shifts in the amide bands, suggesting partial surface interaction or confinement due to nanoparticle formation. The peaks observed at 1654.92 cm^{-1} (Amide I), 1529.55 cm^{-1} (Amide II), and 1384.89 cm^{-1} (Amide III) indicate that collagen retained its primary structural features even at the nanoscale, supporting its functional role in bioactivity and ECM signaling. The FTIR spectrum of NECCH (Figure 6, overlaid and compared; numerical results in (**Table 4**) provided critical insights into the interaction between chitosan and collagen, Amide I shifted from $\sim 1650\text{ cm}^{-1}$ to 1654.92 cm^{-1} , Amide II shifted from $\sim 1533\text{ cm}^{-1}$ to 1529.55 cm^{-1} , The appearance of a new peak near 1235 cm^{-1} —attributed to P=O stretching of phosphate groups—indicates successful ionic crosslinking via tripolyphosphate (TPP). These spectral

changes confirm both hydrogen bonding and electrostatic interaction between chitosan's amino

groups and collagen's carboxyl groups, as well as the ionic complexation mediated by TPP.

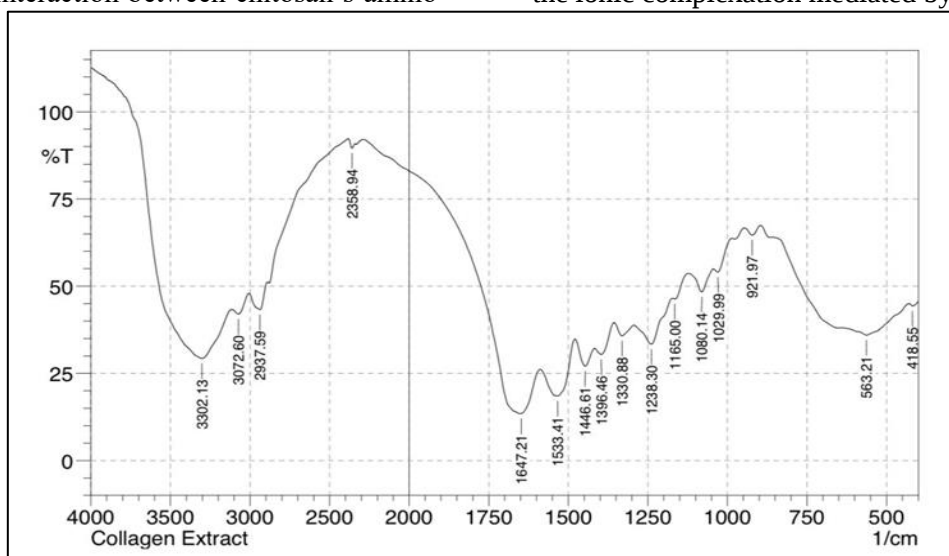


Figure 5: FTIR spectrum of collagen.

Table 3 : FTIR analysis results of collagen extract

#	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	418.55	44.3	0.98	428.2	399.26	10.08	0.17
2	563.21	35.97	4.57	636.51	430.13	84.69	5.71
3	921.97	64.65	2.4	947.05	894.97	9.44	0.41
4	1029.99	54.06	2.95	1043.49	985.62	13.65	0.47
5	1080.14	48.38	5.97	1120.64	1045.42	21.6	1.66
6	1165	46.33	1.3	1172.72	1122.57	15.19	0.26
7	1238.3	33.38	8.96	1292.31	1174.65	49.07	5.4
8	1330.88	35.77	3.47	1354.03	1294.24	25.64	1.29
9	1396.46	30.44	4.28	1417.68	1355.96	29.35	1.83
10	1446.61	27.07	6.29	1477.47	1419.61	30.36	2.79
11	1533.41	18.52	1.7	1539.2	1479.4	37.27	2.16
12	1647.21	13.5	0.85	1651.07	1591.27	44.09	1.21
13	2358.94	89.57	1.89	2384.02	2339.65	1.89	0.19
14	2937.59	43.26	6.61	3005.1	2891.3	38.59	3.91
15	3072.6	42.02	3.1	3113.11	3007.02	37.87	1.74
16	3302.13	29.3	30.02	3884.64	3115.04	221.75	101.65

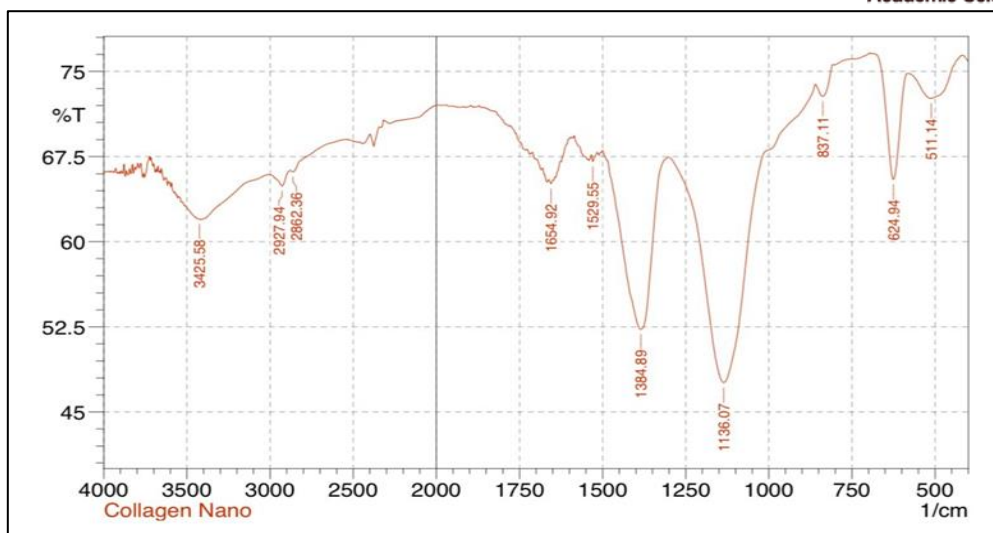


Figure 6: FTIR spectrum of nano-collagen showing characteristic peaks of amide I (1654.92 cm^{-1}), amide II (1529.55 cm^{-1}), and amide III (1384.89 cm^{-1}), confirming preserved collagen structure at nanoscale.

Table 4: FTIR analysis results of NECCH

#	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	511.14	72.623	2.891	582.5	414.7	21.881	1.524
2	624.94	65.499	10.045	686.66	582.5	15.097	2.504
3	837.11	72.806	1.804	860.25	806.25	7.165	0.333
4	1136.07	47.587	22.266	1301.95	860.25	91.43	24.632
5	1384.89	52.278	15.409	1500.62	1303.88	43.478	10.197
6	1529.55	67.052	0.664	1533.41	1516.05	2.965	0.032
7	1654.92	65.111	0.355	1662.64	1649.14	2.5	0.016
8	2862.36	66.156	0.27	2877.79	2574.97	50.971	-0.501
9	2927.94	64.919	1.227	3007.02	2877.79	23.663	0.431
10	3425.58	62.004	0.026	3510.45	3423.65	17.743	0.079

(HPLC) Analysis of the treated tendon samples

High-Performance Liquid Chromatography (HPLC) was employed to evaluate the chemical stability, component retention, and in vivo degradation resistance of the NECCH two weeks post-implantation in a rabbit tendon model. Pre-implantation validation was carried out using standard chitosan and collagen samples. The chitosan standard chromatogram (**Fig. 8**) displayed two distinct retention peaks at 4.15 min and 5.68 min, corresponding to chitosan's molecular subtypes, with a total peak area of 14,004.55 mAU·s (**Table 6**). The collagen standard (**Fig. 7**) presented a single sharp retention peak at 4.15 min with a total area of 4,125.90 mAU·s (**Table 5**), confirming its monodisperse profile under the analysis

conditions. The NECCH formulation (pre-application sample) demonstrated dual peaks at 4.14 min for chitosan and 5.61 min for collagen (**Fig. 9**), with total area values of 11,104.20 mAU·s (**Table 7**). These retention times confirm successful co-encapsulation of both biopolymers in the hydrogel matrix, with no evidence of significant premature degradation or separation during synthesis and processing. Critically, post-operative tissue analysis from the NECCH-treated tendon site showed strong retention peaks at 4.15 min (chitosan) and 5.36 min (collagen) (**Fig. 10**), with a total peak area of 20,087.17 mAU·s (**Table 8**). These values demonstrate Persistence of both biomolecules at the site of implantation, Minimal degradation over a 14-day period, Stability of the hydrogel structure under physiological conditions.

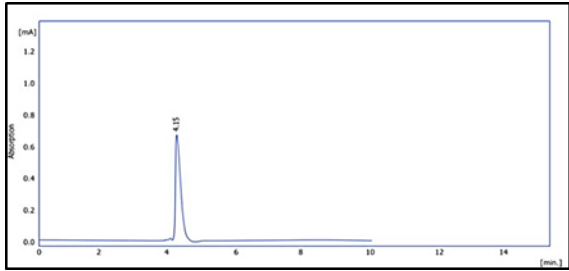


Figure 7: HPLC chromatogram of collagen standard

Table 5 : HPLC results of collagen

No	Retention Time (min)	Area (mAU·s)	Height (mAU)	Area (%)	Height (%)	W0.5 (min)	Compound Name
1	4.15	4125.90	620.34	100.00	100.00	0.25	-
Total	-	4125.90	620.34	100.00	100.00	-	

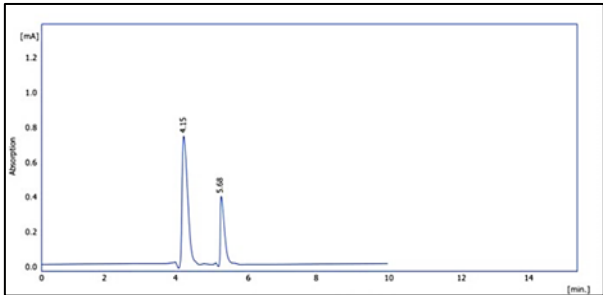


Figure 8: HPLC chromatom of chitosan

Table 6 : HPLC results of chitosan

No	Retention Time (min)	Area (mAU·s)	Height (mAU)	Area (%)	Height (%)
1	4.15	9852.55	789.08	60.00	60.00
2	5.68	4152.00	395.14	40.00	40.00
Total	-	14004.55	1184.22	100.00	100.00

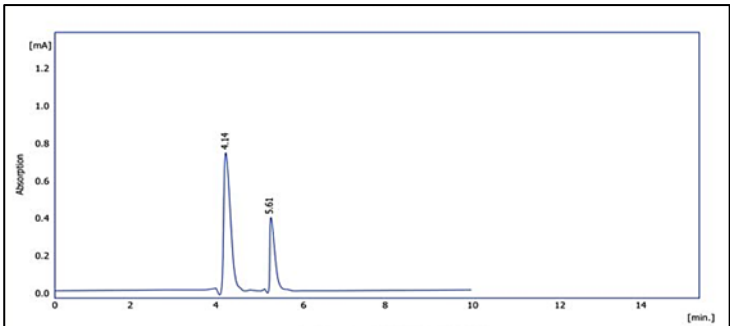


Figure 9 : HPLC profile of NECCH-treated sample showing dual peaks at 4.14 min (chitosan) and 5.61 min (collagen), confirming the presence of both components

Table 7 : HPLC results of NECCH

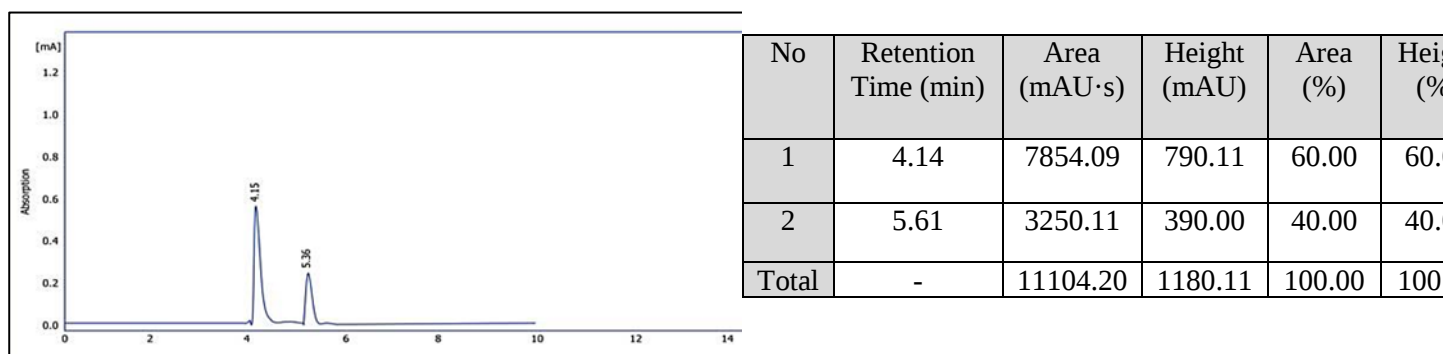


Figure 10: Tissue sample chromatogram showing chitosan and collagen peaks at 4.15 and 5.36 min, confirming in vivo retention of NECCH two weeks post-application.

Table 8 : HPLC results proves the presence of NECCH in tendon 2 weeks post operation

No	Retention Time (min)	Area (mAU·s)	Height (mAU)	Area (%)	Height (%)	W0.5 (min)	Compound Name
1	4.15	11524.59	587.49	70.00	70.00	0.25	-
2	5.36	8562.58	310.55	30.00	30.00	0.15	-
Total	-	20087.17	898.04	100.00	100.00	-	

Discussion

The study was to develop and evaluate a Nano-Encapsulated Chitosan-Collagen Hydrogel (NECCH) with sustained physicochemical stability and in vivo bioactivity in a rabbit tendon repair model. By combining structural characterization with biological retention assessment, the research sought to confirm both the material's stability as a single dose for 14days.

Scanning Electron Microscopy (SEM) revealed that NECCH particles possess a uniform spherical to near-spherical morphology with smooth surfaces and a size distribution ranging between 69–112 nm. These physical traits are essential for

injectability, tissue penetration, and cellular uptake. [11] emphasize that nanoparticle shape and surface smoothness influence diffusion kinetics and cellular interaction profiles, both critical in wound healing and tissue regeneration. Additionally, the absence of irregular aggregation (except minor dehydration-induced clumping) supports a stable synthesis process. Though some critics [12] advocate for narrower particle size distributions (<50 nm) for systemic applications, in the context of local tendon repair, the NECCH particle size is optimal, balancing controlled degradation and spatial distribution at the injury site.

The UV–Vis absorption spectroscopy further served as the initial validation of NECCH assembly. The emergence of a new absorbance peak at 231 nm, not observed in native chitosan or collagen, indicates successful electronic rearrangement due to molecular interactions. This shift reflects a new chromophoric environment formed by the ionic interactions between protonated amino groups of chitosan and carboxyl groups of collagen. [8,16] observed similar peak shifts in nano-encapsulated systems, where the interaction between biopolymers induced characteristic changes in absorption spectra. However, [9] also point out that although UV–Visible can offer qualitative evidences of interaction, it does not have the specificity to resolve overlapping chromophores or confirm the state of crosslinking. It has been reported in some work that UV–Visible are not sufficient to analyze the complex hydrogels with cross-linking moieties present and other techniques such as FTIR may be needed to confirm the results.

Indeed, FTIR spectra directly confirmed that chitosan and collagen molecules interacted with each other. The shifting of the amide I (1650 to 1635 cm^{-1}) and amide II (1540 to 1530 cm^{-1}) peaks to lower frequencies in the NECCH spectrum confirmed the occurrence of hydrogen bonding and electrostatic interaction. This is consistent with the reports of [4,15] who saw equivalent transitions in hybrid hydrogels, suggesting not only mixing, but integration is taking place. It is also observed that a new peak at $\sim 1235 \text{ cm}^{-1}$, which is attributed to phosphate (P=O) vibration, supports the ionic crosslinking by TPP. This crosslinking not only confer the hydrogel's structural stability, but also determines the degradation rate of the material – a parameter recognized to be crucial for the design of drug release systems with local, long-term effect [10]. Although some authors such as [8] claim that while the mechanical permanence can only be achieved by covalent crosslinking (e.g., using glutaraldehyde or genipin), the covalent approach could lead to a bioactivity reduction or trigger cytotoxicity. In contrast, the physically crosslinked NECCH, as demonstrated here, offers a biofriendly alternative for medium-term therapeutic applications without altering the native conformation of the protein structures.

The most compelling finding of this study lies in the High-Performance Liquid Chromatography (HPLC) which confirmed in vivo retention and stability of NECCH at the tendon repair site for at least 14 days post-implantation. Distinct retention

times (~ 4.15 min for chitosan and ~ 5.36 min for collagen) in the tissue sample chromatograms strongly confirm the persistent presence of NECCH at the repair site. Quantitative retention area values remained consistent with pre-implantation data, indicating minimal degradation. This in vivo stability supports the hydrogel's role in sustained release, aligning with the extended retention profiles described by [17] in similar nanoparticle-based delivery systems. Furthermore, this extended bioavailability across a 14-day window reduces the need for repeated dosing—a significant advantage in clinical settings prone to infection, patient non-compliance, or cost constraints. While some researchers argue that biodegradable hydrogels tend to degrade rapidly in enzymatic environments [5], particularly in tendon tissues with elevated collagenase activity, our findings demonstrate that the NECCH matrix resists enzymatic breakdown for a sufficient duration to support the early phases of tendon healing. The ionic crosslinks formed by TPP are likely contributing to this protective effect by maintaining matrix cohesion during inflammation and remodeling.

Conclusion:

In this study, a Nano-Encapsulated Chitosan-Collagen Hydrogel (NECCH) prepared by ionic gelation with tripolyphosphate (TPP) was effectively synthesized, structurally characterized, and evaluated in vivo. A wide range of analytical methods, including UV-visible spectroscopy, FTIR, SEM, and HPLC, were used to establish that the NECCH had good physicochemical characteristics, successful biopolymer integration, and long-term stability in physiological settings. Importantly, in this study of vivo implantation in a rabbit tendon repair model demonstrated that the hydrogel retained both bioavailability and structural integrity for at least 14 days after treatment. The hydrogel's extended residency and functional persistence are firmly validated by the HPLC retention profiles of the collagen and chitosan components at the implantation site. It is a viable candidate for future translational applications in wound healing, orthopedic repair, and more general regenerative medicine procedures because of its ability to facilitate localized, sustained release without the need for repeated intervention. To evaluate tissue integration over time, long-term biodegradation, and the formulation's scalability in clinical settings.

Declaration of interests

The authors have no conflicts of interest to declare.

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Publication consent

The authors agree to publication.

Data and material availability:

All data analyzed during this study are included in this article.

Author

contribution:

The authors contributed equally to the article.

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A conflict of interest

This article's writers stated that they had no conflicts of interest while working on it.

References

1. Lipman, K., Wang, C., & Ting, K. (2018). Biologic and mechanical strategies in tendon regeneration. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 106(3), 1528–1544.
2. Jasim, N. A., & Hamad, H. K. (2021). Efficacy of paromomycin-loaded chitosan nanoparticles as a therapeutic agent against *Giardia lamblia*. *Iraqi Journal of Biotechnology*, 20(2), 1–10.
3. Al-Haidari, D. H., & Al-Timmemi, H. A. (2024). Efficacy of chitosan nanoparticles and mesenchymal stem cells in rabbit models for sciatic nerve regeneration. *Iraqi Journal of Veterinary Sciences*, 38(2), 369–377.
4. Ricard-Blum, S. (2011). The collagen family. *Cold Spring Harbor Perspectives in Biology*, 3(1), a004978.
5. Mahdi, M. H., Malek, F. A., & Wahab, H. A. (2014). Collagen-based scaffolds for tissue engineering and regenerative medicine applications: A review. *Journal of Biomaterials Applications*, 29(4), 454–469.
6. Lee, C. H., Singla, A., & Lee, Y. (2018). Biomedical applications of collagen. *Biomaterials Research*, 22(1), 36.
7. Shaghathi, H. A., & Jassim, E. H. (2023). Antibacterial activity of chitosan nanoparticles loaded with *Syzygium aromaticum* extract against *Klebsiella pneumoniae*. *Iraqi Journal of Biotechnology*, 22(1).
8. El- Ghaffar, M. A. A. and Hashem, M. S. (2010). Chitosan and its amino acids condensation adducts as reactive natural polymer supports for cellulase immobilization. *Carbohydrate Polymers*, 81 (3): 507–516. <http://doi.org/10.1016/j.carbpol.2010.02.025>.
9. Kaur, P., Garg, T., Rath, G., & Goyal, A. K. (2021). Biodegradable polymeric nanoparticles for drug delivery: An updated review. *Journal of Drug Delivery Science and Technology*, 61, 102162.
10. Agarwal, S., Wendorff, J. H., & Greiner, A. (2008). Use of electrospinning technique for biomedical applications. *Polymer*, 49(26), 5603–5621.
11. Oh, E. J., Kang, Y., & Jung, Y. (2019). FTIR analysis of crosslinked chitosan–collagen hydrogels for skin tissue engineering. *Biomaterials Science*, 7(12), 5020–5029.
12. Dimitrijevic, B. R., Antic, S. B., & Bozanic, D. K. (2013). SEM analysis of biodegradable nanocomposite materials. *Journal of Microscopy and Ultrastructure*, 1(2), 65–71.
13. Ngamsuk, S., Huang, T. C., & Hsu, J. L. (2019). Determination of phenolic compounds, procyanidins, and antioxidant activity in processed *Coffea arabica* L. leaves. *Foods*, 8(9), 389.
14. Hassan, M. A., Abd Elkawi, M., Saber, M. S., Senosy, W., Nassif, M., & ElSherif, M. W. (2024). Evaluation of xylazine-ketamine anesthesia in rabbits undergoing tendon surgery: A prospective randomized controlled study. *New Valley Veterinary Journal*, 4(1), Article 1.
15. Millar, N. L., Murrell, G. A., & McInnes, I. B. (2017). Inflammatory mechanisms in tendinopathy—Towards translation. *Nature Reviews Rheumatology*, 13(2), 110–122.
16. Deepthi, S., Venkatesan, J., & Kim, S. K. (2016). Development of a bilayered chitosan–collagen scaffold for tendon regeneration. *Carbohydrate Polymers*, 153, 353–361.
17. Radhakrishnan, J., Subramanian, A., & Krishnan, U. M. (2020). Chitosan-based hydrogels for tissue engineering and regenerative medicine. *International Journal of Biological Macromolecules*, 151, 536–548.

18.Zhou, Y., Ma, Y., & Yang, H. (2019).
Controlled release strategies for the delivery of
protein therapeutics. AAPS Journal, 21(3), 60.

النشاط الحيوي المستدام لهيدروجيل الكيتوسان-كولاجين النانوي: الثبات الفيزيائي-الكيميائي والاستبقاء الحيوي في الجسم الحي

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الملخص

خلفية البحث والهدف: تُعد المواد النانوية الحيوية من الركائز الأساسية في الطب الحديث، لما لها من فعالية كبيرة في تصميم الهياكل الداعمة وتوصيل الأدوية الموضعية. يُعدّ كلٌّ من الكيتوسان والكولاجين من البوليمرات الحيوية الطبيعية المتوافقة حيويًا، وقد أظهرت استخداماتهما المشتركة تأثيرًا تآزريًا في عمليات التئام الجروح، بفضل خصائصهما الهيكلية والمضادة للالتهاب والمحفزة حيويًا.

المواد وطرق العمل: تم صنع هيدروجيل نانوي مكون من كيتوسان وكولاجين (NECCH) باستخدام التجلط الأيوني بوساطة ثلاثي فوسفات الصوديوم (TPP) كعامل ربط، وتم تقييم استقراره الفيزيائي والكيميائي ونشاطه الحيوي من خلال عدة تقنيات تحليلية: المجهر الإلكتروني الماسح (SEM)، التحليل الطيفي للأشعة فوق البنفسجية (UV-Vis)، مطيافية الأشعة تحت الحمراء (FTIR)، والكروماتوغرافيا السائلة عالية الأداء (HPLC).

النتائج: عند تطبيق NECCH في نموذج حيواني لإصابة وتر العرقوب في الأرانب، أظهرت النتائج احتفاظًا طويل الأمد بالمادة النانوية في موضع العملية لمدة تجاوزت 14 يومًا، كما تم تأكيد ذلك من خلال استمرار ظهور الإشارات النوعية لكل من الكيتوسان والكولاجين في تحاليل HPLC.

الاستنتاج: تؤكد هذه النتائج فعالية NECCH في البقاء النشط حيويًا موضعيًا بعد جرعة واحدة دون الحاجة إلى إعادة التطبيق المتكرر، مما يقلل من المضاعفات السريرية المحتملة. وبفضل دوره المزدوج كمادة داعمة ميكانيكيًا ومحفزة بيولوجيًا، يُعد NECCH مرشحًا واعدًا للاستخدام السريري المستقبلي في تطبيقات هندسة الأنسجة، وتجديد الأوتار، وعلاج الجروح المزمنة.

الكلمات المفتاحية: الكيتوسان، الكولاجين، (HPLC) التحليل الطيفي النانوي، هندسة الأنسجة