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Research Article

Evaluation of bioactive gel and spray containing Shilajit-synthesised silver nanoparticles, fish collagen, and L-lysine for accelerated healing of burn and diabetic wounds in rabbit models

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Abstract

Wounds associated with burns and diabetes pose significant clinical challenges due to persistent inflammation, disrupted angiogenesis, increased susceptibility to infection, and abnormal extracellular matrix (ECM) remodelling. Hence, there is a critical need for multifunctional wound dressings that incorporate both antimicrobial and regenerative benefits. Green-synthesised silver nanoparticles (AgNPs), fish collagen, and L-lysine independently promotes wound repair; however, their combined effectiveness and the impact of delivery format remain poorly understood. Here, we formulated a gel and a spray incorporating Shilajit-synthesised AgNPs, fish collagen, and L-lysine, and assessed their cytotoxicity, hemocompatibility, physicochemical behaviour, and antimicrobial effectiveness toward *Escherichia coli* and *Staphylococcus aureus*. Both formulations were non-cytotoxic to mouse fibroblast cell lines (L929), as demonstrated by Fluorescein Diacetate (FDA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays, and exhibited outstanding hemocompatibility with insignificant hemolysis. Rheological analysis confirmed the elastic behaviour of the gel (G2>G3), supporting its structural stability. Shilajit-synthesised AgNPs showed strong antibacterial action at a concentration of 50 µg/mL. *In vivo* studies showed significant improvement in wound contraction in AgNPs–Fcol–Lys–treated groups, particularly the spray, relative to control and standard treatments. Histological, immunohistochemical, and biochemical assessment of tissue samples further confirmed improved angiogenesis, increased hydroxyproline content, and dense, well-aligned collagen and elastin fibre deposition, demonstrating enhanced ECM remodeling and epithelial regeneration in both burn and diabetic wounds. Collectively, AgNPs–Fcol–Lys formulations, particularly in spray form, represent a safe and effective multifunctional therapeutic option for accelerating burn and diabetic wound healing by leveraging combined antimicrobial and regenerative effects.

Keywords: Burn, Diabetic, Fish collagen**,** Rabbit model, Silver nanoparticles

Highlights

- AgNPs–Fcol–Lys spray and gel enhanced burn and diabetic wound remodelling via synergetic action.
- Spray formulation outperformed the gel due to uniform coverage, moisture retention, and improved gaseous exchange.
- Synergetic action of green-synthesised AgNPs, fish collagen, and L-lysine promoted tissue remodeling and reduced inflammation.

INTRODUCTION

In modern wound care therapy, diabetic and burn wounds impose a major challenge in the healthcare system. High risk of infection, impaired wound healing, prolonged inflammatory phase, and minimal extracellular matrix depositionare significant hurdles associated with this malady. Thermal injuries cause a wide range of tissue necrosis, accompanied by microbial colonisation and oxidative stress in a large number of populations (Chen et al., 2025). Hyperglycaemic conditions resulting from diabetes inhibit fibroblast proliferation, collagen synthesis, and angiogenesis, resulting in delayed wound closure (Kunkemoeller & Kyriakides, 2017).

Nanoparticles, particularly silver nanoparticles, have been used in wound repair for a decade owing to their potent antimicrobial ability, oxidative stress and destruction of biofilm in a large number of bacterial species (Kaya et al., 2025a). Dressing can be prepared from the integration of AgNPs into hydrogels, nanofiber mats, and composite materials to develop a localized delivery system to prevent bacterial activity and promote tissue regeneration by controlling dose and concentration, while preserving cytocompatibility (Kolarijani et al., 2025a). Furthermore, green synthesis of AgNPs using plants, bioorganic waste, bacteria, and fungi increases their functional activity due to the addition of numerous functional groups in the synthesis procedure (Gwada et al., 2025).

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Collagen derived from marine sources, particularly fish skin, has gained significant attention in recent times as a bio-friendly wound scaffold in tissue regeneration. It plays a crucial role in wound healing by encouraging fibroblast proliferation, extracellular matrix deposition, and neovascularization with minimal scarring (Zhang et al., 2022). Fish skin rich in type I collagen is found to be similar to mammalian collagen with low immunogenicity and excellent biodegradability. The role of L-lysine (an essential amino acid) in tissue remodelling cannot be overlooked, as it plays a critical role in cross-linking and collagen formation, leading to the construction of stable tissue architecture (Yamauchi & Sricholpech, 2012).

To acquire a multidimensional platform for healing in burn and diabetic wounds, integration of AgNPs with fish collagen and L-lysine can be assessed to obtain a synergistic action to combat infection, encourage wound healing, granulation tissue formation, and remodelling. Previous studies reported the sole application of AgNPs, fish collagen, and L- lysine based formulation in wound healing. The current study accentuates the synergistic potential of Shilajit-synthesised silver nanoparticles, Bhetki fish (*Lates calcarifer*) collagen, and L-lysine in gel and spray form for effective wound remodelling. This maiden work shall outline the synergistic action of these components in wound healing applications. The efficacy of AgNPs–Fcol–Lys formulations will be evaluated through in vitro studies and *in vivo* trials in rabbit burn and diabetic wound models, alongside a detailed analysis of extracellular matrix components to explore their core biological mechanism in tissue regeneration, structural reorganization, and neovascularization

MATERIALS AND METHODS

Development of gel and spray incorporating Shilajit-synthesised silver nanoparticles, fish collagen, and L-lysine: Biological synthesis of silver nanoparticles was carried out by using Shilajit extract from Shilajit resin by a reduction process of $AgNO_3$ solutions (Ahmed et al., 2016). The skin of Bhetki fish (*Lates calcarifer*) was acquired from the local fish market, and collagen was extracted as per the standard technique (Liu et al., 2015). A 0.1% (w/v) L-lysine solution was also used for the formulation. Based on the MTT assay results (Van Meerloo et al., 2011), AgNPs, fish collagen, and L-lysine were designed into a Carbopol-based gel and a glycerol-based spray as described in standard pharmaceutical procedures (Rowe et al., 2006).

Cell culture and cell viability assay: The L929 cell line (Mouse fibroblast) was used for an in vitro cytotoxicity assay. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay measured the percentage cell viability after 1st, 3rd, and 7th day of treatment. Cell morphology was studied using the fluoresce in diacetate (FDA) assay and correlated with the MTT assay data.

Hemocompatibility study of Shilajit- synthesised AgNPs, fish collagen, and L-lysine-based gel and spray: The hemocompatibility of the fabricated composites was assessed using a standard hemolysis assay with minor modifications (Naskar et al., 2017). The percentage of haemolysis was assessed using the equation below, based on three independent replicates.

Percentage of hemolysis= $(A_{sample} - A_{sample\,control}) \times 100 / (A_{positive}\ control - A_{negative\ control})$.

where A denotes the absorbance recorded at 545 nm

Antimicrobial assay of Shilajit-synthesised AgNPs: The antimicrobial activity of the Shilajit-synthesised silver nanoparticles (AgNPs) was carried out against *Escherichia coli* (strain ATCC 25922) and *Staphylococcus aureus* (strain ATCC 25923) by means of the agar well diffusion technique.

Oscillatory Rheology of gel: This study was conducted using the AR-G2 Magnic Bearing Rheometer (TA Instruments Ltd). Amplitude sweep test determines the linear-viscoelastic (LVE) region of the material by recording the measurements corresponding to 0.1% to 100% strain at a constant angular frequency of 10 rad/s. Shear stress is incremented throughout the experiment until the sample structure yields. Subsequently, all the measurements relating to the frequency sweep test (oscillation frequency varied from 0.1 to 100 rad/s) were performed under direct strain control at 10 mrad in a 40-mm-diameter, 2° cone-and-plate measuring system, and a gap of 1000 μm (between the Peltier plate and the probe) was maintained.

Animal model and *in vivo* experimental design

Full-thickness burn and diabetic wounds were created in 24 rabbits of either sex, twelve in each experiment, under anaesthesia with ketamine hydrochloride (33 mg/kg body weight) and xylazine hydrochloride (5 mg/kg), and the anaesthesia was administered via the intramuscular route (Oguntoye & Oke, 2014). An area was prepared aseptically lateral to the dorsal mid-thoracic-lumbar

junction in either side. Five wounds were created in each animal, corresponding to each experimental group: (1) control, (2) STD. Gel, (3) STD. spray, (4) AgNPs-Fcol-Lys (Gel), and (5) AgNPs-Fcol-Lys (Spray). Topicure gel (Natural Remedies) and Topicure spray (Natural Remedies) were used as standard gel and spray, respectively. Burn wounds were induced in which a heated metal rod was pressed gently over it for 20–30 s, to achieve burn injury at a temperature of about 80°C (Bečić et al., 2003) to create full-thickness burn wounds. Diabetes was induced by the intraperitoneal (I/P) infusion of Alloxan monohydrate (Sigma Aldrich) @150 mg/kg by dissolving in normal saline (Kim, 2024). To avoid acute hypoglycemia, the rabbits were then given oral glucose water (12 g/L) for 48 hours. Glucometer reading was taken after 48 hours of induction, and a blood glucose value exceeding 250 mg/dL was measured as diabetic (Ibrahim et al., 2023). In the diabetic animals, the wounds were produced by using a 15 mm biopsy punch. Injection Meloxicam (0.3 mg/kg, Melambic, Alembic, India) was given post operatively once daily for three consecutive days as an analgesic in both treatment groups. During the study period, the animals were housed individually and closely monitored. No abnormalities were detected during the study period. All the experimental protocols were undertaken in compliance with the guidelines provided by the Institutional Animal Ethics Committee of the West Bengal University of Animal and Fishery Sciences (Approval No. 763/GO/ReS/ ReRc-L/03/CCSEA/68/68/2023-2024).

Quantification of wound contraction rate: The rate of wound contraction was assessed at different time points by using the Imito-measure software according to the standard procedure across all five wounds in both the treatment groups, at 0, 3rd, 7th, 10th, 14th, and 21st days of healing.

Histopathological examination of the extracellular matrix: The tissue samples were collected on days 7, 14, and 21 post-operatively for histopathological analysis. H&E staining was conducted according to a standard technique,and the slides were evaluated to detect the microscopic changes in the tissue sections with the help of a bright-field microscope (Schimanski et al., 2025). Masson's trichrome staining was carried out to identify the deposition of the collagen fibres (Yan et al., 2023). The identification of the elastin fibres was completed using Weigert's elastic staining technique (Hernández-Morera et al., 2017).

Immunohistochemistry: Full-thickness skin tissue was collected from the wound site at 7 and 14 days for CD-31 expression and 14 and 21 days for type I collagen estimation and was examined by the Vectastain ABC Kit (Vector Laboratories, U.K.) according to the standard protocol.

Biochemical assessment of hydroxyproline in tissue: Quantitative estimation of hydroxyproline in the wound area acts as a well-established indicator for collagen deposition. Tissue samples were obtained on days 7, 14, and 21 post-treatment from all the groups, i.e., AgNPs-Fcol-Lys-based gel and spray-treated groups, standard treatment groups, and the untreated group. The hydroxyproline content of the retrieved tissue samples was quantified using a Hydroxyproline (HPY) Colorimetric Assay Kit (Elabscience, China) based on the manufacturer's instructions.

Statistical Analysis: Four samples (n=4) were analyzed for each experiment unless otherwise quantified. Data were analysed statistically with the help of Origin 2021b SR2 software. A two-way ANOVA followed by Tukey's multiple comparisons test was used to compare the data between the groups, with $p<0.05$ considered statistically significant.

RESULTS

Cell culture and cell viability assay: The MTT assay revealed no significant difference in absorbance values (Fig. 1B) when comparing cell viability in treated wells to that in control wells. On days 1 and 3, no substantial difference was noticed between the control and treated group. Between the AgNPs-Fcol-Lys- gel-based group and the control group on day 7, a statistically significant difference in cell viability was noticed. Notably, the difference between the treated groups remained statistically nonsignificant on day 7. The FDA assay (Fig. 1A) additionally validated the MTT findings, showing a comparable degree of cell proliferation in all the groups from day 1 to day 7. But the control group exhibited moderate improvement in cell density over time, whereas both the treated groups revealed higher cellular density with uniform cell distribution, particularly on day 7.

Hemocompatibility study of Shilajit- synthesised AgNPs, fish collagen, and L-lysine-based gel and spray: As demonstrated in Fig. 2D, the positive control induced 100% haemolysis of RBCs, while AgNPs-Fcol-Lys-based gel (1.31±0.335%) and spray (1.43±0.130%) formulations showed negligible

haemolysis comparable to the negative control. A highly significant difference was noticed between all other groups and the positive control, signifying the superior hemocompatible nature of the prepared formulations. Moreover, the absence of a substantial difference between the formulated gel and spray confirmed their hemocompatible nature and biological safety.

Antimicrobial assay of Shilajit -synthesised AgNPs: In the current investigation, green-synthesised silver nanoparticles were found to be highly effective against both *E. coli* (Gram-negative) and *S. aureus* (Gram-positive). Notably, larger inhibition zones were observed against *E. coli* at a 50 µg/mL concentration, resulting in greater antibacterial activity than at 100 µg/mL, demonstrating a concentration dependent response.

Oscillatory Rheology of gel: Rheological analysis in oscillation mode helps us to better understand the simultaneous viscous and elastic characteristics of the gel. Furthermore, oscillatory tests are preferred when determining the shelf-life stability of gels because their microstructure is preserved upon application of force in the LVE range (Mezger, 2020). From the amplitude sweep test, elastic and viscous behaviour of the gel reflected by G′ and G″, respectively, are presented in Fig. 1(C). The crossover point of the two moduli indicates the change from solid-like (elastic-dominant) to liquid-like (viscous-dominant) behaviour of the gel. The region of constant G′ and G″ indicates the LVE region and is followed by the region of permanent deformation. LVE region is observed for the shear strain values ranging from 0.01% to 10%, thereby the graphs deviate from their constant values and tend to intersect as the shear strain reaches 100%.

***In vivo* study**

Quantification of wound contraction rate: Measurement of wound area reduction rate (Fig. 3 & Fig. 4) showed that both prepared formulations significantly enhanced the wound closure rate in burn and diabetic models, outperforming the standard treatments and control groups. During the burn wound repair, it was noticed that the spray formulations showed a significant development in wound contraction rate among all groups and attained almost complete wound closure by 21 days post-operatively (97.46±1.75%) (Fig. 3A & 3B). Similarly, in the diabetic wound model (Fig. 4A & 4B), both AgNPs–Fcol–Lys gel and spray formulations reported significant enhancement in wound contraction from day 3 to day 10, with the spray formulation showing relatively faster wound closure and achieving the highest contraction rate at day 21 post-operatively. The standard groups showed modest improvements in contraction rate but exhibited lower efficacy than the AgNPs–Fcol–Lys formulations.

Histopathological examination of the extracellular matrix

Full thickness burn wound: The histological analysis of H&E staining (Fig. 5A) for burn wound healing exhibited slow progression in healing in the control group, with extensive inflammatory cell infiltration, sparse, thin, and loosely arranged collagen fibres, and incomplete epithelialization even by Day 21. Standard gel and spray improved healing, with the gel performing slightly better in collagen organization and epithelial coverage. On the contrary, AgNPs–Fcol–Lys treatments markedly accelerated repair, with dense, parallel collagen fibres, complete epithelial regeneration resembling normal dermal architecture at 14 days post-treatment. Both AgNPs-Fcol-Lys gel and AgNPs-Fcol-Lys spray showed accelerated healing, with the spray form consistently outperforming all groups-demonstrating earlier reduction in inflammation, faster granulation tissue maturation, denser and more organized collagen deposition, and complete epithelial regeneration with keratinization by Day 21.

Evaluation of collagen fibre (Fig. 6A) deposition and remodelling was assessed by Masson's Trichrome staining, which indicated a gradual rise in collagen deposition and maturation in all the treatment groups, with the best results noticed in the AgNPs-Fcol-Lys-based gel and spray groups with the spray formulation promoting the earliest and well-organised deposition by Day 7 and achieving a complete, fully compact collagen architecture by Day 21, closely resembling normal skin connective tissue.

AgNPs–Fcol–Lys treatment, particularly the spray (Fig. 8A), confirmed the highest elastin content with thick, elongated fibres arranged in parallel with slight inflammation, signifying early remodelling activity. On day 14, compared to all the treatment groups, the AgNPs-Fcol-Lys formulation, particularly the spray form, resulted in mature and thick elastin fibres with a highly organised parallel arrangement and minimal inflammation, closely resembling normal dermal elastin architecture. AgNPs–Fcol–Lys treatment, especially the spray, achieved near-complete elastin restoration compared to control and standard treatment groups, with complete, highly organized bundles by

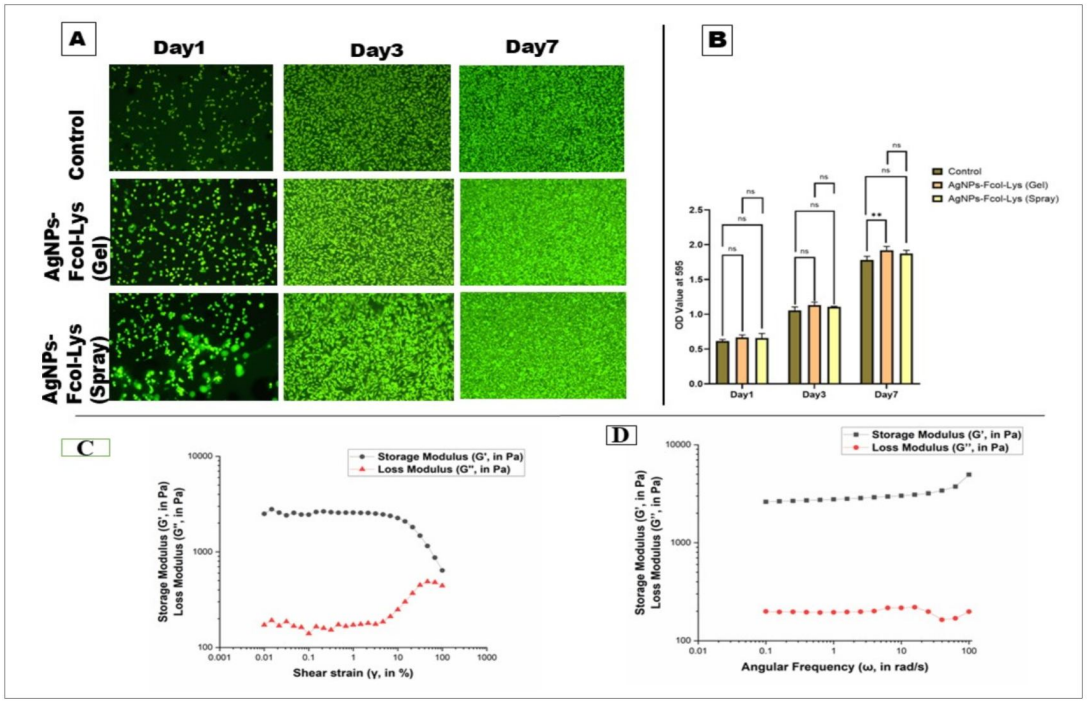


Fig.1 (A) Fluorescence microscopy images of the FDA assay in the L929 cell line; (B) MTT assay of AgNPs-Fcol-Lys gel and spray on L929 cells at day 1, 3 and 7; Rheological characterization of AgNPs-Fcol-Lys gel using (C) amplitude sweep test, and (D) frequency sweep test

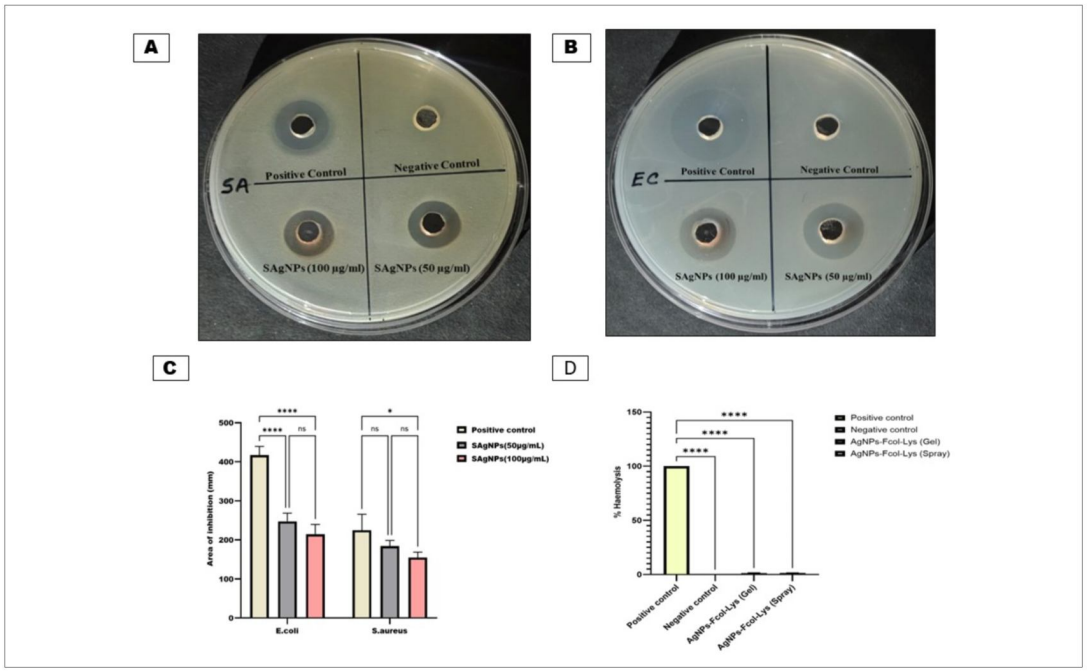


Fig. 2. Antimicrobial activity of Shilajit -synthesized AgNPs against (A) *S. aureus*, and (B) *E. coli*, (C) Graphical representation showing the level of significance between the treatment groups in antimicrobial assay, (D) Graphical data showing hemocompatibility assay, signifying haemocompatible nature of the formulations

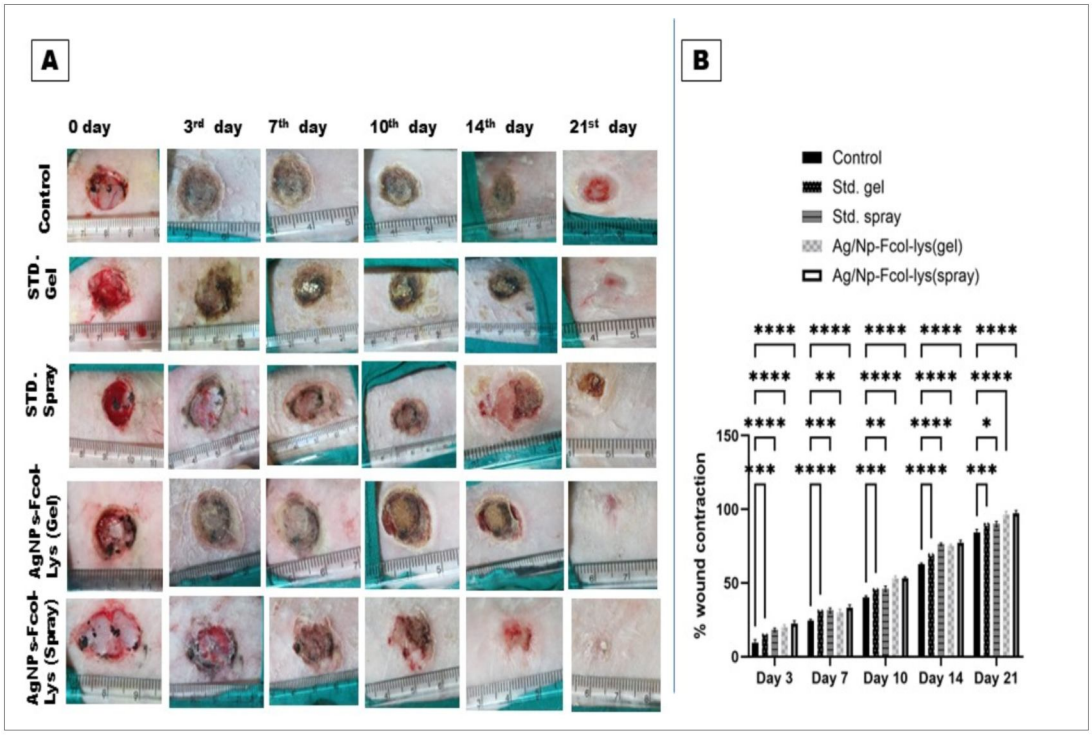


Fig. 3. (A) Macroscopic evaluation of gross wound contraction in burn wounds at 0, 3, 7, 10, 14, and 21 days. (B) Graphical illustration of wound closure percentage over time

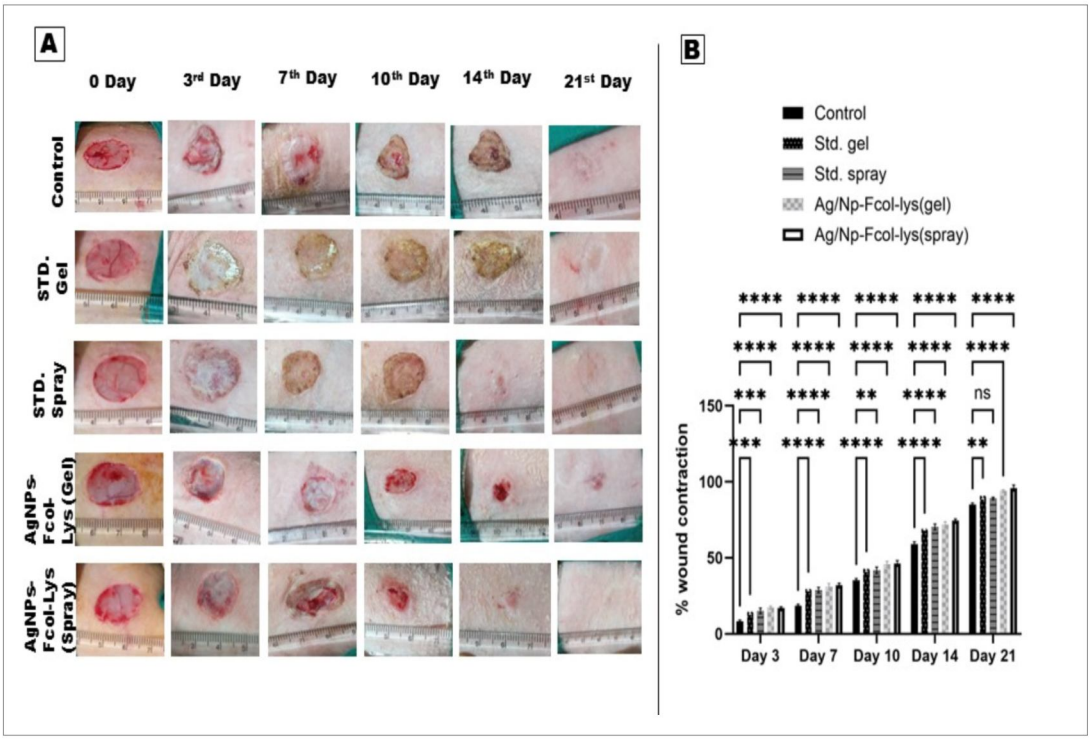


Fig. 4. (A) Macroscopic images of wound contraction rate in diabetic wound at days 0, 3, 7, 10, 14, and 21; (B) Graph showing wound contraction percentage over time

Day 21, histologically approaching normal dermal elastic architecture.

Diabetic wound: AgNPs-Fcol-Lys treatments (Fig. 5B) confirmed superior healing throughout the study period, with the spray form remarkably showing early inflammatory resolution with denser and organised collagen deposition and more advanced epithelial regeneration compared to the gel. By Day 21, the wound treated with AgNPs-Fcol-Lys (spray) exhibited regenerative skin with a complete histological architecture similar to normal skin, featuring a perfectly keratinised epithelium and a restored dermal-epidermal junction, surpassing all other groups.

Both AgNPs-Fcol-Lys formulations (Fig. 7A) showed compact, well-organised collagen deposition with reduced inflammation. By day 21, these formulations markedly enhanced collagen synthesis and remodelling, with the spray formulation constantly producing the densest, best-aligned collagen bundles and near-complete dermal regeneration.

Among experimental groups, both AgNPs-Fcol-Lys gel and spray (Fig. 8B) distinctly improved elastin deposition, fibre alignment, and integration, with the spray formulation showing superior effects at each stage. By Day 21, AgNPs-Fcol-Lys - spray group achieved the highest elastin density and most organised architecture, suggesting it provided the most efficient stimulation of elastogenesis and structural remodelling during diabetic wound healing.

Immunohistochemistry analysis

Burn wound: CD31 immunohistochemistry in burn wounds (Fig. 9A) revealed a time-dependent and treatment-dependent angiogenic response. By day 14, AgNPs-Fcol-Lys gel formulations exhibited regularly distributed and well-matured vessels. The presence of well-defined endothelium with stable lumen structure confirmed successful angiogenesis and remodelling in the treated group. While the groups received AgNPs–Fcol–Lys spray treatment displayed a restored dermal vascular pattern with reduced CD31 immunoreactivity, demonstrating successful vascular maturation and completion of remodelling. In summary, AgNPs–Fcol–Lys treated groups principally the spray, elicited the strongest, structurally organized, and well-regulated angiogenic response, compared to standard treatments and control, reflecting its advanced wound healing potential.

The evaluation of the collagen-by-Collagen type-I marker (Fig. 10A) further supported the healing efficiency of the AgNPs–Fcol–Lys formulations. Immunohistochemical estimation of the Collagen type-I demonstrated gradual enhancement in matrix deposition and remodelling among the treatment groups. Earlier investigations have reported delayed collagen maturation in wounds treated with conventional dressings relative to nanoparticle-based formulations (Mohseni et al., 2019). On post-operative day 14, AgNPs-Fcol-Lys–treated groups, notably those that received spray formulation, revealed strong CT-I immunopositivity with thick, dense and well-organised collagen fibres, signifying strong collagen synthesis and early wound healing. On the contrary, AgNPs-Fcol-Lys-based gel and spray displayed uniformly oriented, tightly packed, dense collagen bundles with insignificant inter-fibre spaces by day 21.

Diabetic wound: Immunohistochemical analysis of CD-31 (Fig. 9B) exhibited a clear and time-dependent angiogenic response in the diabetic model. AgNPs-Fcol-Lys–treated groups, notably the spray group, showed intense CD-31 positivity with well-organised and compactly packed and well-distributed endothelial-lined vasculature reflecting strong angiogenesis. Although there is a slight increase in CD-31 expression by day 14 in control groups, it remained sparse with incomplete vascular organization. Standard treatments exhibited moderate but incomplete vascular reorganisation. In addition to enhancing angiogenesis, our formulations also significantly improve collagen deposition and remodelling. AgNPs-Fcol-Lys gel-treated groups showed densely packed, strong and evenly arranged collagen fibres, while groups that received AgNPs-Fcol-Lys -Spray treatment, exhibited the most compact and well-organised collagen fibres, reflecting advanced remodelling. On day 21, the control group showed poorly organized irregular fibres with incomplete remodelling. Standard gel and spray groups revealed gradual improvement in fibre continuity and alignment with strong collagen expression (Fig. 10B). Whereas AgNPs-Fcol-Lys–treated wounds, predominantly the spray, displayed intense, evenly dispersed and well-structured fibres, resembling normal dermis, indicating progressive collagen deposition and nearly complete matrix remodelling.

Biochemical assessment of hydroxyproline in tissue: The evaluation of hydroxyproline content on days 7, 14, and 21 post-operatively showed an increased quantity from days 7-21 in both burn and diabetic wound models, indicating a gradual progression in collagen deposition. Among the two prepared

formulations, the AgNPs–Fcol–Lys -spray, particularly in the burn model, exhibited a considerable increase in hydroxyproline levels from the early stage of healing, which was preserved up to day 21 compared to the standard treatments and control (Fig. 6B). In the diabetic wound model, though there was an improvement in early collagen synthesis on day 7 in AgNPs–Fcol–Lys gel and spray-treated groups, a negligible variation was detected by day 14. By day 21, the AgNPs–Fcol–Lys spray formulations showed the maximum hydroxyproline level, signifying their role in collagen synthesis (Fig. 7B).

DISCUSSION

The outcomes of the MTT assay confirm the non-cytotoxic nature of the AgNPs-Fcol-Lys gel and spray formulations on L929 cells after 1st, 3rd and 7th days of treatment. The results of MTT and FDA strongly support the active role of AgNPs-Fcol-Lys formulation in enhancing wound healing. The improved cellular viability and even distribution highlighted the collaborative effects of Shilajit green-synthesised AgNPs, fish collagen, and L-lysine in supporting the L929 cell lines' growth and their proliferation. Our findings are consistent with the earlier results, which highlighted the similar pro-healing and biocompatible nature of AgNPs and collagen composites in wound healing (Rath et al., 2016; Stefan et al., 2021; Iurilli et al., 2025). Haemocompatibility findings are in agreement with a recent report, which found that biologically synthesised AgNPs can induce minimal lysis of erythrocytes and exhibit high biocompatibility in haemolytic assays when synthesised optimally. Composites prepared from AgNPs have exhibited minimal haemolysis with biological safety in advanced wound care formulations, highlighting their importance in clinical applications (Chahardoli et al., 2025; Kolarijani et al., 2025b). AgNPs have the potent ability to damage the bacterial cell wall by producing Reactive Oxygen Species. Moreover, it releases silver ions, which play a significant role in inhibiting replication of bacterial DNA and cellular respiration, supporting previous literature (Khalifa et al., 2025). The higher inhibition zone observed in *E. coli* (2B) relative to *S. aureus* (2A) is attributed to the thicker peptidoglycan layer present in *S. aureus*, which restricts the penetration and interaction with nanoparticles, as emphasized in previous studies (Chapa González et al., 2023). Taken together, the findings of our antimicrobial evaluation highlight the importance of green- synthesised AgNPs against numerous bacterial strains, while stressing the importance of adjusting their dose and formulation to improve efficacy against a wide range of bacterial pathogens. Frequency sweeps establish the characteristic behaviour of the sample as a viscous or viscoelastic fluid, a gel-like paste or a fully cross-linked material (Meyer, 2015). In other words, it predicts whether the material is elastic-dominant or viscous-dominant. In the frequency sweep test (Fig. 1D), G′ exhibited higher values compared to G″, indicating a predominant solid like behaviour (Budai et al., 2023; Gefen et al., 2025). The low fluctuations in G′ and G″ in the LVE region suggest a stable microstructure of the gel. Furthermore, the non-intersection of G′ and G″ in Fig. 1C implies that the material is chemically crosslinked and the affinity is not dependent on angular frequency. The AgNPs–Fcol–Lys spray formulation exhibited superior results amongst all the treatment groups in encouraging wound contraction in burn and diabetic models, underlining its importance as a potential delivery platform in wound healing. These reports are consistent with the recent findings, demonstrating that the incorporation of silver nanoparticles with other biomaterials increases wound contraction and tissue remodelling in vivo studies. Nanoparticles exert their action through anti-inflammatory and antimicrobial activity, which facilitates fibroblast proliferation and ECM remodelling. (Nqakala et al., 2021; Kaya et al., 2025). Collagen extracted from fish acts as a bio-friendly scaffold to support improved granulation tissue formation and enhance wound closure, which strongly supports our findings (Shalaby et al., 2020). Overall, the histopathological examination of the extracellular matrix indicated the healing efficacy of AgNPs-Fcol-Lys-based gel and spray preparations. Although both the preparation has shown potent effects in wound healing, the performance of the spray formulation was found to be slightly better than the gel, which may be credited to its ability to form even, breathable films over the wound bed that maintain moisture balance and enhance gaseous exchange (Afshar et al., 2024). Among the different explored biomaterials, the synergistic action of green -synthesised silver nanoparticles, fish collagen, and L-lysine support wound healing by adjusting the wound environment (Nakamura et al., 2019). Antimicrobial activity of biologically synthesised nanoparticles plays a pivotal role in preventing biofilm development, a key challenge often encountered in burn wound healing (Balaure et al., 2025). Moreover, AgNPs–Fcol–Lys gel and spray formulation significantly enhances collagen deposition in both the models, reflecting their superior remodelling efficacy.

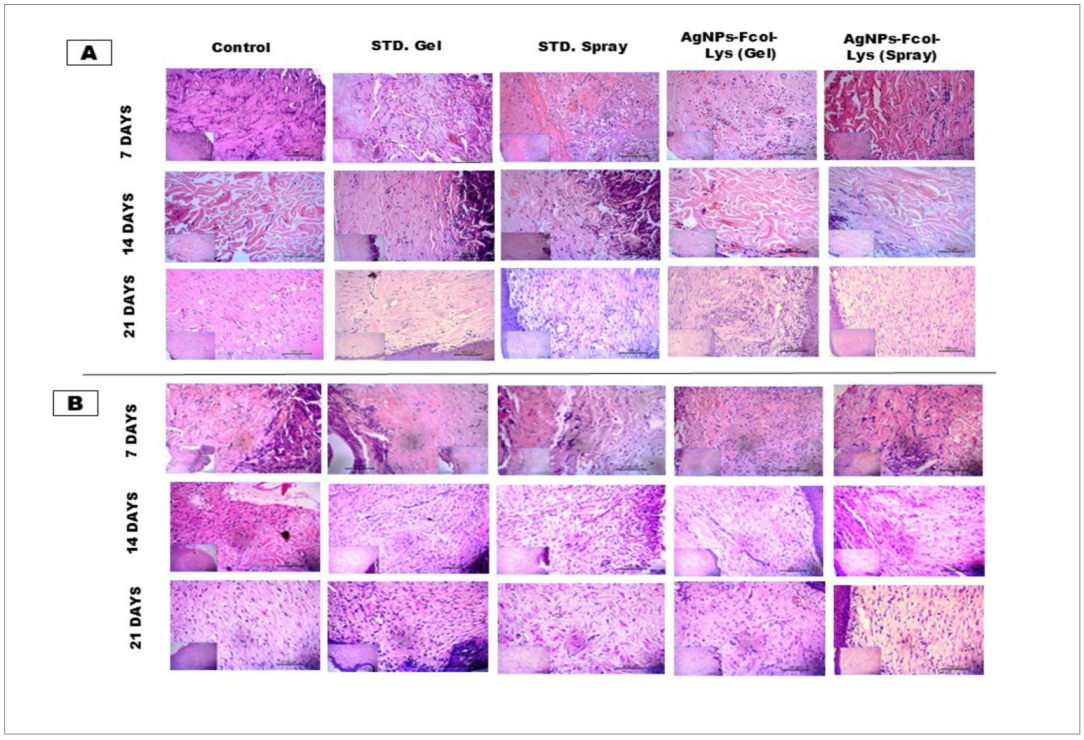


Fig. 5. Histological evaluation of Haematoxylin and eosin-stained sections in rabbit (A) burn and (B) diabetic wound tissues at 7-, 14-, and 21-days post-treatment

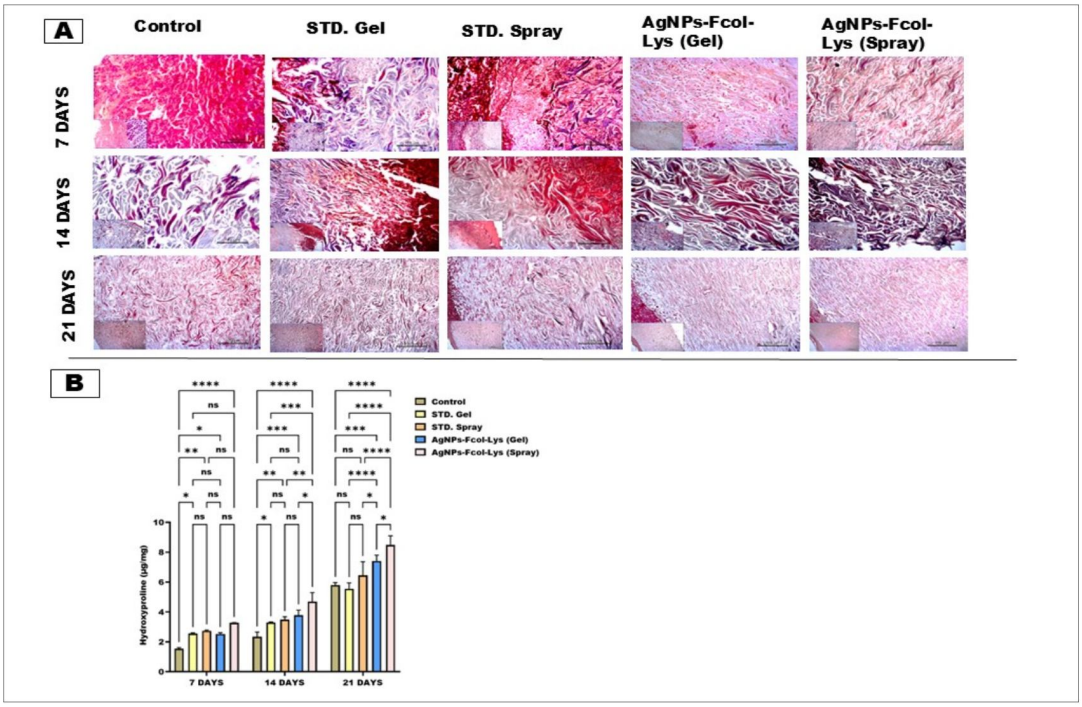


Fig. 6. (A) Histological representation of Masson's Trichrome-stained sections, (B) Graphical data showing hydroxyproline level in burn wounds at day 7th, 14th, and 21st

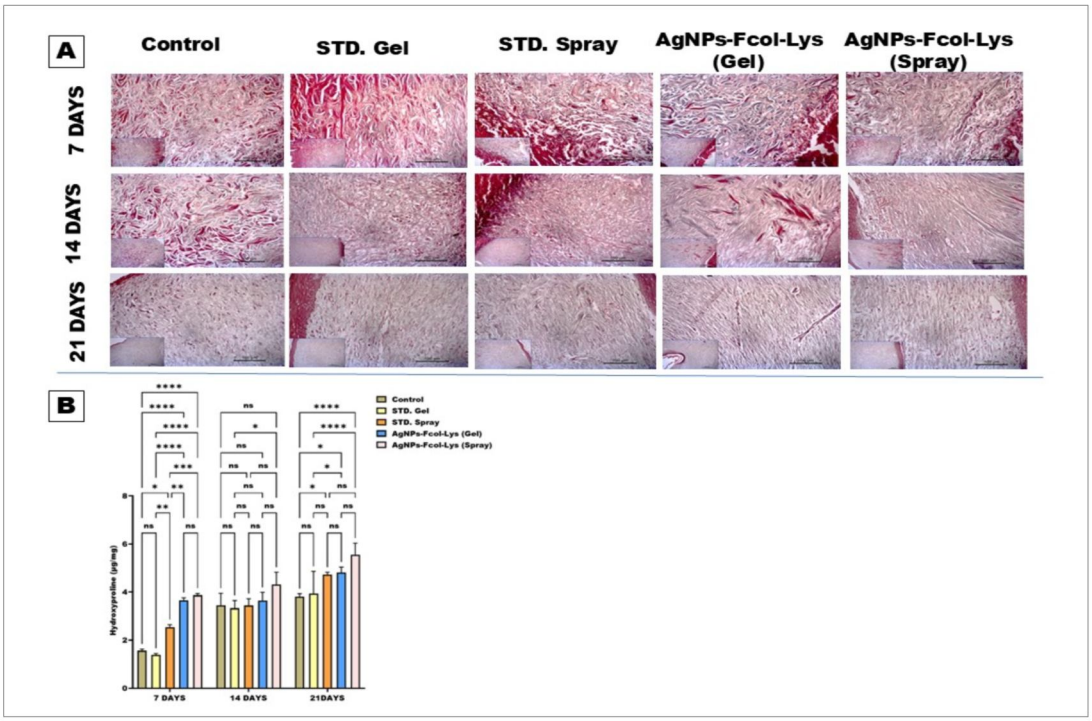


Fig. 7. (A) Histological representation of Masson's Trichrome-stained sections, (B) Graphical data showing hydroxyproline level in diabetic wounds at day 7th, 14th, and 21st

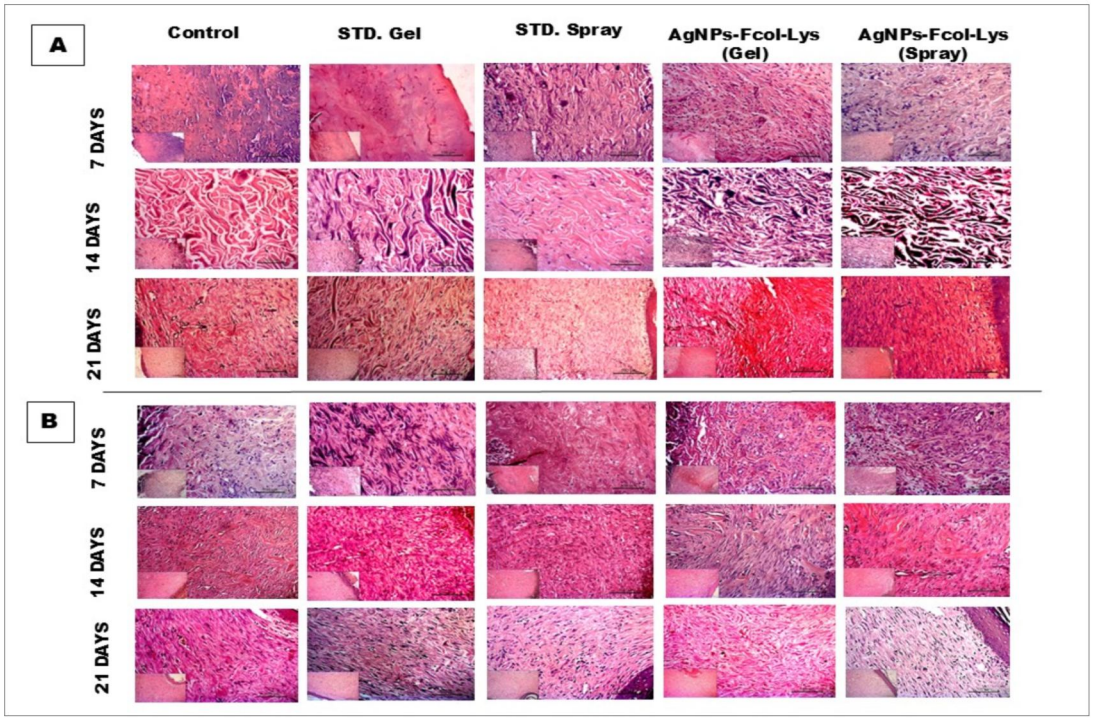


Fig. 8. Histological sections showing highly organised, mature and thick elastin fibres in (A) burn and (B) diabetic wound tissues

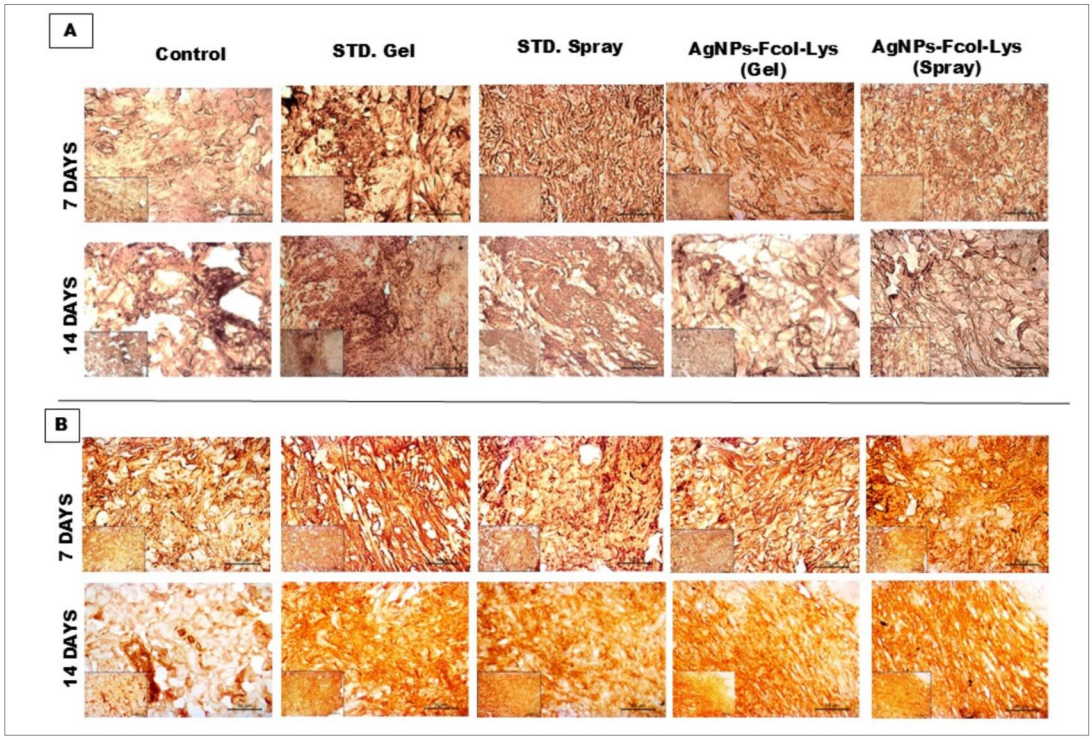


Fig. 9. Immunohistochemistry analysis of CD-31 antibody in (A) burn and (B) diabetic wound tissues

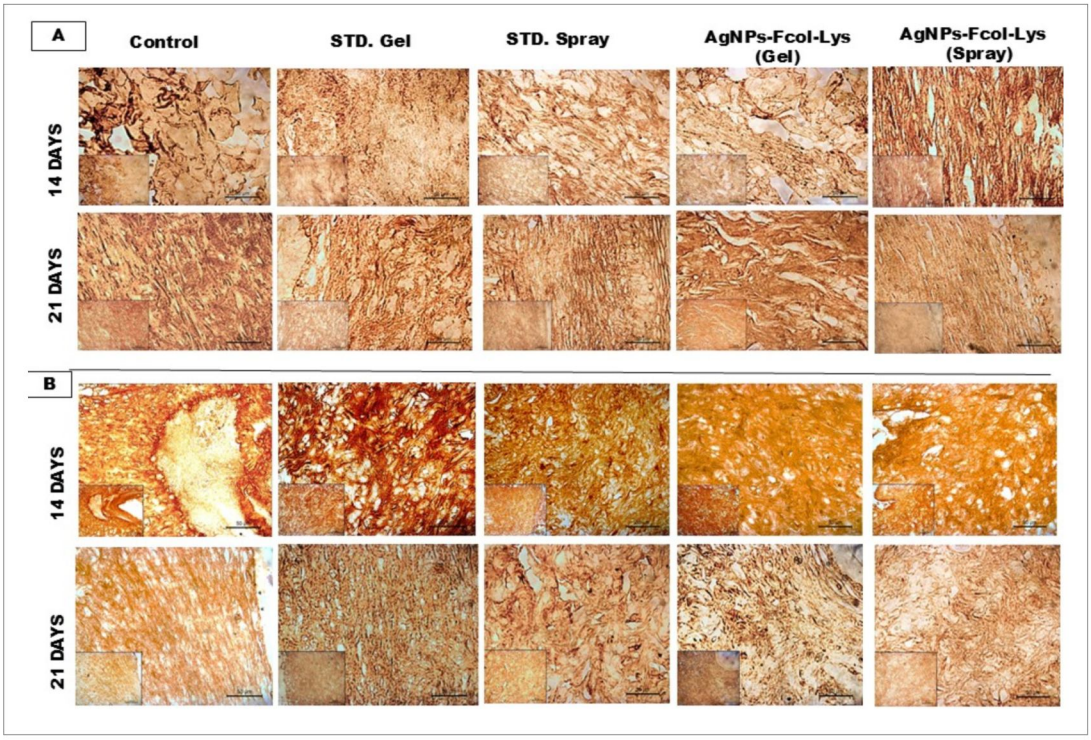


Fig. 10. Representative image of collagen type-I IHC sections of (A) burn and (B) diabetic wound tissues at 14 and 21 days

Furthermore, this study also reported enhanced elastin deposition with a well-organised structure, which reflects the progressive wound remodelling, which is in accordance with earlier findings (Lima et al., 2022). In addition, L-lysine present in our formulation is critical for tissue fibroblast production and enhances tensile strength of the healed epithelium, which is in accordance with previous findings (Albaugh et al., 2017).

AgNPs–Fcol–Lys–treated wounds, particularly the spray, demonstrated strong CD31 immunopositivity with plenty of microvascular network and active endothelial lining, reflecting accelerated angiogenesis, consistent with the previous findings highlighting the pro-angiogenic effects of dressing materials based on silver nanoparticle (Tian et al., 2007). These results are consistent with earlier studies that conventional dressings alone fail to induce neovascularization in diabetic models, likely due to prolonged microenvironmental dysfunction (Zhang et al., 2023). In contrast, AgNPs-Fcol-Lys gel and spray confirmed improved and extensive CD-31 immunoreactivity with thick, mature vessels that were distributed. The spray-treated wounds restored normal vascular architecture, demonstrating the strong and sustained angiogenetic progression in diabetic models. The enhanced vascularization reported with AgNPs–Fcol–Lys formulations demonstrate the role of nanoparticles based wound dressing in promoting angiogenesis and neovascularization in diabetic wounds by endothelial function and reducing inflammatory barriers (Meng et al., 2024). Moreover, vascularisation, collagen deposition and re-epithelialization are also supported by fish collagen as reported earlier (Wang et al., 2023). Formulations containing silver nanoparticles, which encourage collagen synthesis and deposition, have been extensively studied in wound healing models (Keleştemur et al., 2012). These results are aligned with earlier reports, highlighting the significant role of silver nanoparticles in endothelial interactions and modulation of angiogenic signalling pathways and enhancement of angiogenesis and wound remodelling (Kang et al., 2011). These findings are consistent with the reports that nanoparticle based wound formulations enhance collagen deposition, organisation, and ECM remodelling in diabetic models, surpassing traditional treatments (Sethuram & Chandrasekaran, 2025).

The superior angiogenic and collagen remodeling responses observed with AgNPs–Fcol–Lys formulations are mediated by the synergistic interaction of silver nanoparticles, fish collagen, and L-lysine (Rath et al., 2016). Bhetki fish collagen additionally acts as a biological scaffold for encouraging fibroblast proliferation and matrix deposition (Panggabean et al., 2023). Furthermore, L-lysine plays an important role in wound healing by enhancing collagen cross-linking and stabilization, thus collectively improving tissue remodelling and structural organization (Cai et al., 2017).

The quantification of hydroxyproline content showed superior collagen deposition in AgNPs–Fcol–Lys groups compared to control and standard treatments, in line with recent literature that silver nano particle dressings improve collagen synthesis and remodelling by regulating inflammation and extracellular matrix deposition (Subashini et al., 2025). Moreover, the role of L-lysine in collagen synthesis and matrix remodelling cannot be overlooked, as it supports wound closure in chronic wounds, which agrees with the results (Holeček, 2025).

The AgNPs–Fcol–Lys formulations, exclusively in spray form, significantly encourage healing of burn and diabetic wounds by fostering continued and early collagen and elastin deposition, angiogenesis, well-structured ECM formation, and complete epithelial restoration. The spray formulation showed superior results to the gel, which may be attributed to its ability to form uniform, breathable layers that preserve skin moisture and increase gaseous exchange. In comparison to control and standard treatments, formulation prepared with AgNPs, fish collagen and L-lysine constantly reduced inflammation, supported fibroblast movement, and enhanced remodelling of tissue across all evaluated time points. Collectively, the findings emphasize the therapeutic efficacy of green-synthesised silver nanoparticles in combination with fish collagen and L-lysine as a multifunctional approach to wound management.

Conflict of interest: The authors have no conflict of interest in this study.

Author's contribution: RD, PG, SR: Acquisition of data and writing the draft; PD, SKN: Conception and design; CBR, AS, SB: Investigation and performed statistical analysis of data; SM, TA: Performed interpretation of results; SKN: Acquisition of funds and finally edited and corrected the manuscript.

Data availability statement: All data supporting the research findings are provided in the paper. The corresponding author is willing to provide the raw data upon reasonable request.

Ethical approval: All the experimental protocols were undertaken in compliance with the guidelines provided

by the Institutional Animal Ethics Committee of the West Bengal University of Animal and Fishery Sciences (Approval No. 763/GO/ReS/ReRc-L/03/CCSEA/68/68/2023-2024).

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